Self-Organized Glycoclusters Along DNA: Effect of the Spatial Arrangement of Galactoside Residues on Cooperative Lectin Recognition

Kazunori Matsuura,[b] Miki Hibino,[a] Takayasu Ikeda,[a] Yoshinao Yamada,[a] and Kazukiyo Kobayashi*[a]

Abstract: We describe herein the relationship between the spatial arrangement of self-organized galactose clusters and lectin recognition. β-Galactose-modified deoxyuridine phosphoramidite was synthesized and applied to solid-phase synthesis to provide 18-, 20-, and 22-mers of site-specifically galactosylated oligodeoxynucleotides (Gal-ODNs). These Gal-ODNs were self-organized through hybridization with the corresponding 18-, 20-, and 22-mers of half-sliding complementary ODNs (hsc-ODNs) to give periodic galactoside clusters. The self-organization of ODNs was confirmed by size exclusion chromatography and gel electrophoresis. The binding of the Gal-clusters to the FITC-labeled RCA_{120} lectin was analyzed by monitoring the change in fluorescence intensity. The assembly of 20-mer Gal-ODN with the 20-mer hsc-ODN was strongly and cooperatively recognized by the lectin. The 18-mer assembly was bound more weakly and less cooperatively, and the 22-mer assembly was minimally bound to the lectin. RCA_{120} lectin recognized not only the density of galactoside residues, but also the spatial arrangement. The size of the Gal cluster was estimated from the association constant of Gal-ODN with hsc-ODN. The relationship between lectin-recognition and Gal-cluster size is also discussed.

Keywords: cooperative effects · glycoclusters · lectin recognition · oligonucleotides · self-organization

Introduction

Oligosaccharide chains on cell surfaces have been regarded as an essential substance for the expression of various biological phenomena.[1] Molecular recognition concerned with such oligosaccharides is greatly enhanced by multivalency or the glyco cluster effect.[2] For example, it has been reported that glycolipids on cell surfaces can form glycolipid clusters named rafts or patches, that are strongly recognized by saccharide-binding proteins and complementary oligosaccharides.[3] Recently, various glyco conjugate polymers,[4,5] dendrimers,[6] calixarenes,[7] cyclodextrins,[8] nanoparticles,[9] and transition metal complexes[10] have been developed as glyco cluster models. However, there has been no report on successful control of the intervals and directions of the carbohydrate ligands.

[a] M. Hibino, T. Ikeda, Y. Yamada, Prof. Dr. K. Kobayashi
Department of Molecular Design
Graduate School of Engineering, Nagoya University
Chikusa, Nagoya 464-8603 (Japan)
Fax: (+81)52-789-2528
E-mail: kobayash@mol.nagoya-u.ac.jp

[b] Prof. Dr. K. Matsuura
Department of Chemistry and Biochemistry
Graduate School of Engineering
Kyushu University, Hakozaki, Fukuoka 812-8581 (Japan)

Oligodeoxynucleotides (ODNs) have attracted much attention as a "molecular glue" for supramolecular architectonics based on the self-organization concept.[11] ODNs are promising construction materials that can provide an array of functional components such as chromophores,[12] gold nanoparticles,[13] and proteins,[14] in controlled space and direction, since DNA forms a linear, rigid double-strand helix by complementary hydrogen-bonded base-pairing. Self-organized DNAs have also been applied to the molecular machines[15] and DNA-computing.[16]

Glycosylated nucleic acids occur rarely in nature, and their biological roles have not yet been sufficiently elucidated.[17] Inspired by the interesting structures of glycosylated nucleic acids, we [18] and other groups[19] have investigated the synthesis and functions of various types of artificial DNA–carbohydrate conjugates. In the course of the study, we have proposed a novel concept for construction of periodic glycoclusters by DNA self-organization strategy as shown in Figure 1.[20] Site-specific galactosylated ODNs (Gal-ODNs) were synthesized in an automated solid-phase DNA synsthesizer,[18d] and were hybridized with the "half-sliding" complementary ODNs (hsc-ODNs) to afford self-organized glycoclusters along the gapped DNA duplex. The right half-sequence (red) and the left half-sequence (blue) of hsc-ODNs are complementary to the left half-sequence (red) and the right half-sequence (blue) of the target *n*-mer

DOI: 10.1002/chem.200305465

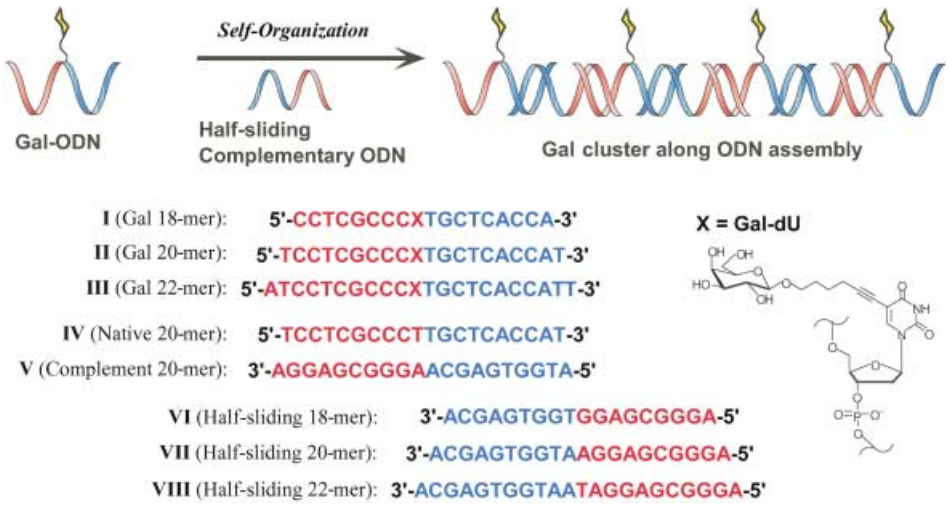


Figure 1. Schematic illustration of Gal clusters constructed by sequentially addressed self-organization of galactosylated ODNs with the half-sliding complementary ODNs. The red and blue sequences on **I**, **II**, and **III** are respectively complementary to the red and blue sequences on **VI**, **VII**, and **VIII**.

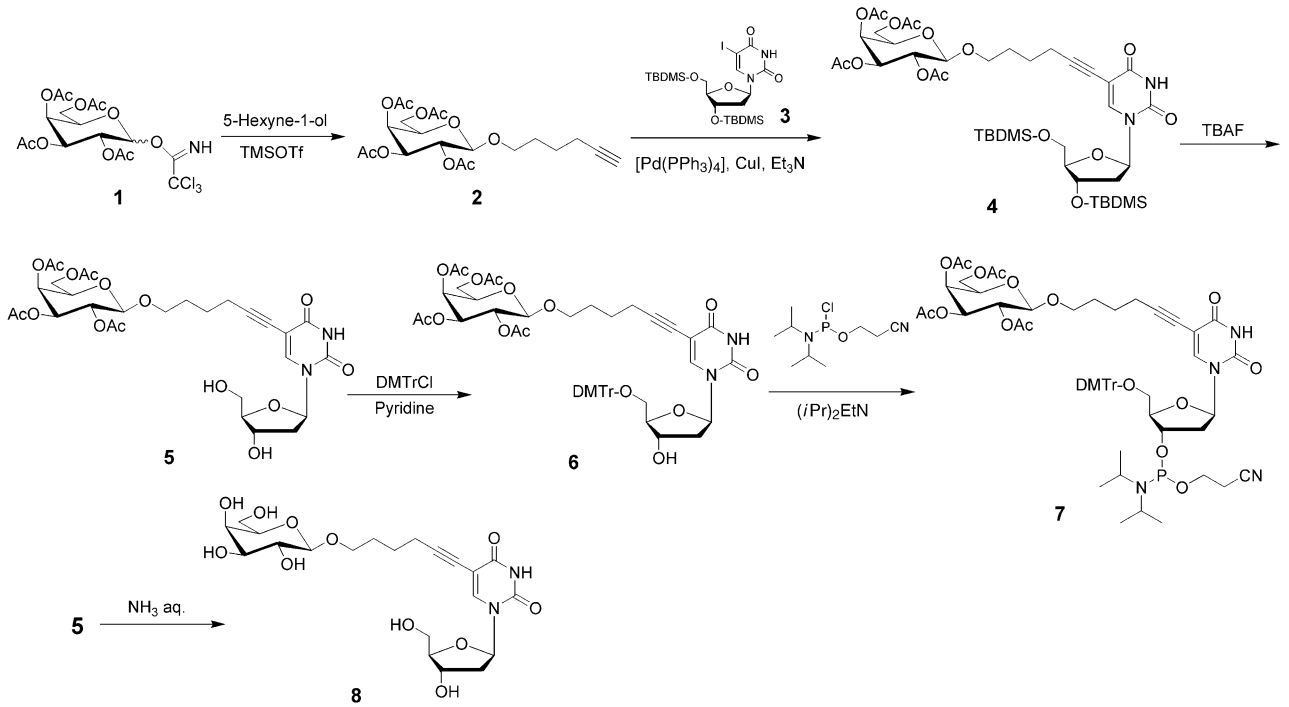
Gal-ODNs respectively (Figure 1). The relative space and direction between carbohydrates can be regulated by the self-organized DNA-duplex. This system will be valuable in studying the relationships between the spatial arrangement of carbohydrates and lectin recognition.

This paper describes a detailed study of the construction of galactoside-clusters based on the hybridization of 18-, 20-, and 22-mer Gal-ODN (termed **I**, **II**, and **III**,) with the corresponding hsc-ODNs (termed **VI**, **VII**, and **VIII**, respectively, in Figure 1), and the relationship between the spatial arrangement of galactosides along DNA and lectin recognition.

Results and Discussion

Synthesis and characterization of galactosylated ODNs (Gal-ODNs): Scheme 1 shows the synthesis of a β-galactose-modified deoxyuridine phosphoramidite derivative used for solid-phase synthesis. Solid-phase synthesis of Gal-ODNs was performed on an automated Expedite Nucleic Acid Synthesis System from the 3′ to the 5′ end by using benzimidazolium triflate[21] as an activator (1.0 μmol scale). The ODNs were deprotected and cleaved from the resin with aqueous ammonia at room temperature, then purified by Sephadex G-25 cartridge column and reversed-phase HPLC. The Gal-ODNs were produced as the main product with 60–80 % coupling efficiency.

Figure 2 shows an HPLC chart of nucleosides obtained by enzymatic digestion (nuclease P1, DNase I, and bacterial alkaline phosphatase) of the β-galactosylated ODN **II**. In addition to the peaks assigned to dA, T, dG, and dC, a peak assignable to β-galactosylated deoxyuridine **8** from comparison with the authentic sample was detected at retention time = 1.8 min. The ratio of each peak area (Gal-dU **8**: 0.9; dC: 10.2; dG: 2.4; T: 4.8; dA: 1.7) was in agreement with the calculated nucleotide composition of ODN **II** (Gal-dU



Scheme 1. Synthesis of β-galactose-modified deoxyuridine derivatives.

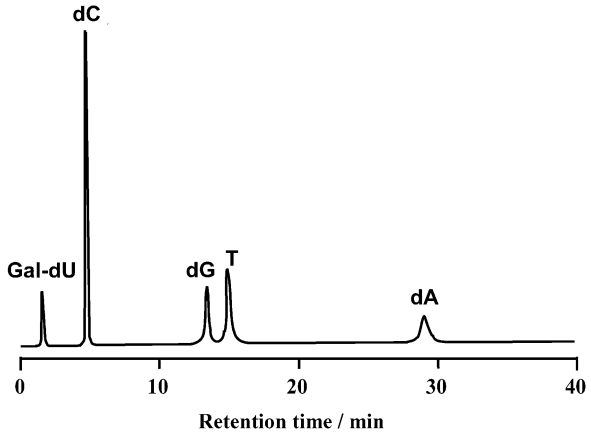


Figure 2. Reversed-phase HPLC chart of enzymatic digest of ODN **II** with DNase I, nuclease P1, and BAP.

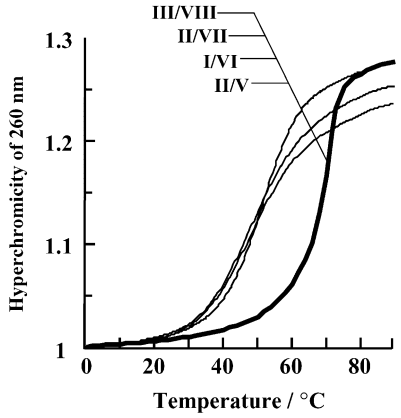


Figure 4. Melting behavior of duplexes of galactosylated ODNs with full complementary or half-sliding complementary ODNs at [ODN] = 1.13 μM in PBS (pH 7.4).

8: 1.0; dC: 10.0; dG: 2.0; T: 5.0; dA: 2.0). In addition, MALDI-TOF-MS showed that the molecular ion mass of the ODNs (*m*/*z* 6179.73) was consistent with that calculated (6183.24). Thus we confirmed the production of the galactosylated ODNs.

Figure 3 shows that the CD spectra of the gapped duplexes of the Gal-ODNs with the corresponding hsc-ODNs (**I**/**VI**, **II**/**VII**, and **III**/**VIII**) were similar to those of the full duplexes of the Gal-ODN and the unmodified ODN with the full complementary ODN (**II**/**V** and **IV**/**V**). The formation of typical B-type duplexes is suggested regardless of the galactoside modification and, hence, the array of carbohydrates as a glycocluster along DNA is discussed on the basis of structural data of B-type DNA duplexes.

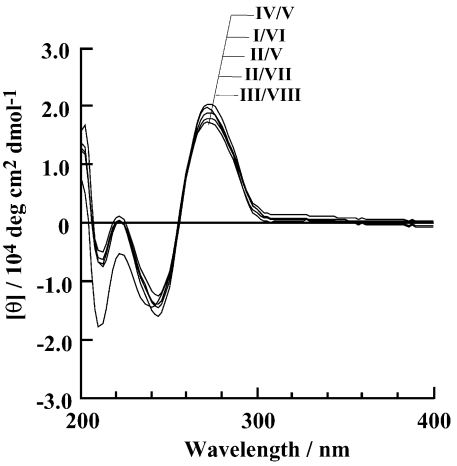


Figure 3. CD spactra of duplexes of galactosylated ODNs with full complementary or half-sliding complementary ODNs at [ODN] = 1.13 μM in PBS (pH 7.4) at 25 °C.

The melting temperatures of these Gal-ODN duplexes were estimated from the hyperchromicity curves shown in Figure 4. The gapped duplexes **I**/**VI** (48.3 °C), **II**/**VII** (48.5 °C), and **III**/**VIII** (51.3 °C) as well as the full duplex **II**/**V** (65.1 °C) can be compared with the corresponding native ODN duplexes **IV**/**VII** (48.4 °C) and**IV**/**V** (68.0 °C). The melting temperatures of the gapped duplexes with hsc-ODN were 20 °C lower than those of the full complementary duplexes. Introduction of the β-galactoside derivative into ODN minimally affected the thermal stability of the gapped duplexes as well as the full duplexes.

Organization of galactosylated ODNs: Self-organization of Gal-ODNs with hsc-ODNs was examined by agarose gel electrophoresis (Figure 5) and size-exclusion chromatography (SEC, Figure 6). The electrophoretic bands of the gapped duplex of 20-mer Gal-ODN (**II**) with hsc-ODN (**VII**) shifted to the shorter mobility and broadened as the concentration increased, whereas the duplex with full complementary ODN (**II**/**V**) indicated almost the same mobility independent of the concentration.

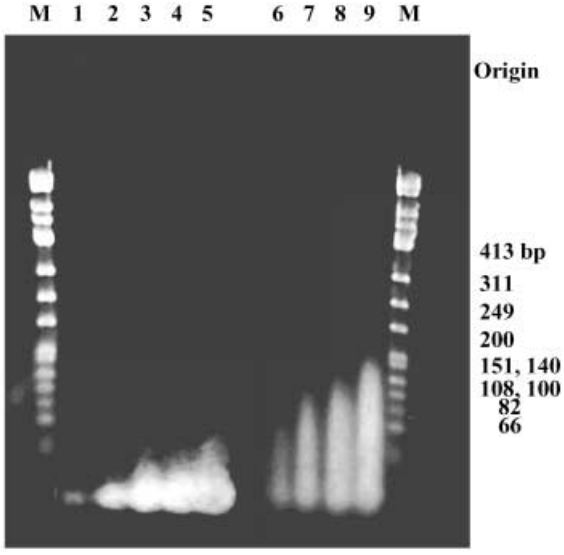


Figure 5. Gel electorophoresis analysis for self-organization of galactosylated ODN with half-sliding cODN. Lane 1: **II** ([ODN] = 150 μM). Lanes 2–5: **II**/**V** ([ODN] = 60, 90, 120, 150 μM, respectively). Lanes 6–9: **II**/**VII** ([ODN] = 60, 90, 120, 150 μM, respectively). M: double-stranded DNA marker.

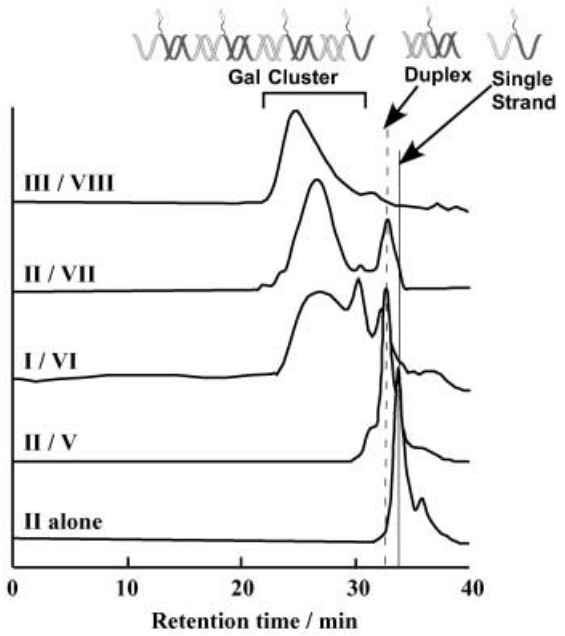


Figure 6. Size exclusion chromatography of galactosylated ODNs.

The SECs of the 18-, 20-, and 22-mer gapped duplexes (**I/VI, II/VII**, and **III/VIII** respectively) were broader and shifted to faster retention time than those of the single strand ODN alone (**II**) and the full complementary duplex (**II/V**). These results suggest that the Gal-ODNs were organized with the hsc-ODN to form Gal clusters. The apparent number average molecular weight compared with pullulan standards were as follows. Single strand 20-mer ODN **II**: $M_n = 7830$ ($M_w/M_n = 1.04$); full complementary duplex **II/V**: $M_n = 12\,000$ ($M_w/M_n = 1.05$); gapped duplex **I/VI**: $M_n = 84\,400$ ($M_w/M_n = 1.22$); **II/VII**: $M_n = 78\,500$ ($M_w/M_n = 1.89$); **III/VIII**: $M_n = 101\,000$ ($M_w/M_n = 1.38$). Thus, about 6.5 galactosides on average were assembled by the half-sliding complementary ODNs; However it is probable that the value is underestimated because the assembly is partially dissociated during SEC and electrophoresis.

Binding affinity of gal clusters with half-sliding complementary ODNs to lectin: Binding affinity was estimated quantitatively by fluorometry with an FITC-labeled β-galactoside specific lectin (FITC-RCA_{120}, *Ricinus communis* agglutinin). Figure 7 shows that addition of an increasing amount of 20-mer Gal cluster **II/VII** to FITC-RCA_{120} decreased significantly the fluorescence intensity at 520 nm (excitation at 490 nm) to give a steep sigmoidal curve. The binding curve of the 18-mer Gal cluster **I/VI** was gently sigmoidal. In contrast, the 22-mer Gal cluster **III/VIII** fluorescence intensity decreased minimally in the present concentration range, similarly to the duplex of Gal-ODN with full complementary ODN (**II/V**) that has only one galactoside.

These results indicate that the 20-mer Gal cluster **II/VII** bound to RCA_{120} most strongly, the 18-mer **I/VI** bound weakly, and the 22-mer **III/VIII** bound minimally. Further more, the sigmoidal dependency of the fluorescence intensi-

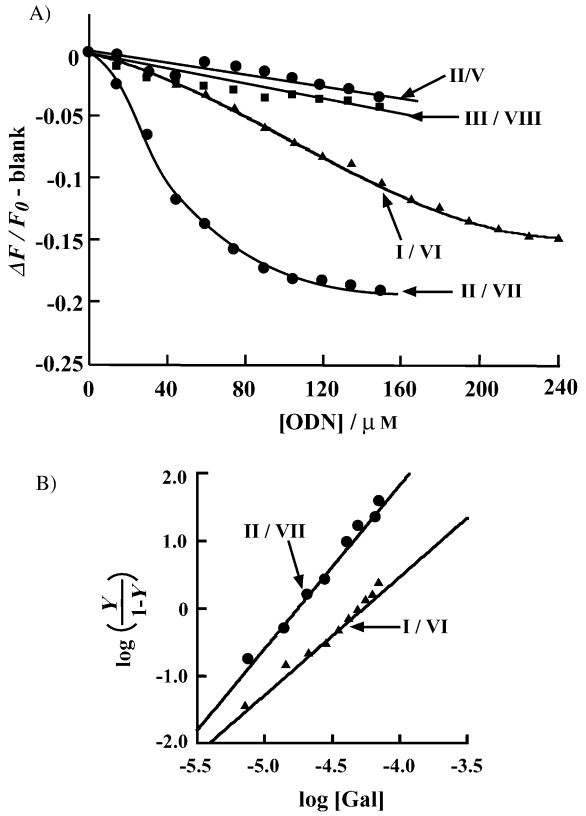


Figure 7. A) Dependence of fluorescence intensity of FITC-RCA_{120} at 520 nm (Ex = 490 nm) on the concentration of ODNs in PBS (pH 7.4) at 25 °C ([RCA_{120}] = 14.6 nM). The ΔF was corrected for the spontaneous breaching of FITC-RCA_{120} in PBS. B) Hill plots of fluorescence intensity change of FITC-RCA_{120} with ODNs.

ty on the concentration of **II/VII** indicates that some galactose residues along DNA were cooperatively bound to RCA_{120}.[22] The sigmoidal curve was treated with the Hill equation [Eq. (1)] to give the apparent affinity constant (K_{af}) and the Hill coefficient (n) as summarized in Table 1.

$$\log\frac{Y}{1-Y} = n\log[\mathrm{Gal}] + n\log K_{\mathrm{af}} \quad \left(Y = \frac{\Delta F}{\Delta F_{\max}}\right) \tag{1}$$

The Hill coefficient of the 18-mer ODN (**II/VII**) is $n = 2.4$, which suggests that an average of 2.4 galactose residues are cooperatively bound to some of the four binding sites on RCA_{120} by organizing the conjugate **II** with **VII**. The ap-

Table 1. Apparent affinity constants (K_{af}) and Hill coefficients (n) in binding of Gal clusters along DNA to RCA_{120} at 25 °C.

Gal cluster	K_{af} [M^{-1}][a]	n
I/VI (18-mer)	1.9×10^4	1.8
II/VII (20-mer)	5.5×10^4	2.4
III/VIII (22-mer)	–	–

[a] K_{af} was calculated using molarity [mol L^{-1}] of the Gal unit.

parent affinity constant and the Hill coefficient of the Gal cluster along the 18-mer ODN (**I/VI**) to RCA_{120} were lower than those of the 20-mer ODN. The fluorescence change of

the Gal cluster along the 22-mer ODN (**III/VIII**) was too small to be treated with the Hill equation.[23]

Since the B-type DNA duplex has an average 10.5 base pairs and 34 Å pitch per turn of the helix, the galactoside residues along the 20-mer and 22-mer ODN assemblies (**II/VII** and **III/VIII**) were displayed at 68 and 75 Å regular intervals, respectively, and their two adjacent residues were twisted 34 degrees each. In contrast, the galactoside residues along the 18-mer ODN assembly (**I/VI**) were displayed at 63 Å regular intervals with larger dihedral angle of 103° (Figure 8). It is probable that the relative arrangement of the Gal-cluster based on the 20-mer ODN (**II/VII**) allows cooperative binding to the binding sites on RCA_{120}, whereas that of the Gal-cluster based on the 18-mer (**I/VI**) is unfavorable to bind cooperatively to $RCA_{120,}$ due to its twisted arrangement of galactosides. The extended interval of 22-mer **III/VIII** seems to hamper the binding to RCA_{120}. We can conclude that RCA_{120} lectin recognized the spatial arrangement of the galactoside residues along DNA.

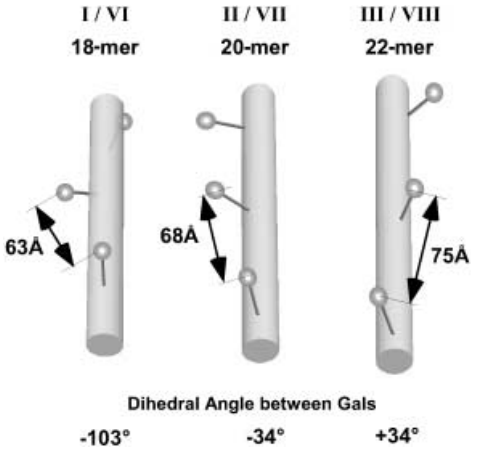


Figure 8. Illustration of the predicted arrangement of Gal cluster based on galactosylated 18-, 20-, and 22-mer ODNs with their half-sliding complementary ODNs.

Estimation of the degree of association between ODNs: In order to discuss the relationship between lectin recognition and Gal cluster size, we attempted to estimate the association constant between ODNs and then the degree of association of the Gal cluster. When two ODNs form an assembly with apparent association constant K_{as} [M^{-1}] at total ODN concentration C [M], the degree of association (DA) is given by Equation (2).[24]

$$DA = \frac{K_{as}C}{\sqrt{K_{as}C+1}-1} \quad (2)$$

The T_m of the gapped duplex (**II/VII**) was measured at several concentrations and plotted according to Equation (3) in Figure 9. The linear relationship gave $\Delta H° = -44$ kcal mol^{-1} and $\Delta S° = -120$ cal $mol^{-1} K^{-1}$ for gapped duplex formation. The apparent affinity constant at 25 °C was calculated to be $K_{as} = 4.2 \times 10^6\ M^{-1}$ using $\Delta G° = \Delta H° - T\Delta S° = -RT\ln K_{as}$. Substituting the ΔG value into Equation (2) afforded dependence of the *DA* of ODNs on the concentration as shown in Figure 10.

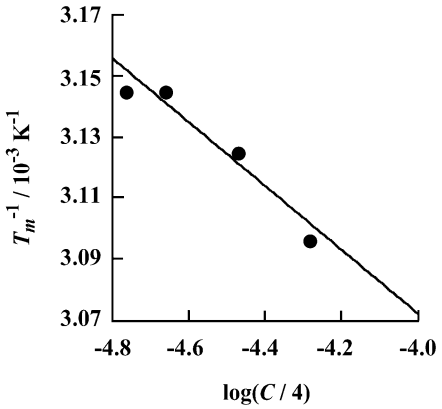


Figure 9. Plot of T_m^{-1} versus log (C/4) for melting of the gapped duplex **II/VII** in PBS (pH 7.4).

$$\frac{1}{T_m} = \frac{2.303R}{\Delta H^o}\log\left(\frac{C}{4}\right) + \frac{\Delta S^o}{\Delta H^o} \quad (3)$$

Figure 7 shows that the binding ability of the Gal cluster **II/VII** to RCA_{120} is enhanced significantly at [ODN] = 40 μM. The DA of ODNs was estimated to be about six, which corresponded to about three galactosides (Figure 10).

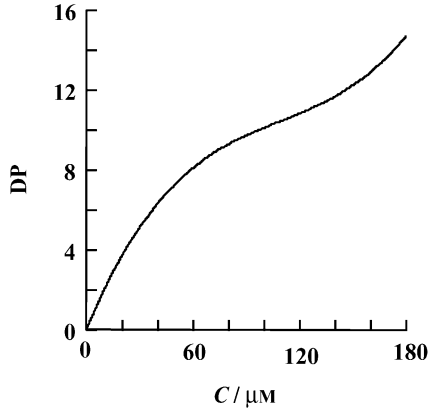


Figure 10. Estimated dependence of degree of association (DA) on ODN concentration in self-organization of the gapped duplex **II/VII** in PBS (pH 7.4) at 25 °C.

This value is comparable to the Hill coefficient 2.4, for binding of the Gal cluster **II/VII** to RCA_{120}. This suggests that the degree of association of about three galactosides on **II/VII** lead to the cooperative binding with RCA_{120}. Thus, about three galactosdes on the 20-mer gapped duplex assembly (**II/VII**) simultaneously recognize three of four binding sites of RCA_{120}.

Conclusions

We have demonstrated that periodic, spatial arrangement of galactoside residues could be attained by self-organization of galactosylated ODN with the corresponding half-sliding

complementary ODN. The affinity of the Gal cluster organized on DNA to RCA_{120} lectin was significantly dependent on this spatial arrangement. The Gal cluster based on the 20-mer ODN, which displays the galactoside residues at 68 Å regular intervals with a small dihedral angle, was strongly and cooperatively bound to the lectin. On the other hand, the largely twisted Gal cluster based on the 18-mer ODN assembly was bound more weakly and less cooperatively, and the 22-mer ODN assembly, bearing an extended interval, was minimally bound to the lectin. The degree of association between Gal-ODN and hsc-ODN was estimated by SEC and thermodynamic analysis of the concentration dependency of T_m, suggesting that about three galactosides on the 20-mer gapped duplex lead to the cooperative binding with RCA_{120}. Therefore, it has been revealed that RCA_{120} lectin recognizes not only the density of galactoside residues, but also the spatial arrangement and degree of association of Gal clusters. Such regular spatial arrangement of carbohydrate residues can not be attained with synthetic glycoconjugate polymers such as polystyrene and polyacrylamide backbones. The glycocluster system with DNA self-organization will be useful to elucidate recognition properties of various carbohydrate-binding proteins. We propose an "assembly-amplified cooperative molecular recognition strategy", which has high potential for applications to various molecular systems.

Experimental Section

Materials: Reactions sensitive to moisture and air were performed under nitrogen or argon atmospheres using anhydrous solvents and reagents. The following five types of oligodeoxynucleotides were purchased from Rikaken (Nagoya): native **IV** (5′-TCCTCGCCCTTGCTCACCAT-3′), full complement 20-mer **V** (5′-ATGGTGAGCAAGGGCGAGGA-3′), half sliding complement 18-mer **VI** (5′-AGGGCGAGGTGGTGAGCA-3′), half sliding-complement 20-mer **VII** (5′-AGGGCGAGGAATGGTGAGCA-3′), half sliding complement 22-mer **VIII** (5′-AGGGCGAGGAATATGGTGAGCA-3′). Fluorescein isothiocyanate (FITC) labeled *Ricinus communis* agglutinin (RCA_{120}), nuclease P1 (from *Penicillium citrinum*, EC 3.1.30.1), DNase I (from bovine pancreas, EC 3.1.21.1), and bacterial alkaline phosphatase (BAP; from *Escherichia. coli*, EC 3.1.3.1) were purchased from Sigma . Benzimidazolium triflate was prepared according to the previously reported procedure.[21] 2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite were prepared at Y. Hayakawa's Laboratory, Graduate School of Human Informatics, Nagoya University.

Instruments: ^{1}H NMR spectra were recorded on Varian Gemini-500 spectrometer at ambient temperature with tetrametylsilane or residual solvent peaks as internal references. ^{31}P NMR spectra were recorded on JEOL α-400 spectrometer at ambient temperature using 85 % H_3PO_4 as external references. Infrared (IR) spectra were recorded with a JASCO Fourier transform IR-230 spectrophotometer. Optical rotation was determined with a JASCO DIP-1000 digital polarimeter using a water-jacketed 1 dm cell at 25 °C. Absorption spectra were recorded on a JASCO V-530 UV/VIS spectrophotometer equipped with a thermal controller. CD spectra were taken in a 10 mm quartz cell with a JASCO J-720 L spectrophotometer. Fluorescence spectra were recorded on a JASCO FP-777 spectrophotometer equipped with a thermal controller. Fast-atom-bombardment (FAB) mass spectra were obtained on a JEOL JMS-AX505HA mass spectrometer operating in positive mode. MALDI-TOF mass spectra were obtained on a PE Applied Biosystem Voyager MALDI-TOF type mass spectrometer with 3-hydroxy-2-picolinic acid as matrix. Solid-phase syntheses of ODNs were conducted on an Expedite Nucleic Acid Synthesis System. Size exclusion chromatography (SEC) was recorded at 25 °C with a JASCO Guliver SB-804HQ to SB-803HQ columns and an RI detector (RI-930). Reversed-phase HPLC was recorded at 25 °C with Shiseido Capcell Pak C18 UG120 (4.5 × 150 mm) and a JASCO Guliver UV/VIS detector (UV-975).

Synthesis of galactosylated deoxyuridine derivatives: **1-(5-Hexyn-1-yl)-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (2)**: 5-Hexyn-1-ol (100 μL, 9.06 mmol) and TMSOTf (535 μL, 2.77 mmol) under nitrogen at −40 °C were added to a solution of **1** (4.54 g, 10.87 mmol) and MS4A in CH_2Cl_2 (10 mL) . The resulting mixture was stirred for 45 min at 0 °C. After removal of the molecular sieves by filtration, the filtrate was extracted with CH_2Cl_2 and the organic layer was washed with $NaHCO_3$(aq) and H_2O. After drying over anhydrous $MgSO_4$, CH_2Cl_2 was evaporated to provide a thick oil. Purification by silica gel column chromatography eluting with ethyl acetate/hexane (3:4) provided pure **2** as a light yellow oil (3.80 g, 98 % yield). $[\alpha]_D^{25} = -37.0°$ ($c = 0.1$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 5.39$ (d, $J = 3.3$ Hz, 1H), 5.20 (dd, $J = 8.0$, 10.5 Hz, 1H), 5.02 (dd, $J = 3.0$, 10.0 Hz, 1H), 4.60 (d, $J = 8.0$ Hz, 1H), 4.21–4.11 (m, 2H), 3.95–3.89 (m, 5-H, 2H), 3.49–3.54 (m, 1H), 2.21–2.16 (m, 2H), 2.14, 2.07, 2.05, 2.00 (4 s, 12H), 1.88 (t, $J = 2.2$ Hz, 1H), 1.70–1.60 (m, 2H) and 1.59–1.48 ppm (m, 2H). IR (KBr): $\tilde{\nu} = 3284$, 2947, 1751, 1435, 1371, 1223, 1057 cm^{-1}; FAB-MS: m/z : 427 [M^++H].

5-[1-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-5-hexyn-6-yl]-2′-deoxyuridine 3′,5′-di-*tert*-butyldimethylsilyl ether (4): $Pd(PPh_3)_4$ (14 mg, 0.012 mmol), CuI (4.6 mg, 0.024 mmol), Et_3N (24 mg, 0.24 mmol), and **2** (154 mg, 0.36 mmol) were added under nitrogen to a solution of **3** (70 mg, 0.12 mmol) and MS4A in dry THF (1.5 mL) . The mixture was stirred for three days at room temperature. After removal of the molecular sieves by filtration, the filtrate was extracted with ethyl acetate and washed with $NaHCO_3$(aq) and H_2O. After drying over anhydrous $MgSO_4$, ethyl acetate was evaporated to provide a thick oil. Purification by silica gel column chromatography eluting with ethyl acetate/hexane (3:5 to 5:3) provided pure **4** as a light yellow oil (61 mg, 58 % yield). $[\alpha]_D^{25} = -8.4°$ ($c = 0.1$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 8.29$ (s, 1H), 7.91 (s, 1H), 6.29 (t, $J = 6.9$ Hz, 1H), 5.39 (d, $J = 3.0$ Hz, 1H), 5.20 (dd, $J = 8.1$, 10.5 Hz, 1H), 5.03 (dd, $J = 3.6$, 10.8 Hz, 1H), 4.50 (d, $J = 8.0$ Hz, 1H), 4.42–4.40 (m, 1H), 4.20–4.09 (m, 2H), 3.98–3.74 (m, 5H), 3.56–3.54 (m, 1H), 2.41–2.26 (m, 4H), 2.15, 2.06, 2.05, 1.98 (4 s, 12H), 1.78–1.64 (m, 2H), 1.32–1.20 (m, 2H), 0.92 (s, 9H), 0.88 (s, 9H), 0.12 (s, 6H), and 0.10 ppm (s, 6H); IR (KBr): $\tilde{\nu} = 2945$, 1751, 1709, 1460, 1371, 1227, 1065 cm^{-1}; FAB-MS: m/z: 883 [M^++H].

5-[1-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-5-hexyn-6-yl]-2′-deoxyuridine (5): A solution (1.0 M) of tetrabutylammonium fluoride (1.359 mL, 1.359 mmol) was added under nitrogen in the dark to a solution of **4** (400 mg, 0.453 mmol) in dry THF (15 mL) . The mixture was stirred for 3 h at room temperature. After removal of THF under reduced pressure, the crude residue was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH (25:1 to 25:4) to provide pure **5** as a colorless oily solid (256 mg, 86 % yield). Mp: 88–91 °C; $[\alpha]_D^{25} = -16.6°$ ($c = 0.1$ in $CHCl_3$); . ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.59$ (br s, 1H), 8.17 (s, 1H), 6.26 (t, $J = 6.5$ Hz, 1H), 5.40 (d, $J = 3.5$ Hz, 1H), 5.18 (dd, $J = 8.0$, 10.5 Hz, 1H), 5.05 (dd, $J = 3.5$, 10.5 Hz, 1H), 4.63–4.61 (m, 1H), 4.49 (d, $J = 8.0$ Hz, 1H), 4.22 (dd, $J = 7.0$, 11.0 Hz, 1H), 4.11(dd, $J = 7.0$, 11.0 Hz, 1H), 4.04–3.89 (m, 5H), 3.56–3.52 (m, 1H), 3.01 (br s, 1H), 2.58 (br s, 1H), 2.44–2.31 (m, 4H), 2.16, 2.09, 2.06, 2.00 (4 s, 12H), 1.76–1.59 ppm (m, 4H). IR (KBr): $\tilde{\nu} = 3487$, 3070, 2945, 1751, 1462, 1371, 1227, 1051 cm^{-1}; FAB-MS: m/z: 655 [M^++H]

5-[1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-5-hexyn-6-yl]-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)uridine (6): 4,4′-Dimethoxytritylchloride (78 mg, 0.229 mmol) was added under nitrogen in dark to a solution of **5** (50 mg, 0.076 mmol) in dry pyridine (2 mL). The mixture was stirred for 16 h at 50 °C. After cooling to room temperature, the mixture was extracted with ethyl acetate and washed with $NaHCO_3$(aq) and H_2O. After drying over anhydrous $MgSO_4$, ethyl acetate was evaporated to provide a thick oil. Purification by silica gel column chromatography eluting with $CHCl_3$:MeOH (50:1 to 50:4) provided pure **6** as a colorless oily solid (61 mg, 83 % yield). Mp: 100–102 °C; $[\alpha]_D^{25} = -2.9°$ ($c = 0.1$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.49$ (br s, 1H), 8.01 (s, 1H), 7.37–7.29 (m, 9H), 6.86 (d, $J = 8.0$ Hz, 4H), 6.25 (t, $J = 6.5$ Hz, 1H), 5.40 (d, $J = 3.5$ Hz, 1H), 5.17 (dd, $J = 8.0$, 10.5 Hz, 1H), 5.03 (dd, $J = 3.5$, 10.5 Hz, 1H), 4.54–4.51 (m, 1H), 4.47 (d, $J = 8.0$ Hz, 1H), 4.22–4.12 (m, 2H), 4.09–4.05 (m, 1H), 3.96–3.93 (m, 1H), 3.82 (s, 6H), 3.79–3.75 (m, 1H), 3.46 (dd, $J = 3.5$, 10.5 Hz, 1H), 3.42–3.38 (m, 1H), 3.35 (dd, $J = 3.5$, 11.0 Hz, 1

H), 2.51–2.28 (m, 2H), 2.16–2.00 (m, containing 4 singlets at $\delta=2.16$, 2.09, 2.06, 2.00, 14H), 1.55–1.52 (m, 2H), 1.36–1.33 ppm (m, 2H). IR (KBr): $\tilde{\nu}=3475$, 2943, 1751, 1614, 1508, 1456, 1371, 1227, 1045 cm^{-1}; FAB-MS: m/z: 957 [M^++H].

5-(1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-5-hexyn-6-yl)-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)uridine-3′-*O*-(2-cyanoethyl-*N*,*N*′-diisopropyl)-phosphoramidite (7): Diisopropylethylamine (211 mL, 1.21 mmol) and 2-cyanoethyl-*N*,*N*-diisopropyl chlorophosphoramidite (188 mL, 0.84 mmol) under argon at −78°C were added to a solution of **6** (52 mg, 0.054 mmol) and MS3A in dry THF (5 mL). The mixture was stirred for 45 min at room temperature. After removal of the molecular sieves by filtration, the filtrate was extracted with ethyl acetate and washed with $NaHCO_3$(aq) and brine. After drying over anhydrous $MgSO_4$, ethyl acetate was evaporated to provide a thick oil. Purification by silica gel column chromatography eluting with ethyl acetate/hexane (1:1 to 2:1 to 4:1) containing 0.5 wt% Et_3N provided two diastereoisomers of **7** as light yellow foam (884 mg, 95% yield). Mp: 85–88°C; $[\alpha]_D^{25}=+4.52°$ ($c=0.1$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) **higher R_f**: $\delta=8.90$ (br s, 1H), 8.06 (s, 1H), 7.50–7.20(m, 9H), 6.84 (d, $J=8.5$ Hz, 4H), 6.30 (t, $J=6.5$, Hz, 1 H), 5.38 (d, $J=3.5$ Hz, 1H), 5.16 (dd, $J=8.0$, 10.5 Hz, 1H), 5.02 (dd, $J=$ 3.5, 10.5 Hz, 1H), 4.60–4.56 (m, 1H), 4.44 (d, $J=8.0$ Hz, 1H), 4.23–4.09 (m, 3H), 3.93–3.89 (t, $J=6.5$ Hz, 2H), 3.79 (s, 6H), 3.84–3.76 (m, 1H), 3.73–3.53 (m, 4H), 3.36–3.27 (m, 2H), 2.62 (t, $J=6.5$ Hz, 2H), 2.56–2.51 (m, 1H), 2.33–2.26 (m, 1H), 2.13–1.92 (m, containing 4 singlets at $\delta=$ 2.13, 2.04, 2.02, 1.98, 14H), 1.52–1.40 (m, 2H), 1.32–1.22 (m, 2H), 1.18 (, d, $J=6.5$ Hz, 6H), 1.16(, d, $J=6.0$ Hz, 6H); **lower R_f**: $\delta=8.90$(br s, 1H), 8.01 (s, 1H), 7.50–7.20 (m, 9H), 6.83 (d, $J=8.5$ Hz, 4H), 6.31 (t, $J=$ 6.5 Hz, 1H) , 5.38 (d, $J=3.5$ Hz, 1H), 5.16 (dd, $J=8.0$, 10.5 Hz, 1H), 5.02(dd, $J=3.5$, 10.5 Hz, 1H), 4.60–4.56 (m, 1H), 4.43 (d, $J=8.0$ Hz, 1H), 4.23–4.09 (m, 3H), 3.93–3.89 (t, $J=6.5$ Hz, 2H), 3.79 (s, 6H), 3.84–3.76 (m, 1H), 3.73–3.53 (m,4H), 3.36–3.27 (m, 2H), 2.43 (t, $J=6.5$ Hz, 2H), 2.56–2.51 (m, 1H), 2.33–2.26 (m, 1H), 2.13–1.92 (m, containing 4 singlets at $\delta=2.13$, 2.04, 2.02, 1.98, 14H), 1.52–1.40 (m, 2H), 1.32–1.22 (m, 2H), 1.18 (d, $J=6.5$ Hz, 6H), 1.16 ppm (d, $J=6.0$ Hz, 6H). ^{31}P NMR: $\delta=$ 149.61 and 149.21 ppm; IR (KBr): $\tilde{\nu}=2968$, 1753, 1705, 1614, 1510, 1456, 1371, 1227, 1043, 827 cm^{-1}; FAB-MS: m/z: 1157 [M^++H].

5-(β-D-Galactopyranosyl)-5-hexyn-6-yl)-2′-deoxyuridine (8): 28% NH_3(aq) (2.5 mL) was added to a solution of **5** (50 mg, 0.076 mmol) in MeOH (2.5 mL) . The mixture was stirred for 90 min at room temperature. After a removal of NH_3(aq) under reduced pressure, the crude residue was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH/H_2O (16:4:1 to 8:4:1 to 6:4:1) to provide pure **8** as a white foam (78.8 mg, 93% yield); ^{1}H NMR (500 MHz, CD_3OD): $\delta=8.10$ (s, 1 H), 6.16 (t, $J=6.5$ Hz, 1H), 4.32 (ddd, $J=6.5$, 3.5, 3.25 Hz, 1H), 4.14 (d, $J=8.0$ Hz, 1H), 3.88–3.83 (m, 2H), 3.82–3.80 (m, 1H), 3.72–3.63 (m, 4 H), 3.56–3.48 (m, 1H), 3.38–3.42 (m, 3H), 2.30–2.35 (m, 2H), 2.25–2.20 (m, 1H), 2.18–2.12 (m, 1H), 1.70–1.62 (m, 2H), and 1.60–1.57 ppm (m, 2 H); UV (in water): $\lambda_{max}=292.5$ nm ($\varepsilon=3700$); FAB-MS: m/z 487 [M^+ +H].

Solid-phase synthesis of glycosylated oligonucleotides: Oligonucleotides (1.0 μmol scale) were automatically synthesized on Expedite Nucleic Acid Synthesis System using β-cyanoethylphosphoramidite chemistry and benzimidazolium triflate as activator.[21] The galactoside-modified phosphoramidite **7** was dissolved in dry acetonitrile and loaded onto the machine. The coupling efficiency was monitored by the quantity of trityl cation released. The modified phosphoramidite was introduced to ODNs on the resin and the coupling time was prolonged. After completion of the synthetic cycles, the ODNs were deprotected and cleaved from the resin by treating the resin with aqueous ammonia overnight at room temperature. Then the ODNs were desalted (Sephadex G-25 cartridge column NAP-10), and purified by HPLC (eluent : 0.1 M ammonium acetate and CH_3CN).

HPLC analysis of the enzymatic digest: TES (3 μmol), $MgCl_2$ (0.3 μmol), $CaCl_2$ (1.5 μmol), DNase I (150 units), bacterial alkaline phosphatase (0.38 μg), and nuclease P1 (4.5 units) were added to the solution of purified ODN (30 mg) in water (30 μL) , and then the total volume of the solution was adjusted to 60 μL (pH7.4). The mixture was incubated at 37 °C for 24 h. The enzymatic digests of ODN were analyzed on reverse-phase HPLC eluting with a linear gradient of 0.1 M ammoniumacetate containing 1–3% CH_3CN over 60 min at 25°C (flow rate: 1 mL min^{-1}). The each peak of nucleosides was assigned with these authentic samples as follows: retention time of Gal-dU (**8**) = 1.8 min; dC = 4.7 min; dG = 13.0 min; T = 14.1 min; dA = 33.0 min. The composition of nucleosides in a ODN was determined from the peak area using extinction coefficient of each bases (dA : 15.4×10^3, dG : 11.7×10^3, dC : 7.5×10^3, T : 9.2×10^3, Gal-dU (**8**): 3.7×10^3).

CD spectra: Double-stranded ODN samples were prepared by dissolving galactosylated ODN with an equimolar amounts of full complementary or half-sliding complementary ODN at [ODN] = 1.13 μM for 1 h in PBS (pH7.4). CD spectra of double strands were recorded at 25°C under equilibrium conditions.

Melting behavior: Double-stranded ODN samples were prepared by dissolving galactosylated ODN with an equimolar amounts of full complementary or half-sliding complementary ODN at [ODN] = 1.13 μM concentrations in PBS (pH7.4). The absorbance was recorded at 260 nm as a function of increasing temperature at 1.0°C min^{-1} on a spectrophotometer equipped with a thermal controller.

Estimation of organization by size exclusion chromatography: Double-stranded ODN samples were prepared by mixing galactosylated ODN with equimolar amounts of full complementary or half-sliding complementary ODN at [ODN] = 85 μM concentrations in PBS (pH7.4) at 25°C. After 1 h, SEC analysis of the double- and single-strand samples (20 μL) was performed at 25°C eluting with PBS buffer (flow rate: 0.5 mL min^{-1}). Pullulan was used as the molecular weight standard.

Estimation of organization by gel electrophoresis: Double-stranded ODN samples were prepared by mixing galactosylated ODN with equimolar amounts of full complementary or half-sliding complementary ODN at several concentrations (30–150 μM) in PBS (pH7.4) at 25°C for 1 h. The double-strand samples (10 μL) were loaded onto a 3% agarose gel, and electrophoresis was performed in 0.5×TBE buffer (90 mM Tris and 90 mM boric acid) at 25°C at 4 V cm^{-1}. DNA bands were stained in a 6 μg mL^{-1} ethidium bromide solution and visualized by transillumination with ultraviolet light (310 nm) by using a DC40 camera equipped with 1D Image Analysis Software MAC ver 2.0 (Kodak Digital Science). The length of DNA relative to the *Hinf* I restriction enzyme digest of ϕX174 was determined by agarose gel electrophoresis.

Fluorometric assay of lectin-binding: Aliquots (1 μL) of a stock solution of 9.0 mM ODN sample were added every 25 min to a solution of FITC-labeled RCA_{120} (14.6 nM, 600 μL) in PBS (pH7.4). Fluorescence spectra (500–600 nm) were recorded with excitation at 490 nm at 25°C. The change in fluorescence intensity at 520 nm (ΔF) was corrected for the spontaneous quenching of FITC-labeled RCA_{120} in PBS.

Acknowledgement

We are grateful to Prof. Yoshihiro Hayakawa of Nagoya University for his useful suggestions and measurement of the MALDI-TOF mass spectra. This research was supported by Grants-in-Aid for Scientific Research on Priority Areas, "Biomolecular Design for Biotargeting" (296/12019230), from the Ministry of Education, Science, Sports and Culture and for Encouragement of Young Scientists (11750763) from the Japanese Society for the Promotion of Science.

[1] a) M. Fukuda, O. Hindsgaul, *Molecular Glycobiology* IRL, Oxford, **1994**; b) A. Varki, R. Cumming, J. Esko, H. Freeze, G. Hart, J. Marth, *Essentials of Glycobiology* Cold Spring Harbor Laboratory Press, New York, **1999**.

[2] a) Y. C. Lee, *FASEB J.* **1992**, *6*, 3193; b) L. L. Kiessling, N. L. Pohl, *Chem. Biol.* **1996**, *3*, 71; c) M. Mammen, S.-K. Choi, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 2908; *Angew. Chem. Int. Ed.* **1998**, *37*, 2754; d) J. J. Lundquist, E. J. Toone, *Chem. Rev.* **2002**, *102*, 555.

[3] a) K. Simons, E. Ikonen, *Nature* **1997**, *387*, 569; b) K. Simons, D. Toomre, *Nat. Rev. Mol. Cell Biol.* **2000**, *1*, 31; c) S. Hakomori, K. Handa, K. Iwabuchi, S. Yamamura, A. Prinetti, *Glycobiology* **1998**, *8*, 11; d) M. Hashizume, T. Sato, Y. Okahata, *Chem. Lett.* **1997**, 669.

[4] a) S. Nishimura, Y. C. Lee, in *Polysaccharides* (Ed: S. Dumitriu) Marcel Dekker, New York, **1998**, p 523; b) R. Roy, *Trends Glycosci. Glycotechnol.* **1996**, *8*, 79; c) N. V. Bovin, *Glycoconjugate J.* **1998**, *15*,

431; d) G. B. Sigal, M. Mammem, G. Dahmann, G. M. Whitesides, *J. Am. Chem. Soc.* **1996**, *118*, 3789; e) D. D. Manning, X. Hu, L. L. Kiessling, *J. Am. Chem. Soc.* **1997**, *119*, 3161; f) H. Kamitakahara, T. Suzuki, N. Nishigori, Y. Suzuki, O. Kanie, C.-H. Wong, *Angew. Chem.* **1998**, *110*, 7607; *Angew. Chem. Int. Ed.* **1998**, *37*, 1524.

[5] a) K. Kobayashi, A. Tsuchida, T. Usui, T. Akaike, *Macromolecules* **1997**, *30*, 2016; b) K. Kobayashi, E. Tawada, T. Akaike, T. Usui, *Biochim. Biophys. Acta* **1997**, *1336*, 117; c) T. Hasegawa, S. Kondoh, K. Matsuura, K. Kobayashi, *Macromolecules* **1999**, *32*, 6595; d) A. Tsuchida, K. Kobayashi, N. Matsubara, T. Muramatsu, T. Suzuki, Y. Suzuki, *Glycoconjugate J.* **1998**, *15*, 1014; e) H. Dohi, Y. Nishida, M. Mizuno, M. Shinkai, T. Kobayashi, T. Takeda, H. Uzawa, K. Kobayashi, *Bioorg. Med. Chem.* **1999**, *7*, 2053; f) Y. Nishida, H. Uzawa, T. Toba, K. Sasaki, H. Kondo, K. Kobayashi, *Biomacromolecules* **2000**, *1*, 68; g) K. Matsuura, H. Kitakouji, N. Sawada, H. Ishida, M. Kiso, K. Kitajima, K. Kobayshi, *J. Am. Chem. Soc.* **2000**, *122*, 7406; h) K. Matsuura, H. Kitakouji, R. Oda, Y. Morimoto, H. Asano, H. Ishida, M. Kiso, K. Kitajima, K. Kobayshi, *Langmuir* **2002**, *18*, 6940.

[6] a) K. Aoi, K. Itoh, M. Okada, *Macromolecules* **1995**, *28*, 5391; b) K. Aoi, K. Tsutsumiuchi, A. Yamamoto, M. Okada, *Tetrahedron* **1997**, *53*, 15415; c) K. Aoi, K. Itoh, M. Okada, *Macromolecules* **1997**, *30*, 8072; d) D. Zanini, R. Roy, *J. Am. Chem. Soc.* **1997**, *119*, 2088; e) H. C. Hansen, S. Haataja, J. Finne, G. Magnusson, *J. Am. Chem. Soc.* **1997**, *119*, 6974.

[7] a) T. Fujimoto, C. Shimizu, O. Hayashida, Y. Aoyama, *J. Am. Chem. Soc.* **1997**, *119*, 6676; b) T. Fujimoto, C. Shimizu, O. Hyashida, Y. Aoyama, *J. Am. Chem. Soc.* **1998**, *120*, 601; c) O. Hayashida, M. Kato, K. Akagi, Y. Aoyama, *J. Am. Chem. Soc.* **1999**, *121*, 11597; d) R. Roy, J. M. Kim, *Angew. Chem.* **1999**, *111*, 380; *Angew. Chem. Int. Ed.* **1999**, *38*, 369; e) D. A. Fulton, J. F. Stoddart, *Bioconjugate Chem.* **2001**, *12*, 655; f) O. Hayashida, K. Mizuki, K. Akagi, A. Matsuo, T. Kanamori, T. Nakai, S. Sando, Y. Aoyama, *J. Am. Chem. Soc.* **2003**, *125*, 594.

[8] a) J. J. García-López, F. Hernández-Mateo, J. Isac-García, J. M. Kim, R. Roy, F. Santoyo-González, A. Vargas-Berenguel, *J. Org. Chem.* **1999**, *64*, 522; b) D. A. Fulton, J. F. Stoddart, *Org. Lett.* **2000**, *2*, 1113; c) N. Yasuda, N. Aoki, H. Abe, K. Hattori, *Chem. Lett.* **2000**, 706; d) T. Furuike, S. Aiba, S. Nishimura, *Tetrahedron* **2000**, *56*, 9909.

[9] a) A. Yoshizumi, N. Kanayama, Y. Maehara, M. Ide, H. Kitano, *Langmuir* **1999**, *15*, 482; b) J. M. de la Fuente, A. G. Barrientos, T. C. Rojas, J. Rojo, J. Canada, A. Fernandez, S. Penades, *Angew. Chem.* **2001**, *113*, 2317; *Angew. Chem. Int. Ed.* **2001**, *40*, 2258; c) H. Otsuka, Y. Akiyama, Y. Nagasaki, K. Kataoka, *J. Am. Chem. Soc.* **2001**, *123*, 8226; d) T. Uchida, T. Serizawa, M. Akashi, *Polym. J.* **1999**, *34*, 970; e) T. Serizawa, S. Yasunaga, M. Akashi, *Biomacromolecules* **2001**, *2*, 469.

[10] a) S. Sakai, T. Sasaki, *J. Am. Chem. Soc.* **1994**, *116*, 1587; b) S. Sakai, Y. Shigemasa, T. Sasaki, *Bull. Chem. Soc. Jpn.* **1999**, *72*, 1313; c) T. Hasegawa, K. Matsuura, K. Kobayashi, *Chem. Lett.* **2000**, 466; d) T. Hasegawa, T. Yonemura, K. Matsuura, K. Kobayashi, *Tetrahedron Lett.* **2001**, *42*, 3989; e) T. Hasegawa, T. Yonemura, K. Matsuura, K. Kobayashi, *Bioconjugate Chem.* **2003**, *14*, 728.

[11] a) N. C. Seeman, *Acc. Chem. Res.* **1997**, *30*, 357; b) N. C. Seeman, *Angew. Chem.* **1998**, *110*, 3408; *Angew. Chem. Int. Ed.* **1998**, *37*, 3220; c) N. C. Seeman, *Nano Lett.* **2001**, *1*, 22; d) N. C. Seeman, A. M. Belchar, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 6451; e) J. J. Storhoff, C. A. Mirkin, *Chem. Rev.* **1999**, *99*, 1849; f) C. M. Niemeyer, *Angew. Chem.* **2001**, *113*, 4254; *Angew. Chem. Int. Ed.* **2001**, *40*, 4128; g) M. Scheffer, A. Dorenbeck, S. Jordan, M. Wüstefeld, G. von Kiedrowski, *Angew. Chem.* **1999**, *111*, 3514; *Angew. Chem. Int. Ed.* **1999**, *38*, 3311; h) K. Matsuura, T. Yamashita, Y. Igami, N. Kimizuka, *Chem. Commun.* **2003**, 376.

[12] Y. Ohya, K. Yabuki, M. Komatsu, T. Ouchi, *Polym. Adv. Technol.* **2000**, *11*, 845.

[13] C. J. Loweth, W. B. Caldwell, X. Peng, A. P. Alivisatos, P. G. Schultz, *Angew. Chem.* **1999**, *111*, 1925; *Angew. Chem. Int. Ed.* **1999**, *38*, 1808.

[14] a) C. M. Niemeyer, W. Bürger, J. Peplies, *Angew. Chem.* **1998**, *110*, 2391; *Angew. Chem. Int. Ed.* **1998**, *37*, 2265; b) C. M. Niemeyer, M. Adler, S. Gao, L. Chi, *Angew. Chem.* **2000**, *112*, 3183; *Angew. Chem. Int. Ed.* **2000**, *39*, 3055; c) C. M. Niemeyer, J. Koehler, C. Wuerdemann, *ChemBioChem* **2002**, *3*, 242.

[15] a) C. Mao, W. Sun, Z. Shen, N. C. Seeman, *Nature* **1999**, *397*, 144; b) B. Yurke, A. J. Turberield, A. P. Mills, Jr., F. C. Simmel, J. L. Neumann, *Nature* **2000**, *406*, 605; c) H. Yan, X. Zhang, Z. Shen, N. C. Seeman, *Nature* **2002**, *415*, 62.

[16] C. Mao, T. H. LaBean, J. H. Reif, N. C. Seeman, *Nature* **2000**, *407*, 493.

[17] a) J. Lichtenstein, S. S. Cohen, *J. Biol. Chem.* **1960**, *235*, 1134; b) I. R. Lehman, E. A. Pratt, *J. Biol. Chem.* **1960**, *235*, 3254; c) M. Ehrlich, K. C. Ehrlich, *J. Biol. Chem.* **1981**, *256*, 9966; d) J. Gommers-Ampt, F. van Leeuwen, A. L. J. de Beer, J. F. G. Viliegenthart, M. Dizdarouglu, J. A. Kowalak, P. F. Crain, P. Borst, *Cell* **1993**, *75*, 1129; e) F. van Leewen, M. C. Taylor, A. Mondragon, H. Moreau, W. Gibson, R. Kieft, P. Borst, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 2366; f) F. van Leewen, R. Kieft, M. Cross, P. Borst, *Mol. Cell. Biol.* **1998**, *18*, 5643.

[18] a) K. Matsuura, T. Akasaka, M. Hibino, K. Kobayashi, *Chem. Lett.* **1999**, 247; b) T. Akasaka, K. Matsuura, N. Emi, K. Kobayashi, *Biochem. Biophys. Res. Commun.* **1999**, *260*, 323; c) K. Matsuura, T. Akasaka, M. Hibino, K. Kobayashi, *Bioconjugate Chem.* **2000**, *11*, 202; d) K. Matsuura, M. Hibino, M. Kataoka, Y. Hayakawa, K. Kobayashi, *Tetrahedron Lett.* **2000**, *41*, 7529; e) T. Akasaka, K. Matsuura, K. Kobayashi, *Bioconjugate Chem.* **2001**, *12*, 776; f) K. Matsuura, K. Hayashi, K. Kobayashi, *Chem. Commun.* **2002**, 1140.

[19] a) M. de Kort, E. Edwin, E. R. Wijisman, G. A. van der Marel, J. H. van Boom, *Eur. J. Org. Chem.* **1999**, 2337; b) M. Adinolfi, G. Barone, L. D. Napoli, L. Guariniello, A. Iadonisi, G. Piccialli, *Tetrahedron Lett.* **1999**, *40*, 2607; c) J. Hunziker *Bioorg. Med. Chem. Lett.* **1999**, *9*, 201; d) T. L. Sheppard, C, -H. Wong, G. F. Joyce, *Angew. Chem.* **2000**, *112*, 3806; *Angew. Chem. Int. Ed.* **2000**, *39*, 3660.

[20] K. Matsuura, M. Hibino, Y. Yamada, K. Kobayashi, *J. Am. Chem. Soc.* **2001**, *123*, 357.

[21] Y. Hayakawa, M. Kataoka, R. Noyori, *J. Org. Chem.* **1996**, *61*, 7996.

[22] a) K. Yamada, M. Minoda, T. Miyamoto, *Macromolecules* **1999**, *32*, 3553; b) K. Matsuura, K. Ariga, K. Endo, Y. Aoyama, Y. Okahata, *Chem. Eur. J.* **2000**, *6*, 1750; c) A. Sugasaki, M. Ikeda, M. Takeuchi, S. Shinkai, *Angew. Chem.* **2000**, *112*, 3997; *Angew. Chem. Int. Ed.* **2000**, *39*, 3839; d) S. Shinkai, M. Ikeda, A. Sugasaki, M. Takeuchi, *Acc. Chem. Res.* **2001**, *34*, 494.

[23] The cooperative binding to RCA_{120} lectin is characteristic of a Gal cluster with hscODN, supported by the following experiment. A Gal cluster bearing three galactose residues was constructed by hybridization of three equivalents of **I** with three times periodic full complementary 60-mer **IX** (5′-(ATGGTGAGCAAGGGCGAGGA)$_3$-3′), and the affinity to FITC-RCA_{120} lectin was estimated. The Gal cluster (3×**I**)/**IX** was bound to the lectin with $K_{af} = 1.1 \times 10^4\,M^{-1}$ and $n = 1.0$, indicating non-cooperative binding.

[24] U. Michelsen, C. A. Hunter, *Angew. Chem.* ***2000,*** *112,* 780; *Angew. Chem. Int. Ed.* **2000**, *39*, 764.

Received: August 19, 2003 [F5465]

