Nanowires

DOI: 10.1002/anie.200604461

The I-Tetraplex Building Block: Rational Design and Controlled Fabrication of Robust 1D DNA Scaffolds through Non-Watson–Crick Interactions**

*Harshad B. Ghodke, Ramya Krishnan, Kasinath Vignesh, G. V. Pavan Kumar, Chandrabhas Narayana, and Yamuna Krishnan**

Nanowires with attractive properties such as high persistence length and structural rigidity as well as with electrical, photonic, and plasmonic properties can act as structural and functional components in complex nanoscale devices. Nanowires have been fabricated by chemical reduction,[1] one-dimensional self-assembly of DNA,[2] from proteins,[3] and even from viral particles.[4] The conductivity of such wires has been enhanced by metalization, where metals have been "coated" or "moulded" onto the outer or inner surfaces of biomolecular templates.[5] Bottom-up assembly of Watson–Crick base-paired duplex DNA has been a powerful and preferred approach thus far to fabricate scaffolds in the field of structural DNA nanotechnology.[6] However, in this regard, with a few exceptions, the potential of non-Watson–Crick base-paired building blocks to yield DNA superstructures has not been similarly exploited.[7,8] We have been interested in developing new four-stranded building blocks[9,10] for applications in structural DNA nanotechnology. Herein we present a strategy to build 1D scaffolds with highly desirable characteristics by using a four-stranded DNA building block called the i motif. The i motif, or i tetraplex, consists of two parallel-stranded duplexes, each held together by $C^+H{-}C$ base pairs, intercalated in an antiparallel orientation.[11] Given that "slippage" in G quadruplexes can lead to formation of an extended quadruplex,[10] we reasoned that this property could be exploited to achieve bidirectional extension in i motifs to give 1D wirelike structures.

We used the poly-cytosine sequence $^{5'}CCCCCCC^{3'}$ ($d(C_7)$) to facilitate the formation of higher order structure (HOS). These sequences excluded non-C bases at their termini, as these have been shown to block HOS formation.[10] Samples of 500 μM $d(C_7)$ in 100 mM phosphate buffer at pH 5.5 were annealed to promote formation of the i motif, incubated, and checked for HOS formation by atomic force microscopy (AFM). Figure 1 shows an AFM image from a typical sample deposited on mica. Importantly, we observe a profusion of 1D structures with lengths on the micrometer scale which we refer to as *I* wires. The lengths of the *I* wires are polydisperse, with average lengths of 0.5–0.6 μm (see the Supporting Information), and some reaching up to 3 μm. Notably, all the *I* wires showed highly uniform diameters ((2.6 ± 0.3) nm) throughout the length of the wires (see the Supporting Information),[12] which were in good agreement with those of i motifs, as obtained by X-ray crystallography[13] and solution NMR spectroscopy.[14] The uniformly high diameters of the *I* wires suggest that they are not single-stranded or duplexed DNA. The remarkable uniformity in the diameter also suggests that the *I* wires could possibly be formed by the ordered self-assembly of a given unit of $d(C_7)$ to yield a 1D superstructure.

To establish the nature of the soluble oligomers that led to the formation of the *I* wires, the self-assembly was followed as a function of time by circular dichroism (CD) spectroscopy (see the Supporting Information). These studies revealed that the soluble oligomers showed molecular signatures corresponding to i motifs. Two possible scenarios may be envisaged for such soluble i-motif-like oligomers to propagate bidirectionally into *I* wires. These would involve: 1) association of "blunt-ended" i motifs stacked end to end, or 2) the formation of "slipped" or imperfectly formed i motifs that could template the association of monomers through overhangs at either end to give rise to an interlocked, extended i motif. The first possibility is entropy disfavored and is less probable. In the second case, the molecular arrangement in an i motif would require the strand polarity to be maintained for further extension. This would imply that the 5′-PO_4 and 3′-OH termini of the *I* wires generated from 5′-PO_4-$d(C_7)$ ($d(pC_7)$) would be positioned contiguously along the length of an *I* wire. Chemical ligation of these termini using *N*-cyanoimidazole (NCI)[15,16] should then lead to the continuous phosphodiester segments with lengths representative of the lengths of the parent I-wires. We carried out chemical ligation of aliquots at different time points from a sample of 500 μM $d(pC_7)$ assembling into *I* wires. We then denatured the wires into their component single strands and analyzed the fragment sizes by gel electrophoresis (Figure 2). It was apparent that the fragment sizes had vastly increased from the starting monomer, thus suggesting that: a) there is an ordered

[*] H. B. Ghodke, R. Krishnan, K. Vignesh, Dr. Y. Krishnan
National Centre for Biological Sciences, TIFR
Bellary Road, Bangalore, 560065 (India)
Fax: (+91) 80-2363-6662
E-mail: yamuna@ncbs.res.in
G. V. P. Kumar, Dr. C. Narayana
Light Scattering Laboratory
Chemistry and Physics of Materials Unit
Jawaharlal Nehru Centre for Advanced Scientific Research
Jakkur, P.O. Bangalore 560064 (India)

[**] We thank G. V. Shivashankar for critical comments, F. M. Hameed for technical assistance, S. S. Indi for TEM images, and the interunit Collaborative project, JNCASR. This work was funded by the Nanoscience and Technology Initiative of the DST, Govt. of India.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

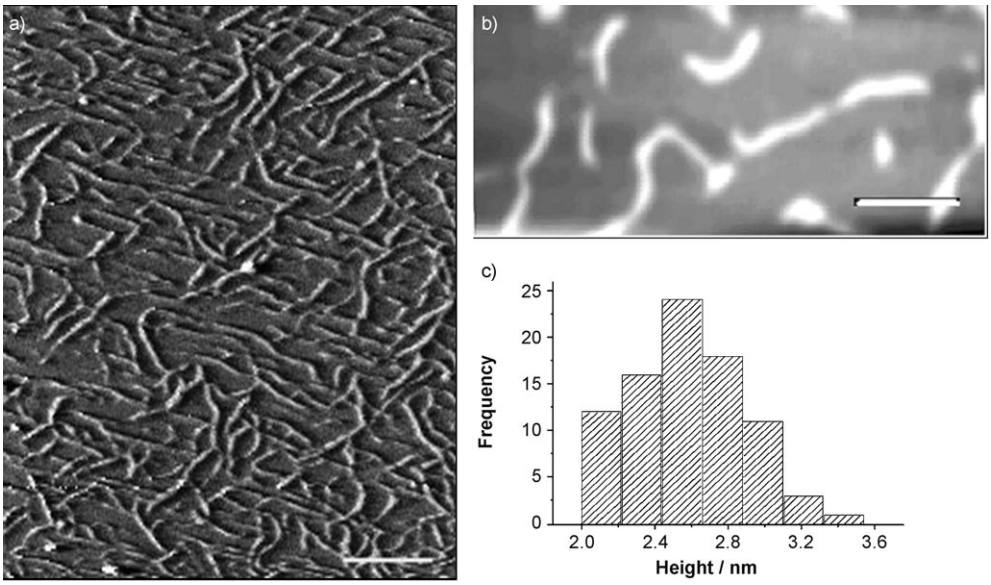

Figure 1. a) and b) Tapping mode AFM images of 500 μM d(C_7) pH 5.5 deposited on mica. Scale bar: a) 1 μm; b) 0.5 μm. c) Histogram showing the height of a sample with 85 distinct wires.

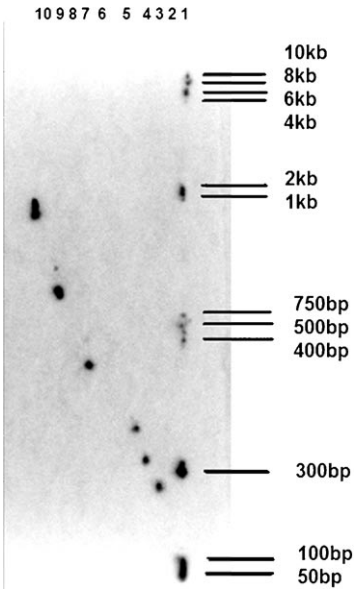

Figure 2. Denaturing polyacrylamide gel electrophoresis (PAGE) analysis of chemically ligated *I* wires as a function of incubation time. The *I* wires were incubated, sampled at various incubation times (t), chemically ligated with NCI, denatured by heating for 3 h at pH 8.0, run on 4% denaturing PAGE for 2.5 h at 400 V, and stained with SYBR Green. Lane 1: Novagen 0.05–10 Kbp marker; lane 2: $t = 3$ days; lane 3: $t = 5$ days; lane 4: $t = 7$ days; lane 6: $t = 9$ days; lane 8: $t = 12$ days; lane 10: $t = 15$ days; lanes 5, 7, and 9 were not loaded with sample.

arrangement of strands on the *I* wire, and b) the strand polarities are preserved in this arrangement. The average length of a sample examined after 15 and 21 days correspond to approximately 1.5 kb and 2 kb of duplex DNA, respectively (see Figure 2 and the Supporting Information).[17] These lengths are consistent with the micrometer-length wires observed by AFM. The growth of an *I* wire is a relatively slow but continuous process, as revealed by the increase in length over a 28-day period (see the Supporting Information).

We investigated these wires using surface-enhanced Raman spectroscopy (SERS) to confirm that the local structure along the length of the *I* wire is indeed an i tetraplex. Raman spectroscopy is a diagnostic tool that has been used to characterize i-motifs,[18] and in the presence of gold nanoparticles, molecules show unusually large Raman signals, widely referred to as SERS.[19] Transmission electron microscopy (see the Supporting Information for details) revealed that when gold nanoparticles were added to a solution of *I* wires, the latter were templated uniformly and preferentially along the *I* wire (Figure 3). Thus, *I* wires in the presence of gold nanoparticles[20] showed an increase in the Raman signal in the modes of vibration characteristic of the DNA_4 i motif as a result of surface-enhanced resonance (Figure 4). The peaks at 1385 and 1542 cm^{-1} are characteristic of hemiprotonation of cytosine residues, as in C-H^+-C base pairs. The peaks at 804, 852, 888, and 972 cm^{-1} are characteristic Raman markers of the unique DNA backbone arrangement found only in i motifs.[18] These results confirm that the local structure along the length of the *I* wire is indeed an i motif. The enhancement in the relevant modes of vibration in SERS reaffirms that the *I* wire is structurally intact, despite association with inorganic substrates such as gold, thus demonstrating its robustness for use as a scaffold to immobilize metal nanoparticles in a 1D arrangement through electrostatic interactions. The free energies of stabilization for every four cytosine and guanidine residues in an i motif and G quadruplex, respectively, are comparable.[21,22] Given that G quadruplexes are not denatured on mica through the pinning effect, it is not surprising that i motifs also retain their structural integrity.

We thus propose that the formation of *I* wires could occur in two stages: nucleation followed by growth. Nucleation involves the possible formation of a nucleus or "slipped" i motif,[23] with imperfect C-H^+-C base pairing and/or incomplete intercalation. Thus the nucleus could have unpaired cytosine residues and/or non-intercalated sites projecting at opposite ends of the i motif core (Figure 3d), which function as "sticky ends". Growth would involve an increase in the length of the nucleus by the templated and sequential assembly of monomer sequences onto both ends of the nucleus to form a highly extended i motif and, eventually, an

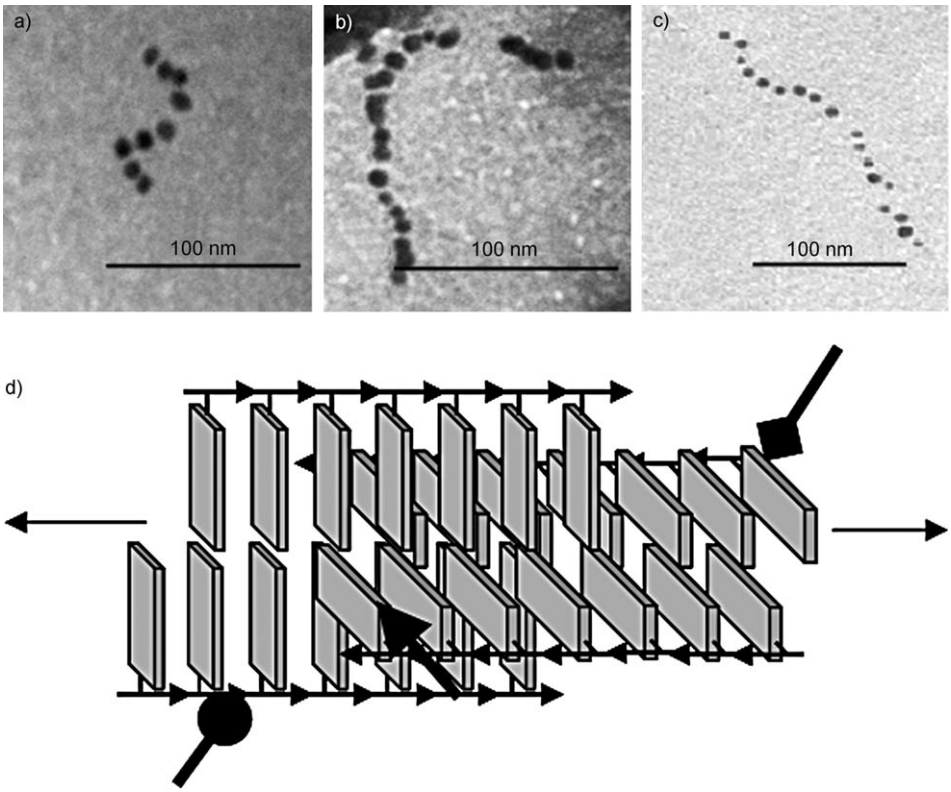

Figure 3. a–c) Transmission electron micrographs showing gold nanoparticles templated on: a) unligated and b), c) chemically ligated *I* wires. d) Schematic representation of the hypothetical "nucleating species" illustrating up to three different types of sites indicated by the different arrowheads: circle: base-pairing present but no intercalation; triangle: intercalation present but no base pairing; diamond: No base pairing or intercalation present. Monomer sequences can assemble on these sites sequentially thereby allowing extension of the nucleus in the direction indicated by the horizontal arrows.

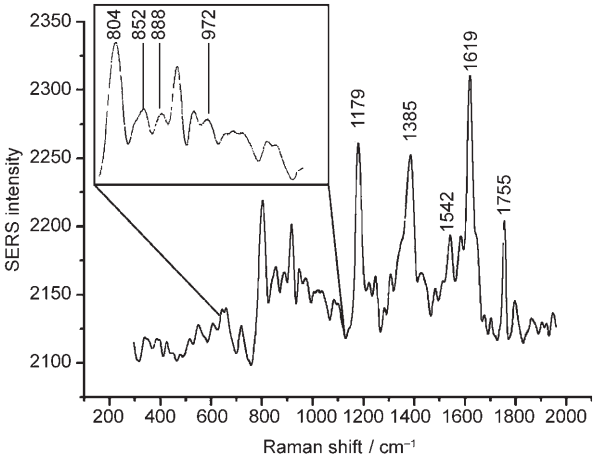

Figure 4. Surface enhanced Raman spectrum of *I* wires in the presence of 30–50 nm gold nanoparticles. Bands characteristic of the DNA_4 i motifs formed from poly-C sequences and corresponding to hemiprotonated cytosines are labeled. The inset shows bands ($\pm 3\ cm^{-1}$) arising from the unique sugar–phosphate arrangement seen only in DNA_4 i motifs.

I wire. By using a cytosine-rich heptanucleotide and a rationally designed self-propagation strategy based on non-Watson–Crick base pairing, we have formed a structurally unique supramolecular scaffold, the *I* wire. Detailed structural characterization of these wires using AFM, CD, SERS, and chemical ligation followed by gel electrophoresis reveal that this scaffold is made up of interlocked and extended i motifs. The structure of the *I* wire is not disrupted on mica surfaces and the *I* wires have aspect ratios ranging from 1000 to 1500. This building block is amenable to external control, which has been demonstrated by controlling the wire lengths through a combination of chemical ligation followed by dilution. This control is aided by its slow and templated growth, which may be locked in place and extension stopped at any time. As the *I* wires retain their structural integrity on surfaces they are ideal for surface applications. Their resistance to thermal denaturation makes them ideally suited for applications at temperatures greater than the melting point of duplex DNA. Furthermore, 1) the *I* wire has a continuous core of protons in a linear arrangement, and 2) an inter-nucleobase spacing of about 1.5 Å as a result of intercalation—which is not found in any other secondary structure adopted by DNA. These features could potentially endow very different properties for both electron and hole transport along DNA. This is a new design strategy, which uses a rational, non-Watson–Crick self-assembly route to create high quality 1D DNA scaffolds with tetraplex building blocks, thereby illustrating the untapped potential of alternative building blocks in structural DNA nanotechnology.

Experimental Section

Sample preparation for AFM: Sample solution (3 μL) was allowed to adsorb onto a freshly cleaved mica surface for 1 min. The sample was then washed with MilliQ water and rapidly dried under a stream of nitrogen (2 min) and under vacuum (20–60 min). A PicoPlus AFM system (Molecular Imaging, Tempe, Arizona) with a multipurpose scanner was used for imaging in air. Silicon nitride (Molecular Imaging) cantilevers with spring constants of $42\ N\,m^{-1}$ and a resonant frequency of 320 KHz were used for intermittent contact imaging (Acoustic AC mode). Contact mode imaging was carried out using cantilevers (Molecular Imaging) with a spring constant of $0.07\ N\,m^{-1}$. All heights were measured using PicoScan software.

SERS measurements: Raman spectra were recorded in solution by depositing the sample in a depression slide. For SERS, citrate-reduced Au nanoparticles (30–50 nm), prepared by a standard protocol,[24] were added to a solution of the *I* wires in a 95:5 ratio (v/v) to achieve a sample volume of 100 μL. The spectral accumu-

lation time was 180 s (see the Supporting Information for set-up details). The spectral resolution for this experiment was 3 cm^{-1}.

Received: October 31, 2006
Published online: March 2, 2007

Keywords: DNA · i motif · nanostructures · scanning probe microscopy · self-assembly

[1] K. K. Caswell, C. M. Bender, C. J. Murphy, *Nano Lett.* **2003**, *3*, 667 – 669.
[2] H.-W. Fink, C. Schönenberger, *Nature* **1999**, *398*, 407 – 410.
[3] a) H. Yan, S. H. Park, G. Finkelstein, J. H. Refi, T. H. LaBean, *Science* **2003**, *301*, 1882 – 1884.
[4] C. Mao, C. E. Flynn, A. Hayhurst, R. Sweeney, J. Qi, G. Georgiou, B. Iverson, A. M. Belcher, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 6946 – 6951.
[5] a) L. Berti, A. Alessandrini, P. Facci, *J. Am. Chem. Soc.* **2005**, *127*, 11216 – 11217; b) E. Braun, Y. Eichen, U. Sivan, G. Ben-Yoseph, *Nature* **1998**, *391*, 775 – 778; c) T. Scheibel, R. Parthasarathy, G. Sawicki, X.-M. Lin, H. Jaeger, S. L. Lindquist, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 4527 – 4532; d) M. Reches, E. Gazit, *Science* **2003**, *300*, 625 – 627.
[6] a) N. C. Seeman, P. S. Lukeman, *Rep. Prog. Phys.* **2005**, *68*, 237 – 270; b) M. Brucale, G. Zuccheri, B. Samorì, *Trends Biotechnol.* **2006**, *24*, 235 – 243; c) K. V. Gothelf, T. H. LaBean, *Org. Biomol. Chem.* **2005**, *3*, 4023 – 4037; d) S. Pitchiaya, Y. Krishnan, *Chem. Soc. Rev.* **2006**, *35*, 1111 – 1121.
[7] a) T. C. Marsh, J. Vesenka, E. Henderson, *Nucleic Acids Res.* **1995**, *23*, 696 – 700; b) A. B. Kotlyar, N. Borovok, T. Molotsky, H. Cohen, E. Shapir, D. Porath, *Adv. Mater.* **2005**, *17*, 1901 – 1905.
[8] P. J. Paukstelis, J. Nowakowski, J. J. Birktoft, N. C. Seeman, *Chem. Biol.* **2004**, *11*, 1119 – 1126.
[9] S. Modi, A. H. Wani, Y. Krishnan, *Nucleic Acids Res.* **2006**, *34*, 4354 – 4363; Y. Krishnan-Ghosh, E. Stephens, S. Balasubramanian, *J. Am. Chem. Soc.* **2004**, *126*, 5944 – 5945; Y. Krishnan-Ghosh, E. Stephens, S. Balasubramanian, *Chem. Commun.* **2005**, 5278 – 5280.
[10] Y. Krishnan-Ghosh, D. Liu, S. Balasubramanian, *J. Am. Chem. Soc.* **2004**, *126*, 11009 – 11016.
[11] K. Gehring, J.-L. Leroy, M. Gúeron, *Nature* **1993**, *363*, 561 – 564.
[12] This is in contrast with mica-immobilized duplex DNA. One hypothesis used to explain the observed collapse on mica surfaces is the pinning effect, where duplex DNA is unwound by surface binding forces.
[13] I. Berger, M. Egli, A. Rich, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 12116 – 12121.
[14] X. Han, J.-L. Leroy, M. Gúeron, *J. Mol. Biol.* **1998**, *278*, 949 – 965.
[15] Chemical ligation was performed by using a standard literature method. Contiguous 5′-phosphorylated and 3′-OH groups in i motifs were ligated using NCI: T. Li, D. Liu, J. Chen, A. H. F. Lee, J. Qi, A. S. C. Chan, *J. Am. Chem. Soc.* **2001**, *123*, 12901 – 12902.
[16] J. P. Ferris, C. H. Huang, W. J. Hagan, Jr., *Nucleosides Nucleotides* **1989**, *8*, 407 – 414.
[17] These should be considered the lower limits of the actual lengths of the wires, as accuracies are limited by efficiencies of a) $d(C_7)$ phosphorylation, b) chemical ligation, and c) binding of the contiguous monomer. Furthermore, the molecular-weight markers are ds-DNA fragments.
[18] J. M. Benevides, C. H. Kang, G. J. Thomas, Jr., *Biochemistry* **1996**, *35*, 5747 – 5755.
[19] K. Kneipp, H. Kneipp, I. Itzkan, R. R. Dasari and M. S. Feld, *J. Phys. Condens. Matter* **2002**, *14*, R597 – R624.
[20] The attractive electrostatic interactions between the positively charged gold nanoparticles and the negatively charged phosphodiester backbones of the wire enable the templated assembly of gold nanoparticles along the backbones of the wire.
[21] J. R. Wyatt, P. W. Davis, S. M. Freier, *Biochemistry* **1996**, *35*, 8002 – 8008.
[22] K. Kanaori, N. Shibayama, K. Gohda, K. Tajima, K. Makino, *Nucleic Acids Res.* **2001**, *29*, 831 – 840.
[23] Slipped tetramolecular structures are known to be favored at high concentrations.[10] This is consistent with the observation that at low concentrations (< 100 μM) $d(C_7)$ wires are not formed (see the Supporting Information).
[24] P. C. Lee, D. Meisel, *J. Phys. Chem.* **1982**, *86*, 3391 – 3395.

