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'Nucleo-nanocages': designed ternary oligodeoxyribonucleotides spontaneously form nanosized DNA cages†

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DNA three-way junctions formed from three 30-mer oligonucleotides that contain single-chained self-complementary sticky ends spontaneously self-assemble into 'nucleo-nanocages': the exo- and endo-nuclease digestion experiments indicate that defects such as the single and double strand end structures are absent on the spherical nano-assemblies, providing clear evidence for the closed nanocage structure.

Biological supramolecular nano-assemblies as exemplified by microtubules, flagella, chromatins and viruses are spontaneously formed from the constituent biopolymers.[1] Inspired by these self-assembling processes, the design and construction of peculiar nanostructures from artificial supermolecules has been an active field of modern chemistry.[2] For example, molecular-sized capsules or cages have been prepared by the use of hydrogen bonding or coordination.[2] However, in contrast to the biological systems, the size of these hollow nanostructures is limited to *ca.* 1–2 nm, and it remains difficult to rationally design such mesoscopic architectures in water. DNA can be employed as tectons for supramolecular nano-architectures in water.[3,4] For example, Seeman and co-workers have demonstrated the stepwise synthesis of DNA cubes with the size of *ca.* 10 nm.[3a,3b,4a] Though their approach allows precision synthesis of DNA nano-architectures, their protocol requires 10 different oligodeoxyribonucleotides (ODNs), multistep reactions and the concomitant purification processes. The complicated and time-consuming procedures render their cubes less suitable for the basis of functional bio-nanomaterials. We describe herein a simple and general method for constructing mesoscopic DNA cages (Nucleo-nanocages) by the self-assembly of suitably designed, three ODN strands.

Fig. 1 shows the sequences of three programmed 30-mer ODNs **1**–**3** and a schematic illustration of their self-assembly into the nucleo-nanocage. The same color sequences are complementary to each other, and the purple single-strands are self-complementary sticky ends. Since the red, blue, and green domains (GC rich region) have higher melting temperatures than the purple domains (AT region), mixing of these three ODNs will afford DNA three-way junctions in the first step. Succeeding hybridization of the self-complementary sticky ends at the 3′-terminals of the three-way junctions will hierarchically afford larger DNA assemblies. When the DNA three-way junctions are organized to consume all of the self-complementary sticky ends, formation of cages is expected.

Equimolar solution of three ODNs **1**–**3** were mixed at room temperature ([total ODN] = 1 μM, [NaCl] = 0.5 M). After heating the mixture at 70 °C for 5 min, it was cooled slowly to 10 °C and then aged at this temperature for 12 h.[5] The mixture was applied to a carbon-coated grid at 10 °C. After drying, the sample was post-stained by uranyl acetate. In transmission electron microscopy (TEM, Hitachi H-7500), spherical assemblies with diameters of 10–50 nm are abundantly seen (Fig. 2a). The average diameter of these nanoparticles as determined by dynamic light scattering (DLS, Otsuka Electronics DLS-7000DL) was 48.0 ± 10.4 nm. The observed size-distribution would originate from the structural flexibility of the DNA three-way junctions.[6] Interestingly, the size of DNA assemblies is affected by their total concentration. When the total concentration of ODNs was raised to 5 μM, larger spherical assemblies were produced (Fig. 2b, average diameter from DLS; 275.8 ± 66.8 nm).[7] It may be that collision between the nanoparticles is increased at higher ODN concentration and it facilitates their growth into larger particles.

The formation of spherical nano-assemblies is disrupted by destabilizing the DNA duplexes. Upon decreasing either the total ODN concentration ([ODN] < 0.4 μM) or the ionic strength ([NaCl] = 0 M), nanoparticles disappeared and only amorphous structures were observed. In addition, these nanostructures are not observed at temperatures above the T_{m1} of an

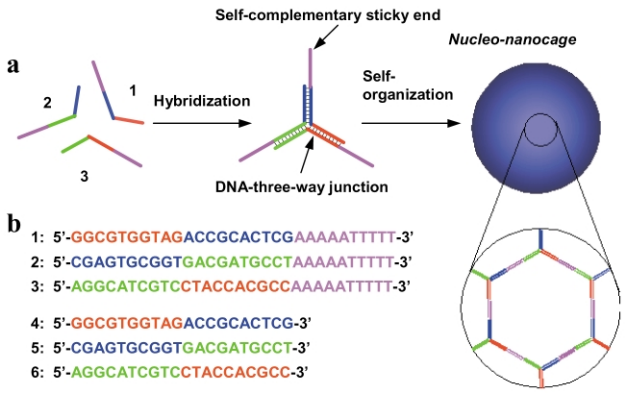

Fig. 1 (a) Schematic illustration of the formation of 'Nucleo-nanocages'. (b) DNA sequences used in this study. The same color sequences are complementary each other.

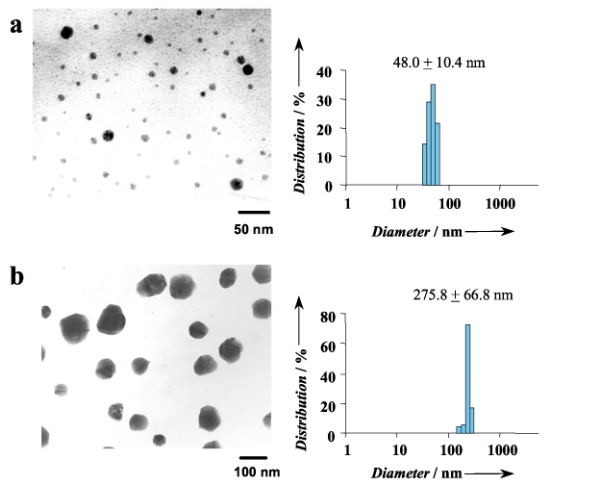

Fig. 2 Transmission electron micrographs and size-distribution obtained from dynamic light scattering of aqueous ODN mixtures. (a) [ODN] = 1 μM, [NaCl] = 0.5 M, 10 °C, (b) [ODN] = 5 μM, [NaCl] = 0.5 M, 10 °C. Samples were post-stained by uranyl acetate.

† Electronic supplementary information (ESI) available: a time course of absorbance increase of the ligated nucleo-nanocages **1**–**3**, due to the enzymatic action of DNase I. See http://www.rsc.org/suppdata/cc/b2/b210139d/

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equimolar mixture of **1**–**3**. Furthermore, neither the equimolar mixture of ODNs **4**–**6** which lacks the self-complementary sticky ends, nor the mixture of two ODNs among the three **1**–**3** ODNs gave the spherical nano-assemblies. These results strongly indicate that ODNs **1**–**3** form the three-way junctions and they are further self-assembled *via* hybridization of the self-complementary sticky ends to the spherical assemblies.

We confirmed that the spherical assemblies of ODNs **1**–**3** are cages, and are not formed from dendritic (starburst-like) structures.[8] The dendritic structure inevitably possesses many single strands on the surface of the spherical assemblies, and they would be digested by single strand-specific nuclease. If the sticky-end-eliminated ODN-assemblies are successively treated with a double strand-specific exonuclease, the whole architecture would be digested. The absence of single- or double-stranded terminals in nano-cages should allow them intact to the enzymatic hydrolysis, and this test provides a decisive evidence for the formation of nano-caged structure. Fig. 3a shows the time course of the absorbance increase (at 260 nm) when **1** alone, ternary ODN mixtures of ligated **1**–**3**[9,10] and **4**–**6** are mixed with mung bean nuclease (a single strand-specific endonuclease, molecular weight, 39 kDa). The ligated spherical assembly of **1**–**3** and three-way junction **4**–**6** were not digested by this nuclease, whereas the single strand **1** was completely digested under the same conditions. The mung bean nuclease-treated samples of **1**–**3** and **4**–**6** were then added into the buffer containing exonuclease III (a double strand-specific 3′ to 5′ exonuclease, molecular weight, 31 kDa). In Fig. 3b, the three-way-junctioned ODN **4**–**6** was readily digested by exonuclease III within 10 min, whereas the ligated assembly **1**–**3** was intact even after 70 min (Fig. 3b). The observed resistance of ligated assembly **1**–**3** towards the enzymatic action of Mung bean nuclease and exonucrease III is not ascribed to their restricted accessibility, since the ligated assembly **1**–**3** was readily digested with a nuclease of similar size (DNase I, molecular weight, 29 kDa. See Supplementary Information†). Therefore, the observed resistance is reasonably explained by the absence of single- and double-strand end structures on the spherical nano-assemblies and not by the steric hindrance between them.

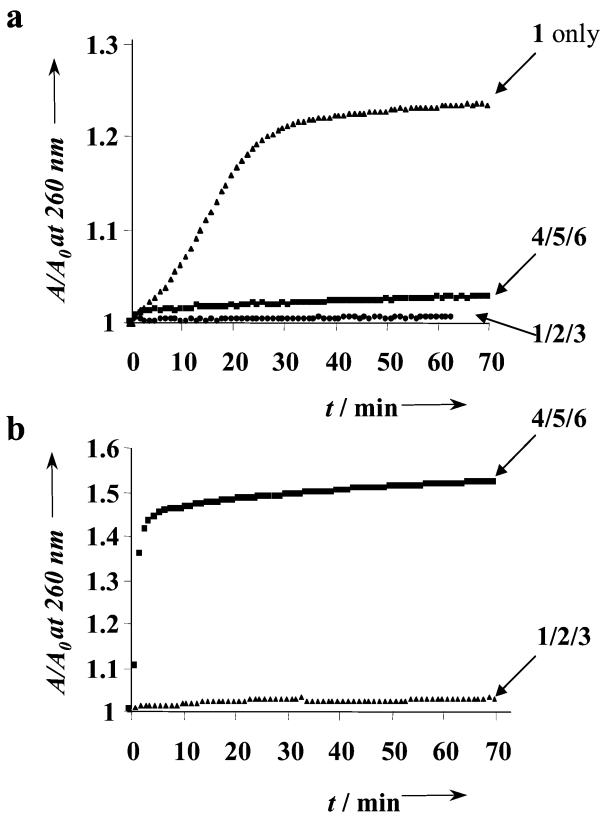

Fig. 3 Time course of absorbance increase due to the digestion of ODNs by nucleases. (a) Digestion with mung bean nuclease. [nucleobase] = 30 μM, enzyme, 70 unit in 400 μL of 0.5 M NaCl aq. at 37 °C. (b) Successive digestion with exonuclease III. [nucleobase] = 30 μM, enzyme, 70 unit in 400 μL of 660 mM Tris-HCl buffer (6.6 mM $MgCl_2$, pH 8) at 37 °C. Absorption changes were monitored at 260 nm.

In conclusion, we have developed a methodology to prepare nanocaged DNA by the self-assembly of suitably designed ODNs. The present approach is simple and would be widely applicable to the design of DNA-based, self-assembling nano-containers. Studies on the capability of these nucleo-nanocages to include nanoparticles or proteins and its application as nano-reaction vessels are currently in progress.

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Notes and references

1 C. Branden and J. Tooze, *Introduction to Protein Structure*, 2nd Ed., Garland Publishing, New York, 1999.
2 (*a*) J. Rebek, *Chem. Soc. Rev.*, 1996, 255; (*b*) M. M. Conn and J. Rebek, *Chem. Rev.*, 1997, **97**, 1647; (*c*) M. Fujita, D. Oguro, M. Miyazawa, H. Oka, K. Yamaguchi and K. Ogura, *Nature*, 1995, **378**, 469; (*d*) T. Kusukawa and M. Fujita, *J. Am. Chem. Soc.*, 1999, **121**, 1397.
3 For recent reviews (*a*) N. C. Seeman, *Acc. Chem. Res.*, 1997, **30**, 357; (*b*) N. C. Seeman, *Angew. Chem., Int. Ed.*, 1998, **37**, 3220; (*c*) J. J. Storhoff and C. A. Mirkin, *Chem. Rev.*, 1999, **99**, 1849; (*d*) C. M. Niemeyer, *Angew. Chem., Int. Ed.*, 2001, **40**, 4128.
4 (*a*) J. Chen and N. C. Seeman, *Nature*, 1991, **350**, 631; (*b*) C. Mao, W. Sun and N. C. Seeman, *Nature*, 1997, **386**, 3655; (*c*) C. A. Mirkin, R. L. Letsinger, R. C. Mucic and J. J. Storhoff, *Nature*, 1996, **382**, 607; (*d*) M. Scheffer, A. Dorenbeck, S. Jordan, M. Wüstefeld and G. von Kiedrowski, *Angew. Chem., Int. Ed.*, 1999, **38**, 3312; (*e*) K. Matsuura, M. Hibino, Y. Yamada and K. Kobayashi, *J. Am. Chem. Soc.*, 2001, **123**, 357.
5 The equimolar mixture of the three ODNs **1**–**3** ([ODN] = 1.0 μM in 0.5 M NaCl aq.) showed a multi-step melting behavior; T_{m1} = 23.6 °C, T_{m2} = 45.9 °C, T_{m3} = 51.5 °C. Therefore, specimens for TEM of assemblies of the three ODNs **1**-**3** were prepared at a temperature below the T_{m1} (at 10 °C).
6 L. S. Shiyakhtenko, V. N. Potaman, R. R. Sinden, A. A. Gall and Y. L. Lyubchenko, *Nucleic Acid Res.*, 2000, **28**, 3472.
7 Roughly estimated numbers of ODN molecules from the size of nanoparticles: 160 and 5300 molecules for the 48 nm and 275 nm diameter particles, respectively.
8 (*a*) T. W. Nilsen, J. Grayzel and W. Prensky, *J. Theor. Biol.*, 1997, **187**, 273; (*b*) J. Wang, M. Jiang, T. W. Nilsen and R. C. Getts, *J. Am. Chem. Soc.*, 1998, **120**, 8281.
9 Since the DNA assembly is unstable at the nuclease reaction temperature of 37 °C and exonuclease III may also digest the assembly from the nick moiety, we ligated the assembly before the nuclease assay.
10 The assembly of 5′-phosphated ODNs **1**–**3** ([**1**] = [**2**] = [**3**] = 2 μM) was ligated with TaKaRa DNA Ligation kit ver. 2 (TAKARA SHUZO Co., Ltd., Kyoto) in 30 μL of TBE buffer (pH = 8.0, [NaCl] = 0.5 M) at 16 °C for 3 h. The ligated DNA assembly was purified by ethanol precipitation. The ligated DNA assembly showed remarkable increase in T_m (above 80 °C in 0.5 M NaCl).