Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 4311–4317
Bioorganic & Medicinal Chemistry

# Binding of lectins to DNA micro-assemblies: Modification of nucleo-cages with lactose-conjugated psoralen

Kwonil Kim, Kazunori Matsuura* and Nobuo Kimizuka*

*Department of Chemistry and Biochemistry, Graduate School of Engineering, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan*

Received 28 December 2006; revised 13 March 2007; accepted 14 March 2007
Available online 18 March 2007

**Abstract**—Spherical DNA micro-assemblies appended with lactose units (Lactose-nucleo-cages, Lac-NC) are newly developed. DNA spherical assemblies self-assembled from suitably designed three oligodeoxyribonucleotides (ODNs) **1**–**3** were cross-linked by lactose-conjugated psoralen derivative **4**. Confocal laser scanning fluorescence microscopy (CLSM) observation of Lac-NC shows that rhodamine labeled peanut lectin (Rho-PNA: a galactose-specific lectin) binds to lactose-modified nucleo-cages with higher affinity compared to that of unmodified nucleo-cages. Binding isotherm experiments indicated that the apparent affinity constant of Rho-PNA to Lac-NC was in the order of $10^5$ $M^{-1}$.


## 1. Introduction

Glycoconjugates on cell surfaces play many important roles in cell biology, such as cell–cell adhesion, parasitic infection, and immunological recognition.[1] Molecular recognition concerned with oligosaccharides is greatly enhanced by multivalency, what is called glycocluster effect.[2] Various kinds of artificial glycoclusters were developed by employing polymers, dendrimers, and nanoparticles as scaffolds and they have been used in protein recognition,[3] cell culturing,[4] and cell targeting delivery.[5]

On the other hand, DNA has been attracting much interest as nanostructure-building blocks because of its programmable secondary and ternary structures.[6] Various DNA nano-architectures, such as arrays,[7] tubes,[8] polyhedrons,[9] have been constructed via self-assembly. DNA has also been utilized as linear scaffold for immobilizing proteins,[10] fluorescent dyes,[11] and gold nanoparticles.[12] Matsuura et al. reported formation of periodically aligned galactose units along DNA through the hybridization of galactose-modified ODNs with half-sliding complementary ODNs.[13]

Recently, we have developed a simple strategy to construct spherical assemblies from $C_3$-symmetric DNA junctions[14] and trigonal β-sheet-forming peptide conjugates.[15] The spherical DNA-assemblies 'nucleo-cages, NC' spontaneously self-assembled from suitably designed three 30-mer ODNs in aqueous NaCl solution, as revealed by transmission electron microscopy (TEM), dynamic light scattering (DLS), and enzymatic digestion experiments.[14a] The presence of spherical nucleo-cage structures in water was also confirmed by using confocal laser scanning fluorescence microscopy (CLSM). It was found that inside of the DNA self-assembly was filled, rather than forming hollow structures.[14b] In this paper, we report a novel glyco-conjugate that is based on nucleo-cages as scaffold. Lactose-modified nucleo-cages (Lac-NCs) are prepared by photo-crosslinking of nucleo-cages with lactose-conjugated psoralen derivative **4**, and their lectin recognition is evaluated by CLSM observation and binding isotherms of lectin.

## 2. Results and discussion

### 2.1. Preparation of Lac-NCs

Figure 1 schematically shows self-assembly of nucleo-cages and their modification with lactose-conjugated psoralen derivative **4**. ODN **1**–**3** were designed to form three-way junctions bearing both of the self-complementary sticky-ends and photo-crosslinking sites

*Keywords*: Self-assembly; Nucleo-cages; Psoralen; Lectin recognition.
* Corresponding authors. Tel.: +81 92 802 2833; fax: +81 92 802 2839 (K.M.); e-mail: ma14tcm@mbox.nc.kyushu-u.ac.jp

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.03.043

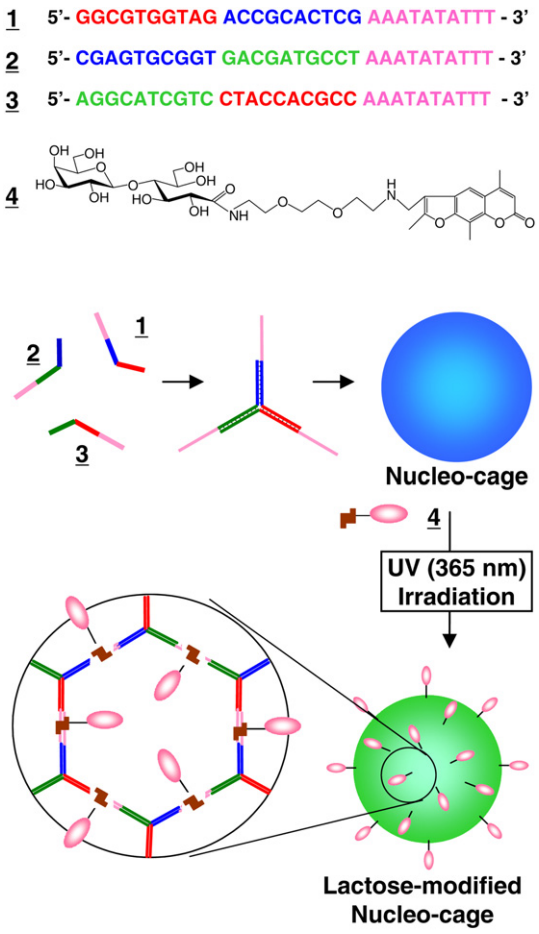



**Figure 1.** Schematic illustration of the self-assembly of nucleo-cages and their modification with lactose-psoralen derivatives.

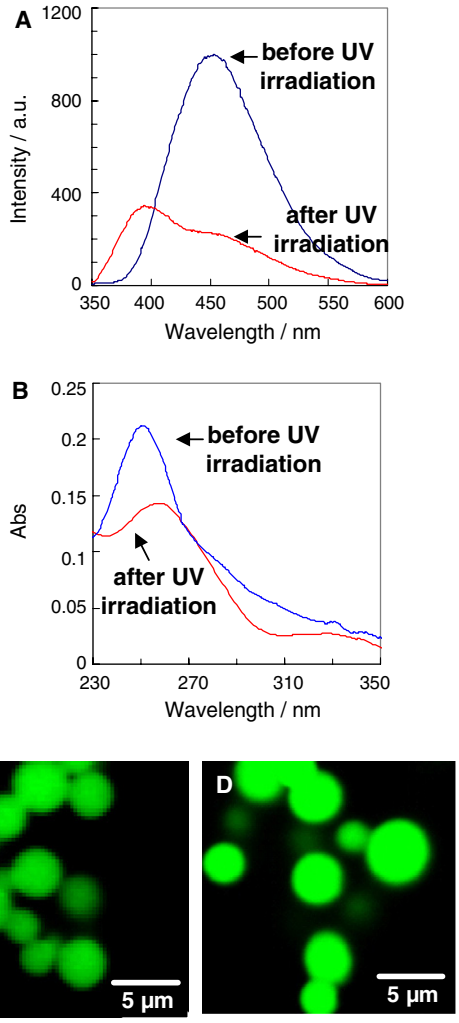
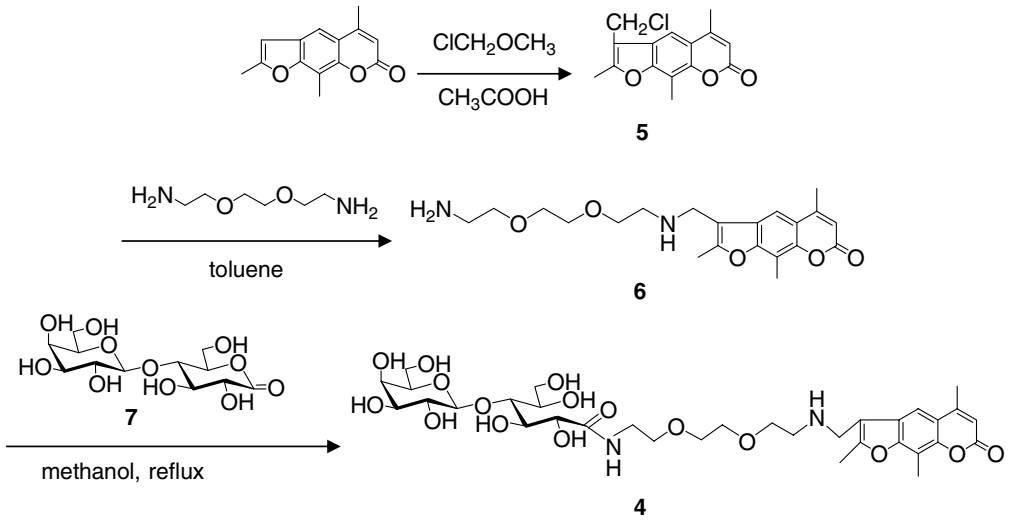



**Figure 2.** (A) Fluorescence spectra ($\lambda_{ex}$, 330 nm) and (B) UV–vis spectra of nucleo-cages aqueous mixtures before and after UV irradiation. CLSM images of (C) unmodified nucelo-cages and (D) Lac-NCs ([4] = 300 μM); $[ODN]_{total}$ = 20 μM, [NaCl] = 0.5 M, [YOYO-1] = 1 μM at 10 °C.

5′-TA-3′. Nucleo-cages ($[ODN]_{total}$ = 20 μM, 0.5 M NaCl) were prepared according to the previous paper[14b] (see Section 4) and these structures were confirmed by CLSM observation (Fig. 2C). Synthetic procedure of lactose-conjugated psoralen derivative **4** is summarized in Scheme 1. Psoralen derivatives are known to crosslink DNA duplexes specifically at the 5′-TA-3′ site,[16] and they dramatically increase the stability of nucleo-cages against heat- and urea-treatment. Lactose-modified nucleo-cages (Lac-NCs) were prepared by UV (365 nm) irradiation of aqueous nucleo-cages in the presence of **4** at 10 °C (see Section 4). The progress of photo-crosslinking was monitored by fluorescence spectra (Fig. 2A, excitation at 330 nm).[17] Upon UV irradiation, fluorescence intensity at 465 nm decreased and a new peak appeared at 390 nm, which reflects the photo-crosslinking of nucleo-cages with **4**. Absorption spectrum of aqueous mixture (Fig. 2B) was changed after UV irradiation probably by the structural change of **4**. This is supported by the intact absorption spectrum of nucleo-cages upon UV (365 nm) irradiation. Figure 2D shows CLSM image of Lac-NCs prepared by photo-crosslinking of nucleo-cages with **4** (300 μM). Apparently, the spherical structure of nucleo-cages is maintained during the lactose modification.

## 2.2. Estimation of lactose contents in Lac-NCs

In order to estimate the lactose content introduced in Lac-NCs, decrease in the bulk concentration of **4** after UV irradiation was investigated by measuring the absorbance at 273 nm. At this wavelength, the absorbance before and after UV (365 nm) irradiation shows no changes (see Fig. 2B). Figure 3 shows the absorption spectra of ultrafiltration (cut off Mw: 3000) filtrate of aqueous mixture of nucleo-cages ([ODN] = 20 μM, [NaCl] = 0.5 M) and **4** (300 μM) before (line A) and after (line B) the UV irradiation. The spectrum in Figure 3A is characteristic to that of **4** in the absence of nucleo-cages, and is ascribed to the unbound species in the bulk. The decrease of absorbance at 273 nm corresponds to the concentration change of unbound **4** from 300 to 266.6 μM. The observed concentration decrease (33.4 μM) reflects the amount of **4** cross-linked to ODNs. The result indicates that one molecule of **4** was introduced per 9.1 bp of nucleo-cages.




**Scheme 1.** Synthesis of lactose-conjugated psoralen **4**.

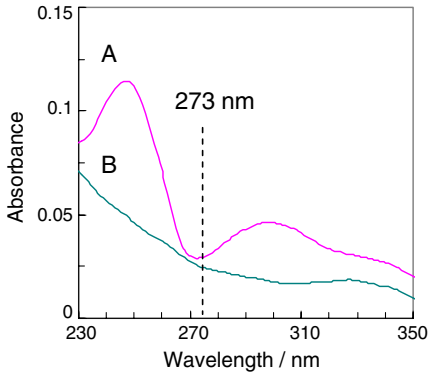



**Figure 3.** UV–vis spectra of ultrafiltration filtrate of nucleo-cages aqueous solutions at 25 °C. (A) Before and (B) after UV irradiation in the presence of **4** (300 μM).

## 2.3. Melting behaviors of Lac-NCs

The melting behaviors of three Lac-NCs samples prepared in the presence of **4** at various concentrations (300, 450, and 600 μM) are shown in Figure 4, together with that observed for unmodified nucleo-cages. The melting curves of Lac-NCs shifted to higher temperature ($T_{m1}$ = ca. 42 °C) compared to the unmodified nucleo-cages ($T_{m1}$ = 39 °C and $T_{m2}$ = 54 °C). This result indicates that nucleo-cages are stabilized by the photo-crosslinking. In the case of Lac-NCs, the slope of melting curves became smaller over the broad temperature range, suggesting that the melting of duplexes in Lac-NCs is less cooperative.

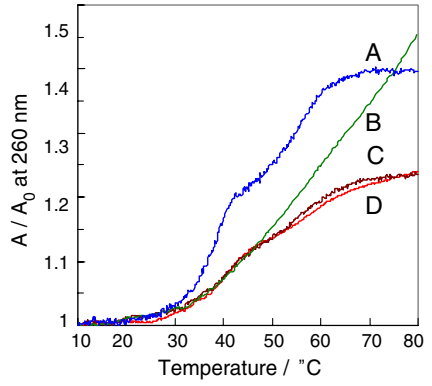



**Figure 4.** Melting curves of nucleo-cages; (A) unmodified nucleo-cages, (B–D) Lac-NCs (concentration of **4** for photo-crosslinking: (B) 600 μM, (C) 450 μM, (D) 300 μM).

## 2.4. CLSM observation of interaction of Lac-NCs with peanut lectin

In order to image the interaction between Lac-NCs and galactose-specific lectin, rhodamine labeled peanut lectin (Rho-PNA, Mw 110 kDa) and YOYO-1-stained Lac-NC were observed by CLSM. Figure 5 shows the CLSM images of YOYO-1-stained Lac-NCs in the presence of Rho-PNA ([Rho-PNA] = 2 μM, in 50 mM sodium phosphate buffer, pH 7.5). Green fluorescence of YOYO-1 indicates the spherical microstructure of Lac-NCs (Fig. 5A, Ex: 488 nm, Detection: 505–530 nm). The red fluorescent ascribed to the rhodamine unit of Rho-PNA (Fig. 5B) was observed as concentric circles, indicating binding of PNA to Lac-NCs. As the fluorescence of Rho-PNA is detected also from the interior of Lac-NC, it is not only bound to the surface but also penetrated into the interior of Lac-NCs.

On the other hand, in the CLSM image of aqueous mixtures consisting of unmodified nucleo-cages and 2 μM Rho-PNA, Rho-PNA was excluded form the interior of the YOYO-1-stained unmodified nucleo-cage (pH 7.5, Fig. 6A and B). CLSM images of Lac-NC in the presence of Rho-PNA (2 μM) and lactose (3 mM) also show the exclusion of Rho-PNA from Lac-NC (Fig. 6C and D). Apparently, interactions between Lac-NC and Rho-PNA are inhibited by excess lactose. These results indicate that the interaction of Lac-NC with Rho-PNA is controlled by specific recognition between lactose and PNA.

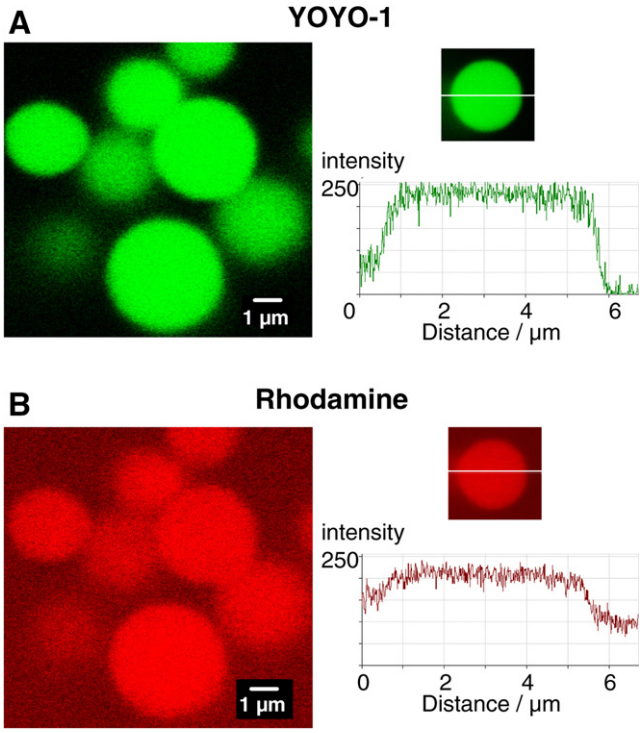



**Figure 5.** CLSM images and fluorescence profiles of Lac-NCs at 10 °C, $[ODN]_{total}$ = 20 μM, [NaCl] = 0.5 M, [YOYO-1] = 1 μM, [Rho-PNA] = 2 μM, in 50 mM sodium phosphate buffer, pH 7.5. (A) Excitation at 488 nm, detection at 505–530 nm; fluorescence of YOYO-1, (B) excitation at 543 nm, detection beyond 560 nm; fluorescence of rhodamine.

### 2.5. Binding isotherm of peanut lectin to Lac-NCs

To evaluate the binding affinity of PNA to Lac-NCs, binding isotherm of Rho-PNA to Lac-NCs was investigated. The concentration of free Rho-PNA in the supernatant after centrifugation of mixtures consisting of Lac-NCs ($[ODN]_{total}$ = 20 μM, [NaCl] = 0.5 M) and Rho-PNAs (0.75–6 μM) was determined by fluorescence intensity of rhodamine. Figure 7 shows the binding isotherm of Rho-PNA to unmodified nucleo-cages and Lac-NCs at pH 7.5 (A) and pH 5.0 (B), respectively. The binding isotherm at pH 5.0 was analyzed by Scatchard plot (Eq. 1) to obtain the association constant ($K_a$) and maximum amount of Rho-PNA bound to Lac-NCs ($[Bound\ PNA]_{max}$).

$$\frac{[\text{Bound PNA}]}{[\text{Free PNA}]} = -K_a[\text{Bound PNA}] + K_a[\text{Bound PNA}]_{max} \quad (1)$$

In pH 7.5 phosphate buffer, specific binding of Rho-PNA to Lac-NC was observed (Fig. 7A), whereas the interaction to unmodified nucleo-cages was negligibly weak. The binding isotherm of Rho-PNA to Lac-NC at pH 7.5 shows a sigmoidal curve. Although the curve cannot be strictly analyzed by Scatchard plot, it indicates that the apparent affinity constant of Rho-PNA to Lac-NC is of the order of $10^5\ M^{-1}$.

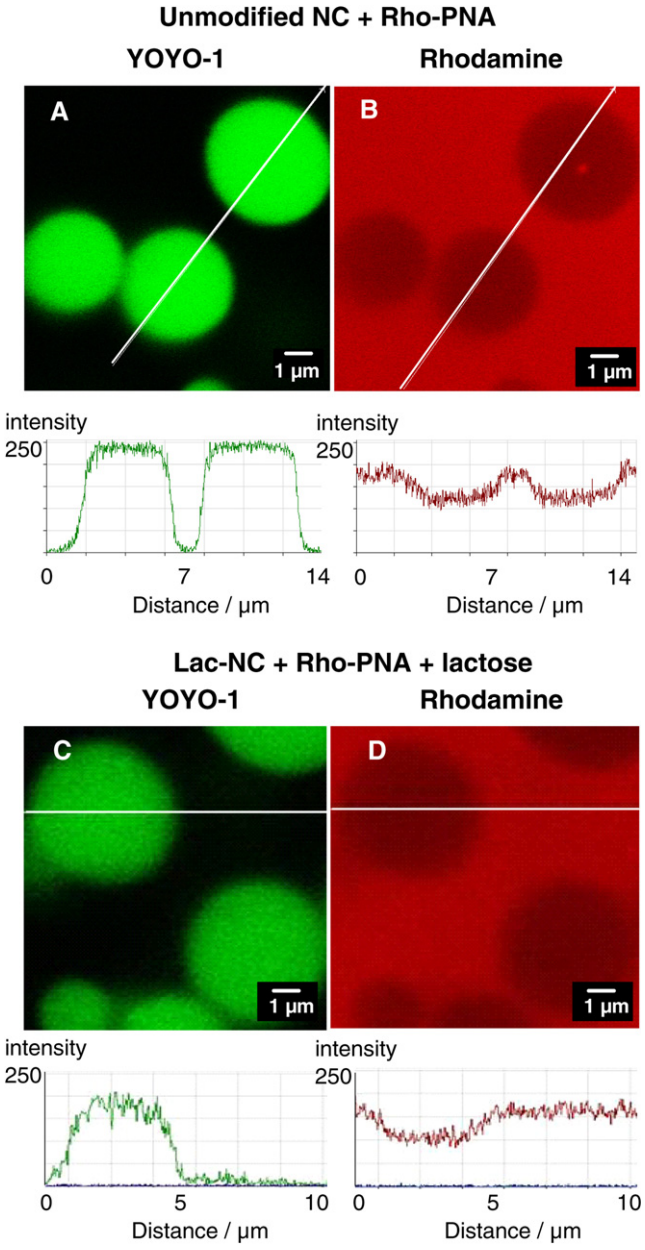



**Figure 6.** CLSM images of nucleo-cages at 10 °C, $[ODN]_{total}$ = 20 μM, [NaCl] = 0.5 M, [YOYO-1] = 1 μM, [Rho-PNA] = 2 μM, pH 7.5, 50 mM sodium phosphate buffer. (A and B) Unmodified nucleo-cages. (C and D) Lac-NCs in the presence of 3 mM lactose, [4] = 300 μM. (A and C) Excitation at 488 nm, detection at 505–530 nm; fluorescence of YOYO-1 (B and D) excitation at 543 nm, detection beyond 560 nm; fluorescence of rhodamine.

In contrast, different binding behavior of Rho-PNA to Lac-NC was observed at pH 5 (Fig. 7B). The affinity of Rho-PNA against Lac-NCs at pH 5.0 was calculated to be $K_a = (9.0 \pm 2.0) \times 10^5\ M^{-1}$ and $[Bound\ PNA]_{max}$ = 1.97 μM, whereas that against unmodified nucleo-cages was calculated to be $K_a = (6.0 \pm 2.1) \times 10^5\ M^{-1}$ and the $[Bound\ PNA]_{max}$ = 1.27 μM. Since PNA is composed of isolectins which have isoelectric points of 5.70–6.70,[18] PNA has positive net charges at pH 5.0. It is presumed that the binding of Rho-PNA to Lac-NCs at pH 5.0 results from both the specific interaction and non-specific electrostatic interactions.

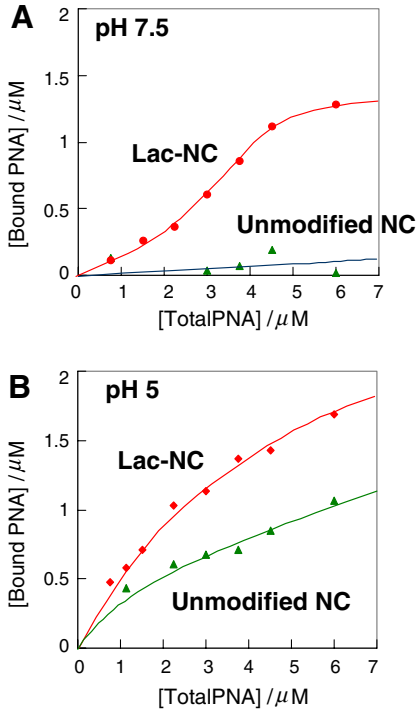



**Figure 7.** Interaction between PNA and nucleo-cages. Dependancy of [bound PNA] on the [total PNA] at (A) pH 7.5 and (B) pH 5 [NaCl] = 0.5 M.

## 3. Conclusion

Lactose-modified nucleo-cages were prepared by photo-crosslinking nucleo-cages with lactose-conjugated psoralens **4**. Rho-PNAs are bound not only on the surface but also into the interior, with the apparent affinity constant of ca. $10^5$ $M^{-1}$. The present lactose-modified nucleo-cages have the following features: first, they are novel micro-sized DNA-assemblies appended with lactose units. Second, proteins can penetrate interior of the nucleo-cages. Together with the biodegradability, Lac-nucleo-cages would be widely applied to the analysis, separation, and inhibition for the lactose specific cell and viruses.

## 4. Experimental

### 4.1. Instrumental

$^1$H NMR spectra were recorded on Bruker DRX 600 spectrometer at ambient temperature with tetramethylsilane or residual solvent peak as internal references. MALDI-TOF mass spectra were obtained on a PE Applied Biosystem Voyager System 1180 MALDI-TOF type mass spectrometer with dithranol and α-cyano-4-hydroxy cinnamic acid (CHCA) as matrix. Infrared spectra were recorded on a Nicolet Magna-IR 840 spectrophotometer. Fluorescence spectra were recorded at 10 °C in a 1.0 mm quartz cell with a F-4500 fluorescence spectrophotometer. UV–vis spectra were recorded in a 1.0 mm quartz cell with a JASCO V-570 spectrophotometer.

### 4.2. Materials

Synthetic ODNs (purified by HPLC) were purchased from Thermo Electron Corporation (Germany) as lyophilized powders and used without further purification. YOYO-1 iodide was purchased from Molecular Probe Co. Ltd as 1 mM DMSO solution. Rhodamine labeled peanut lectin (Rho-PNA, Mw 110 kDa, 3.2 molecules of rhodamine per molecule of PNA) was purchased from Vector.

### 4.3. Synthesis of lactose-conjugated psoralen (Lac-psoralen)

Scheme 1 shows the synthesis of Lac-psoralen **4** from 4, 5′, 8-trimethylpsoralen. Compound **5** was synthesized by chloromethylation of 4, 5′, 8-trimethylpsoralen according to the reported procedure[16] (62%). $^1$H NMR: (600 MHz, $CDCl_3$): $\delta$ 7.60 (s, 1H), 6.27 (s, 1H), 4.75 (s, 2H), 2.57 (s, 3H), 2.53 (s, 6H) ppm. MALDI-TOF-MS (matrix: dithranol): *m/z* 277 ($[M+H]^+$).

Compound **6** was synthesized by nucleophilic substitution of compound **5** by 1,2-bis(2-aminoethoxy)ethane according to the reported procedure[14b] (65%). $^1$H NMR: (600 MHz, $D_2O$): $\delta$ 6.93 (s, 1H), 5.61 (s, 1H), 3.44–3.46 (m, 8H), 3.35 (t, $J$ = 5.3 Hz, 2H), 2.58–2.59 (m, 4H), 2.15 (s, 3H), 1.98 (s, 3H), 1.66 (s, 3H) ppm. IR (NaCl): 3377, 2920, 2871, 1705, 1594, 1183, 1107 $cm^{-1}$. MALDI-TOF-MS (matrix: CHCA): *m/z* 389 ($[M+H]^+$).

Lactonolactone **7** (0.13 g, 0.39 mmol) and compound **6** (0.15 g, 0.39 mmol) were dissolved in methanol (1.5 mL) and the mixture was refluxed with magnetic stirring for 4 h. The precipitate formed was filter and purified by silica gel column chromatography (eluting with gradually changed solution from chloroform/methanol/water (10:6:1) to 100% methanol). Lac-psoralen **4** was obtained as colorless powder (0.045 g, 16%). $^1$H NMR: (600 MHz, $D_2O$): $\delta$ 7.10 (s, 1H), 5.80 (s, 1H), 4.37 (d, $J$ = 7.7 Hz, 1H), 4.20 (d, $J$ = 2.6 Hz, 1H), 4.00 (t, $J$ = 3.1 Hz, 1H), 3.79 (dd, $J$ = 4.3, 6.3 Hz, 1H), 3.74–3.76 (m, 2H), 3.69 (dd, $J$ = 3.3, 11.9 Hz, 1H), 3.49–3.62 (m, 13H), 3.30–3.42 (m, 3H), 3.16–3.19 (m, 2H), 2.66 (t, $J$ = 5.1 Hz, 2H), 2.25 (s, 3H), 2.12 (s, 3H), 1.88 (s, 3H) ppm. IR with a horizontal sampling accessory with diamond internal reflection element (Smith Detection, DuraSample*IR*): 3327, 2922, 2872, 1697, 1637, 1593, 1541, 1400, 1276, 1074, 1045 $cm^{-1}$, MALDI-TOF-MS (matrix: CHCA): *m/z* 729 ($[M+H]^+$). Anal. Calcd for $C_{33}H_{48}N_2O_{16}$ (as dihydrate): C, 51.83; H, 6.85; N, 3.66. Found: C, 51.49; H, 6.35; N, 3.73.

### 4.4. Preparation of nucleo-cages

Nucleo-cages were prepared by mixing equimolar ODNs **1**–**3** ([total ODN] = 20 μM) in 0.5 M aqueous NaCl at room temperature. The mixture was heated to 70 °C and kept at this temperature for 5 min, to secure complete dissociation of ODNs. Succeedingly, the mixture was cooled to 10 °C at a constant cooling rate

of −0.33 °C min$^{-1}$. The preparation of nucleo-cages was confirmed by CLSM observation.

### 4.5. Preparation of lactose-modified nucleo-cages (Lac-NCs)

Aqueous solution of Lac-psoralen **4** (10 mM) was added to a solution of intact nucleo-cages (**1** + **2** + **3**, 200 μL) at the final concentration of [total ODN] = 20 μM, [NaCl] = 0.5 M, [**4**] = 300, 450 or 600 μM. The mixture was photo-irradiated (ca. 8 mW cm$^{-2}$ for 240 min, 5 °C) in a 1.0 mm quartz cell with the 365 nm bright line using high-pressure mercury lamp (USHIO, USH-500SC) and a monochromator (Jasco, CT-10). The progress of photo-crosslinking was monitored by fluorescence spectra. Absorption spectra of the aqueous mixtures were also recorded before and after UV irradiation, by diluting the reaction mixture for five times with 0.5 M NaCl aqueous solution.

### 4.6. Determination of lactose contents in Lac-NCs

An aliquot (200 μL) of Lac-NCs solution in 0.5 M aqueous NaCl was filtered through a Millipore Microcon Centrifugal Filter Devices (cut off Mw 3000) at 10,000 rpm for 90 min. Then filtrates was diluted three times with pure water and the amount of Lac-psoralen unbound was quantified by absorbance of the filtrate at 273 nm.

### 4.7. Melting behaviors of nucleo-cages

Aqueous solution of Lac-NCs and unmodified nucleo-cages were diluted five times with 0.5 M aqueous NaCl. Absorbance of these solutions at 260 nm was recorded as a function of temperature on a spectrophotometer (heating rate, 1.0 °C min$^{-1}$).

### 4.8. CLSM observation of interaction between PNA and nucleo-cages

Confocal laser scanning fluorescence microscopy (CLSM) was conducted on a Carl Zeiss LSM 510 instrument with a 63× oil-immersion objective. Excitation was made with argon and helium–neon lamp and the excitation wavelength was selected with an interference filter. Emission range was selected with interference filter either with transmittance 505–530 nm or beyond 560 nm. An aliquot (30 μL) of nucleo-cages solution was placed on a glass-bottomed dish and observed by CLSM. In order to observe the interaction between Lac-NCs and PNA, rhodamine labeled peanut lectin (Rho-PNA) was employed. After Rho-PNA was added to Lac-NCs solution ([total ODN] = 20 μM, [NaCl] = 0.5 M, [4] = 300 μM, in 50 mM sodium phosphate buffer, pH 7.5), the solution was aged for 1 h and YOYO-1 was added ([YOYO-1] = 1 μM).

### 4.9. Binding curves of Rho-PNA to nucleo-cages

Various volumes of Rho-PNA stock solution (45 μM) were added to Lac-NC solution and the mixtures (final volume, 60 μL) were incubated at 10 °C for 1 h. The mixtures were then centrifuged at 10,000 rpm for 10 min and the supernatants (30 μL) were diluted four times. To determine the amount of unbound Rho-PNA, fluorescence intensity was measured at $\lambda_{em}$ = 570 nm at 10 °C ($\lambda_{ex}$, 530 nm).

## Acknowledgments

We thank Professor I. Hamachi (Kyoto University) for the use of the Carl Zeiss LSM 510. This study was financially supported by research grants from the Association for the Progress of New Chemistry, Grant-in-Aid for Scientific Research (A) (16205028) from the Japan Society for the Promotion of Science, and by a Grant for 21st Century COE Program from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

## References and notes

1. (a) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683; (b) Hakomori, S. I. *Glycoconj. J.* **2000**, *17*, 627; (c) Bertozzi, C. R.; Kiessling, L. L. *Science* **2001**, *291*, 2357.
2. (a) Mammen, M.; Chio, S. K.; Whitesides, G. M. *Angew. Chem. Int. Ed.* **1998**, *37*, 2754; (b) Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555.
3. (a) Aoi, K.; Itoh, K.; Okada, M. *Macromolecules* **1995**, *28*, 5391; (b) Kitov, P. I.; Sadowska, J. M.; Mulvey, G.; Armstrong, G. D.; Ling, H.; Pannu, N. S.; Read, R. J.; Buldle, D. R. *Nature* **2000**, *403*, 669; (c) Vrasidas, I.; de Mol, N. J.; Liskamp, R. M. J.; Pieters, R. J. *Eur. J. Org. Chem.* **2001**, 4685; (d) Otsuka, H.; Akiyama, Y.; Nagasaki, Y.; Kataoka, K. *J. Am. Chem. Soc.* **2001**, *123*, 8226.
4. (a) Kobayashi, A.; Akaike, T.; Kobayashi, K.; Sumitomo, H. *Macromol. Rapid Commun.* **1986**, *7*, 645; (b) Seo, S. J.; Kim, I. Y.; Choi, Y. J.; Akaike, T.; Cho, C. S. *Biomaterials* **2006**, *27*, 1487.
5. (a) Koyama, Y.; Yamada, E.; Ito, T.; Mizutani, Y.; Yamaoka, T. *Macromol. Biosci.* **2002**, *2*, 251; (b) Nakai, T.; Kanamori, T.; Sando, Shinsuke.; Aoyama, Y. *J. Am. Chem. Soc.* **2003**, *125*, 8465; (c) Hasegawa, T.; Umeda, M.; Matsumoto, T.; Numata, M.; Mizu, M.; Koumoto, K.; Sakurai, K.; Shinkai, S. *Chem. Commun.* **2004**, 382.
6. Reviews: (a) Seeman, N. C. *Angew. Chem. Int. Ed.* **1998**, *37*, 3220; (b) Storhoff, J. J.; Mirkin, C. A. *Chem. Rev.* **1999**, *99*, 1849; (c) Seeman, N. C. *Nature* **2003**, *421*, 427; (d) Wengel, J. *Org. Biomol. Chem.* **2004**, *2*, 277; (e) Feldkamp, U.; Niemeyer, C. M. *Angew. Chem. Int. Ed.* **2006**, *45*, 1856.
7. (a) Ding, B.; Sha, R.; Seeman, N. C. *J. Am. Chem. Soc.* **2004**, *126*, 10230; (b) Chelyapov, N.; Brun, Y.; Gopalkrishnan, M.; Reishus, D.; Shaw, B.; Adleman, L. *J. Am. Chem. Soc.* **2004**, *126*, 13924; (c) Liu, Y.; Ke, Y.; Yan, H. *J. Am. Chem. Soc.* **2005**, *127*, 17140; (d) Rothemund, P. W. K. *Nat. Biotechnol.* **2006**, *440*, 297.
8. (a) Mitchell, J. C.; Harris, J. R.; Malo, J.; Bath, J.; Turberfield, A. J. *J. Am. Chem. Soc.* **2004**, *126*, 16342; (b) Rothemund, P. W. K.; Nkodo, A. E.; Papadakis, N.; Kumar, A.; Fygenson, D. K.; Winfree, E. *J. Am. Chem. Soc.* **2004**, *126*, 16344; (c) Endo, M.; Seeman, N. C.; Majima, T. *Angew. Chem. Int. Ed.* **2005**, *44*, 6074.
9. (a) Chen, J.; Seeman, N. C. *Nature* **1991**, *350*, 631; (b) Shih, W. M.; Quispe, J. D.; Joyce, G. F. *Nature* **2004**, *427*, 618; (c) Goodman, R. P.; Schaap, I. A. T.; Tardin, C. F.;

Erben, C. M.; Berry, R. M.; Schmidt, C. F.; Turberfield, A. J. *Science* **2005**, *310*, 1661.
10. (a) Niemeyer, C. M.; Burger, W.; Peplies, J. *Angew. Chem. Int. Ed.* **1998**, *37*, 2265; (b) Winfree, E.; Liu, F.; Wenzler, L. A.; Seeman, N. C. *Nature* **1998**, *394*, 539; (c) Yan, H.; Park, S. H.; Finkelstein, G.; Reif, J. H.; LaBean, T. H. *Science* **2003**, *301*, 1882; (d) Malo, J.; Mitchell, J. C.; Bryan, C. V.; Harris, J. R.; Wille, H.; Sherratt, D. J.; Turberfield, A. *J. Angew. Chem. Int. Ed.* **2005**, *44*, 3057; (e) Park, S. H.; Yin, P.; Liu, Y.; Reif, J. H.; LaBean, T. H.; Yan, H. *Nano Lett.* **2005**, *5*, 729; (f) Park, S. H.; Pistol, C.; Ahn, S. J.; Reif, J. H.; Lebeck, A. R.; Dwyer, C.; LaBean, T. H. *Angew. Chem. Int. Ed.* **2006**, *45*, 735.
11. (a) Ohya, Y.; Yabuki, K.; Hashimoto, M.; Nakajima, A.; Ouchi, T. *Bioconjug. Chem.* **2003**, *14*, 1057; (b) Li, Y.; Cu, Y. T. H.; Luo, D. *Nat. Biotechnol.* **2005**, *23*, 885.
12. (a) Pinto, Y. Y.; Le, J. D.; Seeman, N. C.; Forsyth, K. M.; Taton, T. A.; Kiehl, R. A. *Nano Lett.* **2005**, *5*, 2399; (b) Deng, Z.; Tian, Y.; Lee, S. H.; Ribbe, A. E.; Mao, C. *Angew. Chem. Int. Ed.* **2005**, *44*, 3582; (c) Sharma, J.; Chhabra, R.; Liu, Y.; Ke, Y.; Yan, H. *Angew. Chem. Int. Ed.* **2006**, *45*, 730.
13. (a) Matsuura, K.; Hibino, M.; Yamada, Y.; Kobayashi, K. *J. Am. Chem. Soc.* **2001**, *123*, 357; (b) Matsuura, K.; Hibino, M.; Ikeda, T.; Yamada, Y.; Kobayashi, K. *Chem. Eur. J.* **2004**, *10*, 352.
14. (a) Matsuura, K.; Yamashita, T.; Igami, Y.; Kimizuka, N. *Chem. Commun.* **2003**, 376; (b) Kim, K.; Masumoto, K.; Matsuura, K.; Kimizuka, N. *Chem. Lett.* **2006**, *35*, 486.
15. Matsuura, K.; Murasato, K.; Kimizuka, N. *J. Am. Chem. Soc.* **2005**, *127*, 10148.
16. Issacs, S.; Shen, C. J.; Hearst, J. E.; Rapoport, H. *Biochemistry* **1977**, *16*, 1058.
17. Johnston, B. H.; Hearst, J. E. *Photochem. Photobiol.* **1981**, *33*, 785.
18. Miller, R. L. *Anal. Biochem.* **1983**, *131*, 438.