In Situ Observation of Spherical DNA Assembly "Nucleo-cages" in Water and Their Stabilization by Photocrosslinking

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Formation of spherical microstructures from three-way junction DNA is observed in water by confocal laser scanning fluorescence microscopy (CLSM). Photocrosslinking of nucleo-cages by psoralen derivatives is shown to effectively increase their stability against heat and urea.

Biological systems are replete with exquisite examples of self-assembled nano- to microstructures. Various architectures are spontaneously integrated from biomolecules such as nucleic acids, proteins, carbohydrates, and lipids.[1] Especially, DNA has been attracting much interest as nanostructure-building blocks because of its programmable secondary and ternary structures.[2] Formation of varied DNA nano-architectures, such as 1D-[3] and 2D-arrays,[4] tubes,[5] polyhedrons,[6] and nano-actuator[7] have been reported.

We have developed a simple strategy to construct spherical assemblies from C_3-symmetric DNA junctions[8] and trigonal β-sheet-forming peptide conjugates.[9] The spherical DNA-assemblies "nucleo-cages" were spontaneously self-assembled from suitably-designed three 30-mer oligodeoxyribonucleotides (ODNs) in aqueous NaCl solution, as revealed by transmission electron microscopy (TEM), dynamic light scattering (DLS), and enzymatic digestion experiments.[8] Stabilization of nucleo-cages has been one of important issues, in order to utilize them as carriers or hosts for functional molecules. In this study, we report stabilization of nucleo-cages by photo-crosslinking with psoralen derivatives. Thermal stability of nucleo-cages was observed in situ in water by using confocal laser scanning fluorescence microscopy (CLSM).

Figure 1 schematically shows a scheme for stabilizing nucleo-cages by photo-crosslinking. Psoralens are known to crosslink duplex DNA specifically at the 5′-TA-3′ site, and we therefore designed ODNs **1**–**3** containing photo-crosslinkable sticky-ends (5′-AAATATATTT-3′). Nucleo-cages were prepared by mixing equimolar ODNs **1**–**3** in 0.5 M aqueous NaCl. The mixture was first heated to 70 °C and kept at this temperature for 5 min to secure complete dissociation into monomeric strands. The mixture was then cooled to 10 °C at a cooling rate of −0.33 °C/min. Aqueous solutions of nucleo-cages were stained with 1 μM YOYO-1, which is known as a DNA duplex specific fluorescence probe. Fluorescence images were observed by CLSM (Carl Zeiss LSM 510, excitation at 488 nm, LP505 filter).

Figure 2 shows CLSM images of nucleo-cages at the total ODN concentration of 20 μM.[10] Spherical fluorescence images with diameters of ca. 3.5–5.3 μm were abundantly seen. These observations clearly indicate that spherical nucleo-cages do exist in water. Since the blurring effect is estimated to be about 0.3 μm,[11] the actual size of the spherical assemblies would be 2.9–4.7 μm. It is found that fluorescence is also observed from

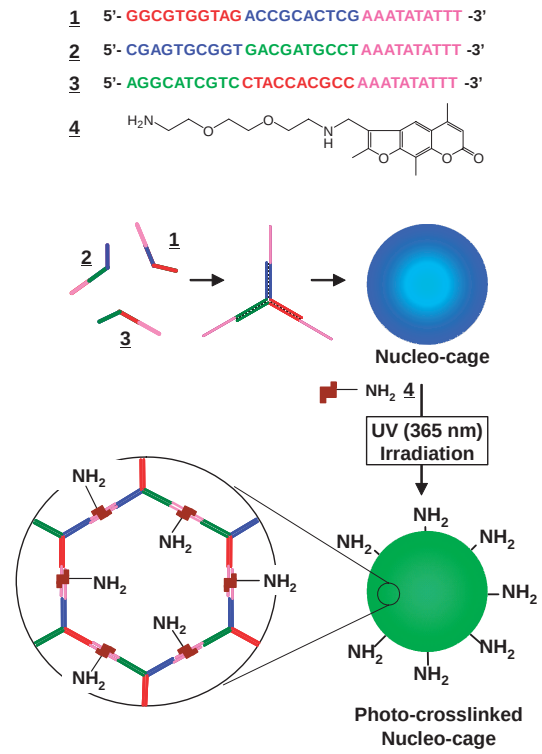

Figure 1. Schematic illustration of the self-assembly of nucleo-cages and their photocrosslinking with psolaren derivatives.

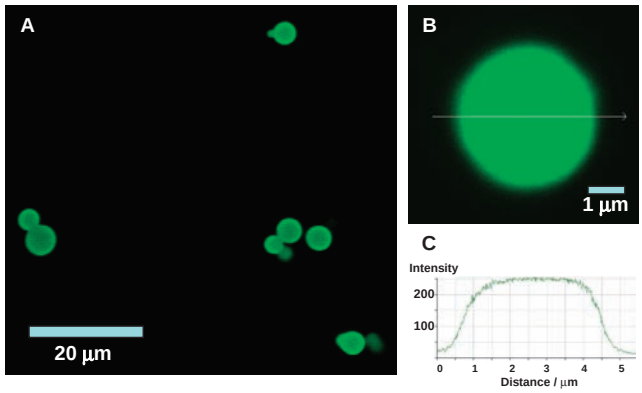

Figure 2. A and B: CLSM images of nucleo-cages in water at 10 °C, [ODN] = 20 μM, [NaCl] = 0.5 M, [YOYO-1] = 1 μM. C: the fluorescence profile for B.

the inside of assemblies, as shown by a fluorescent profile (Figure 2c). It suggests that inside of the assemblies is filled with DNA, and they are not hollow-caged structures.[12]

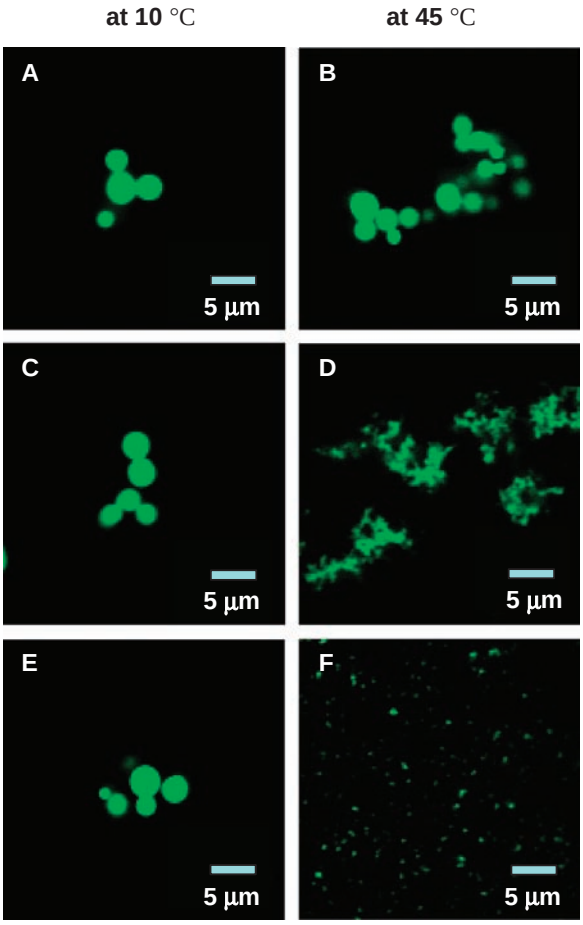

Figure 3. CLSM images of nucleo-cages. A and B: photocross-linked nucelo-cages; C and D: nucleo-cages + **4** (300 μM), no UV light irradiation; E and F: intact nucleo-cages (A, C and E: at 10 °C; B, D and F: after aging for 1 h at 45 °C). [ODN] = 20 μM, [NaCl] = 0.5 M, [YOYO-1] = 1 μM.

To photocrosslink nucleo-cages, water-soluble psoralen derivative **4** (300 μM) was synthesized. It was added to nucleo-cage solutions ([total ODN] = 20 μM, [NaCl] = 0.5 M) and the mixtures were photoirradiated with 365 nm UV-light (ca. 8 mW cm^{-2} for 240 min) at 5 °C. The progress of photocrosslinking was monitored by fluorescence spectra of the solution (Figure S1). The photocrosslinked nuleo-cages gave a melting curve shifted to higher temperature (T_m = ca. 56 °C) compared to that of intact nucleo-cages (T_{m1} = 42 °C and T_{m2} = 55 °C). In addition, slope of the melting curve became gentle over the broad temperature range, suggesting the formation of crosslinks which made the melting of DNA duplexes less cooperative (Figure S2).

Figure 3 shows the CLSM images of photo-crosslinked nucleo-cages (A and B), nucleo-cages in the presence of **4** (without photoirradiation) (C and D), and intact nucleo-cages (E and F) at 10 and 45 °C, respectively. The spherical structure of photo-crosslinked nucleo-cages were maintained even after keeping the mixture at 45 °C for 1 h (B), whereas intact nucleo-cages are collapsed to amorphous or smaller structures (F). CLSM images of the non-irradiated mixture of psoralen derivative **4** and nucleo-cages show agglomerated irregular structures (D). The cross-linked nucleo-cages show enhanced stability against urea, which is a powerful denaturant of DNA duplex. In aqueous 6 M urea, spherical structures of cross-linked nucleo-cages are maintained at 10 °C even after aging for 40 min, at which condition intact nucleo-cages were completely dissociated (Figure S3). These results clearly indicate that the stability of the nucleo-cages is considerably enhanced by the photocrosslinking with psoralen derivative **4**.

In conclusion, CLSM observation of nucleo-cages has revealed that the spherical structure of nucleo-cages actually exists in water. Inside of the DNA self-assembies is filled, rather than forming hollow structures. Photocrosslinking of nucleo-cages with psoralen derivatives dramatically enhanced their stability against heat and urea. As the amino group of psoralen derivative **4** can be modified with various functional groups, we envisage funtionalization of nucleo-cages, for example by arming the cell targeting functionalities on the surface.

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10 The size of nucleo-cages shows a dependence on the total concentration of ODN. When total concentration of ODN is 1 μM, dots of ca. 0.5 μm were found in CLSM. Due to the blurring effect, structural characterization of such small nucleo-cages seems to be difficult. We therefore prepared nucleo-cages at higher concentration of [total ODN] = 20 μM.
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