DNA Terminal-Specific Dispersion Behavior of Polystyrene Latex Microparticles Densely Covered with Oligo-DNA Strands Under High-Salt Conditions

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Abstract

We prepared microspheres densely covered with oligo-DNA strands by immobilizing amino-terminated oligo-DNA strands on the surface of carboxylate polystyrene latex (PS) particles via the amide bond formation. The obtained microspheres (ssDNA-PS) stably dispersed in neutral pH buffer containing high concentrations of NaCl. For the ssDNA-PS ≥1 μm diameter, only 3%–5% of surface-immobilized oligo-DNA could form a duplex with the complementary strands. Nevertheless, the resulting ssDNA-PS showed a distinct duplex terminal dependency in their dispersion behavior under neutral pH and high NaCl conditions; the microspheres with fully-matched duplexes on the surface spontaneously aggregated in a non-crosslinking manner. By contrast, the microspheres with terminal-mismatched duplexes remained dispersed under the identical conditions. These results suggest that the micrometer-scale particles covered with oligo-DNA strands also have high susceptibility to a duplex terminal sequence in their dispersion property, similar to previously reported DNA-functionalized nanoparticles. This property could potentially be used in various applications including analytical purpose.

Key Words: DNA, Microparticles, Dispersion Behavior, Terminal-Sequence Specificity, Non-Crosslinking Aggregation
Introduction

Over the past two decades, DNA-mediated interactions between colloidal particles have received a great deal of attention in both fundamental and applied aspects. We reported a unique dispersion behavior of nanoparticles densely covered with oligo-DNA strands (ssDNA-NP) in aqueous medium with a high salt concentration; the ssDNA-NP can disperse stably even in the high-salt solution due to interparticle electrostatic and entropic repulsion, but the hybridization with complementary DNA to form fully-matched duplexes on the surface causes spontaneous aggregation in a non-crosslinking manner. Interestingly, such an aggregation can be greatly suppressed under the same conditions when the duplex has a single-base mismatch or overhang at the distal end (Fig.1). These observations strongly suggested that the terminal sequence of duplex formed on the surface dominates the interaction between the particles under high-salt conditions.

Similar behavior has been widely observed for the ssDNA-NP of various core materials (e.g., gold nanomaterials, polymer micelles, and polystyrene latexes), sizes (2–300 nm) and shapes (sphere, rod, and plate). With the use of the terminal-sequence specificity of the interaction between the ssDNA-NP, various analytical methods have been examined including the detection of toxic metal ions, aptazyme cofactors, pollen allergens, and genetic polymorphisms or mutations, as well as identification of clear liquors. Furthermore, this unique interaction has also been applied to construct the dynamic nanomaterial assemblies.

Thus, although the influence of the terminal sequence on the dispersion property of ssDNA-NP has been studied extensively, it has not been explored for the larger (i.e., ≥1 μm diameter) ssDNA-NP. In this study, we investigate whether micrometer-scale particles densely covered with oligo-DNA strands exhibit terminal-sequence specificity...
in their dispersion behavior in a medium with a high salt concentration, similar to the nanometer-sized ssDNA-NP. To this end, we first developed a method for densely immobilizing oligo-DNA strands on the surface of commercial carboxylate polystyrene latex (PS) particles of various sizes. We then prepared ≥1 μm diameter PS particles densely covered with oligo-DNA strands (ssDNA-PS) and we conducted the hybridization of complementary DNA strands on the ssDNA-PS surface and examined the effects of the resulting duplex terminals on the particles’ dispersion behavior in a medium with a high salt concentration.

**Experimental**

*Reagents and chemicals*

Surfactant-free carboxyl-functionalized polystyrene latex beads (d = 0.45, 1.0, and 1.3 μm) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). The source of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) was FUJIFILM Wako Pure Chemical Corp. (Tokyo, Japan). The oligo-DNAs were purchased from Tsukuba Oligo Service (Ibaraki, Japan) and Integrated DNA Technologies (IDT, Coralville, IA, USA). The base sequences of the oligo-DNAs used in this study are provided in Table 1. The oligo-DNAs were dissolved in sterile water as a stock solution and stored at −20°C until used. The DNA concentration was determined by measuring the absorbance at 260 nm. The molecular absorbance coefficient of each oligonucleotide was calculated using the software program OligoAnalyzer 3.1 (IDT, Coralville, IA, USA). Other chemicals were commercially available and used without further purification. MilliQ-grade water (>18 MΩ·m) produced with a Direct-Q UV 3 system (Millipore, Bedford, MA, USA) was used throughout the experiments.
Preparation of probe DNA-immobilized polystyrene latex particles (ssDNA-PS)

Figure 2 depicts the preparation scheme of the probe DNA-immobilized PS particles (ssDNA-PS). The PS suspension (d = 1.3 μm, 3.3 × 10¹⁰ particles/mL, 12.5 μL) was added to 2-(N-morpholino)ethanesulfonic acid (MES) buffer (25 mM, pH 7.2, 487.5 μL). The mixture was sonicated and vortexed for several seconds and then centrifuged at 5,000g for 10 min. The supernatant was replaced with MES buffer (25 mM, pH 7.2). The sample was then sonicated and vortexed for several seconds to induce the dispersion of the particles. This cycle was repeated three times. The resulting PS precipitates were dispersed in 500 μL of MES buffer (25 mM, pH 7.2). The dispersion of PS (8.3 × 10⁸ particles/mL) and 5'-aminoethyl-terminated oligo-DNA (probe DNA: 2.6 nmol) were mixed in a 1.5-mL reaction tube.

Next, a freshly prepared solution of DMTMM (100 mg/mL in H₂O, 37.5 μL) was added to the mixture and agitated at 25°C. After 1 hr, another 37.5 μL of DMTMM solution was added to the mixture. After 3 hr and 6 hr, 32.4 μL of NaCl solution (3.0 M) was added (the final NaCl concentration of the reaction medium was 300 mM) and continuously agitated at 25°C overnight. Aqueous ammonia solution (28 wt%, 20 μL) was added and further agitated for 3 hr at 25°C. The reaction mixture was then centrifuged at 5,000g for 10 min and supernatant was replaced with 1.0 mL of MES buffer (10 mM, pH 7.2) containing 10 mM of NaCl. The mixture was vortexed and sonicated for several seconds to disperse the ssDNA-immobilized PS (ssDNA-PS).

The above washing cycle was repeated at least five times. After the washing step, the ssDNA-PS particles were dispersed in 250 μL of MES buffer (10 mM, pH 7.2) containing 10 mM NaCl and 0.01 wt% Tween 20 (1.7 × 10⁹ particles/mL). The ssDNA-PS dispersions were stored in a refrigerator at 4°C until used. ssDNA-PS of
other core sizes and probe lengths were prepared by the same procedure.

Quantification of probe DNA on the PS surface

The number of probe DNA immobilized on the PS surface was determined by a fluorescent method using a microplate fluorometer (SPARK 10M plate reader, TECAN, Männedorf, Switzerland). To this end, the 3’-terminal of probe DNA was labeled with a Cy3-fluorophore (3’-Cy3 probe DNA, Table S1 in Supporting Information). The immobilization of 3’-Cy3 probe DNA on the PS surface was carried out with the protocol described above. After the immobilization reaction, the reaction mixture was centrifuged at 5,000g for 10 min, and supernatant containing unreacted 3’-Cy3 probe DNA was replaced with MES buffer (10 mM, pH 7.2) containing 10 mM NaCl and 0.01 wt% Tween 20. The precipitate was washed repeatedly by resuspension and centrifugation until the fluorescence intensity of the supernatant was reduced to the background level.

The well-washed precipitate (Cy3-ssDNA-PS) was dispersed in MES buffer (10 mM, pH 7.2) containing 10 mM NaCl and 0.01 wt% Tween 20. The particle number concentration of the resulting dispersion was determined by an Attune NxT Flow cytometer (Thermo Fisher Scientific, Waltham, MA, USA). We converted the measured fluorescence intensity ($E_x$: 550 nm, $E_m$: 570 nm) of the Cy3-ssDNA-PS dispersion to the 3’-Cy3 probe DNA concentration ($C_{probe}$) by using the calibration curve derived from the free 3’-Cy3 probe DNA solutions in advance. The number of 3’-Cy3 probe DNA immobilized on the PS particle ($N_{probe}$) was determined from the $C_{probe}$ value and the particle number concentration of Cy3-ssDNA-PS.
Bright-field microscope imaging

Microscope imaging of the dispersion was carried out using a BX53 bright field microscope (Olympus, Tokyo, Japan) equipped with a 100× oil-immersion objective lens. Approximately 5 μL of the dispersion was loaded in a microchamber consisting of two glass coverslips separated by a Parafilm® spacer and sealed with silicone grease. During the observation, the sample temperature was held at 25°C using a KM-1 Microwarm Plate (KITAZATO Co., Shizuoka, Japan). Static images were captured by a DP74 microscope digital camera (Olympus, Tokyo, Japan), and analyzed using ImageJ software (U.S. NIH).

Melting temperature (T_m) measurement

We obtained the melting curves of DNA duplexes in 150 mM NaCl containing MES buffer solution (10 mM, pH 7.2) by measuring the change of absorbance at 260 nm as a function of temperature with a UV-2550 spectrophotometer equipped with a TMSPC-8 temperature controller unit (Shimadzu, Kyoto, Japan). The DNA duplex concentration was set at 4.0 μM. The temperature ramp was 1°C/min. The T_m was determined as an average of the maximum values in the first derivative of the melting curves obtained from the heating and cooling processes.

Surface hybridization analysis

The number of target DNA hybridized on the ssDNA-PS surface was quantified by a fluorescent method. The 5’-FAM-labeled target DNA (5’-FAM target DNA, Table S1 in Supporting Information) was added into the dispersion of ssDNA-PS (1.4 × 10^8 particles/mL) in MES buffer (10 mM, pH 7.2) containing predetermined concentrations of NaCl. The final concentration of 5’-FAM target DNA was 4.0 μM. The mixture was
incubated overnight in the dark at 25°C, after which it was centrifuged at 5,000 g for 10 min to remove unhybridized 5’-FAM target DNA-involved supernatant. The precipitate was washed with incubation buffer repeatedly by redispersion and centrifugation until the fluorescence intensity of the supernatant was reduced to the background level.

The fluorescence intensity of the resulting dispersion (3.1 × 10⁶ particles/mL) was measured with the above-mentioned microplate fluorometer (Eₚ: 488 nm, Eₘ: 530 nm). We converted the fluorescent intensity of the dispersion to the concentration of 5’-FAM target DNA (C_target) by using the calibration curve derived from the free 5’-FAM target DNA solutions in advance. The number of 5’-FAM target DNA that corresponds to the number of duplexes (N_duplex) formed on the ssDNA-PS surface was determined by the C_target value and the particle number concentration of the dispersion.

Zeta potential measurement

We determined the zeta potential of the ssDNA-PS particles by performing electrophoretic light scattering using an ELS-Z zeta-potential and particle size analyzer (Otsuka Electronics Co., Osaka, Japan). The sample temperature was held at 25°C. We converted the measured electrophoretic mobility (ν) into the zeta potential (ζ) by applying the Smoluchowski equation: ζ = 4πην/ε, in which η is the viscosity and ε is the dielectric constant of the solvent, respectively.

Results and Discussion

Preparation of ssDNA-PS

We prepared a series of the ssDNA-PS dispersions by the covalent immobilizing of the 5’-aminohexyl terminated oligo-DNA (probe DNA) to the carboxylate PS surface as shown in Fig.2. To avoid the unfavorable aggregation of the PS particles and the
unintended immobilization of probe DNA via the functional groups other than terminal aminohexyl group, the reaction was conducted in neutral MES buffer (pH 7.2). Under the neutral-pH conditions, the PS particles can be stabilized through the interparticle electrostatic repulsion provided by the surface carboxylate groups (the pKₐ of the carboxyl group on the PS particles ≈ 5).

We used DMTMM, a water-soluble triazine-based condensing agent, to activate the PS surface carboxyl groups. Unlike the conventional water-soluble carbodiimide, DMTMM can adopt to a wide pH range and forms a nucleophile reactive intermediate with a carboxyl group, which promotes the amide linkage formation with an amino group. Moreover, we added NaCl stepwise into the reaction medium to a final concentration of 300 mM. The added sodium ions were expected to reduce the electrostatic repulsion between the negatively charged probe DNAs and also between the probe DNA and the carboxylate PS surface, leading to a dense immobilization of the probe DNA on the PS surface. Under these conditions, the PS particles maintained the dispersed state during the reaction process. After the washing treatment by repeated centrifugation and redispersion, the ssDNA-PS was obtained as a stable dispersion.

**Probe DNA density on the ssDNA-PS surface**

We examined the probe DNA density immobilized on the ssDNA-PS surface by a fluorescence method. We used the probe DNA labeled with Cy3-fluorophore at the 3’-terminal (3’-Cy3 probe DNA, Table S1 in Supporting Information). Cy3 has no potential functional groups that unfavorably react with DMTMM. The immobilization of 3’-Cy3 probe DNAs to the PS surface was carried out by the same procedure as that used for the non-labeled probe DNA. The number of 3’-Cy3 probe DNAs (Nprobe) immobilized on each ssDNA-PS surface was determined by fluorescence intensity. We
then derived the molecular footprint ($S_{\text{probe}}$) and areal density ($D_{\text{probe}}$) of probe ssDNA based on the size of the PS core and the $N_{\text{probe}}$ value. The results are summarized in Table 2.

For the 15-nucleotide (nt)-long-probe ssDNA ($s_{15}$), the areal density was found to be approx. $7.6 \times 10^4$ to $1.2 \times 10^5$ strands/μm$^2$, which corresponds to a molecular footprint of 8.3 to 13 nm$^2$/strand, regardless of the PS core size. This surface density of probe DNA is significantly higher than that in a previous study using the neutravidin-biotin linkage (approx. $3.3 \times 10^3$ to $7.1 \times 10^3$ strands/μm$^2$),$^{31-33}$ and almost comparable to those using the newly developed high reactive particles (approx. $1.8 \times 10^4$ to $7.2 \times 10^4$ strands/μm$^2$).$^{34,35}$ We also confirmed that the 9-nt-long and 30-nt-long probe DNAs were also immobilized at high density on the PS surface. These results support the effectiveness and versatility of the method that we used to immobilize probe DNA described herein.

**Dispersion properties of micrometer-sized ssDNA-PS**

We next studied the dispersion properties of the micrometer-sized ssDNA-PS in NaCl solution. The dispersion state of the ssDNA-PS ≥1 μm diameter in aqueous solution can be observed directly by bright-field microscopy. Microscope images of the ssDNA-PS particles taken after 1-hr incubation at 25°C in MES buffer (10 mM, pH 7.2) containing 750 mM NaCl are shown in Fig.3. For the 1.3 μm of PS particles covered with 15-nt-long probe DNA ($s_{15}$-PS(1.3)), most of the particles in the microscope image are individually separated from each other, reflecting their well-dispersed state at high NaCl conditions (Fig.3a).

In contrast, in the microscope image of the $s_{15}$-PS(1.3) particles in the presence of complementary target DNA (15-comp), some portion of the particles formed aggregates.
under the same condition (Fig.3b). However, such aggregate formation was hardly observed in the presence of the other target DNAs (Figs.3c–e). We quantified the dispersion degree of the s15-PS(1.3) particles at each condition based on the fraction of singlet (i.e., non-aggregated) particles in the corresponding microscope images. Figure 4a shows the mean singlet fraction (SF) for the s15-PS(1.3) dispersion determined at various NaCl concentrations. The s15-PS(1.3) was able to disperse stably in NaCl solutions, and thus the SF value was found to be ≥0.95 in the NaCl concentration range of 150–750 mM. For the dispersion of s15-PS(1.3) in the presence of 15-comp, the SF value was decreased to 0.77 at the NaCl concentration of 300 mM (Fig.4b, X = G). In contrast, for the dispersion of the s15-PS(1.3) in the presence of other target DNAs with a single-base substitution at the 5’-terminal, ≥0.94 as the SF value was obtained even at the NaCl concentration of 750 mM (Fig.4b, X = A, T, or C). Please note that such difference in the SF values for the s15-PS(1.3) dispersions is hard to discriminate by the turbidity change observation with the naked eye.

All of the $T_m$ values of the duplexes between the 15-nt-long probe DNA (s15, not immobilized) and the target DNAs in the presence of 150 mM NaCl were observed to be higher than the experimental temperature (25°C); 65.6°C for s15/15-comp, 64.7°C for s15/15-sub-A, 64.2°C for s15/15-sub-T, and 63.9°C for s15/15-sub-C (Fig.S1–S4 in Supporting Information). It can thus be considered that the duplexes between probe and target DNAs formed on the PS surface are thermodynamically stable in the present experimental conditions.

To understand the hybridization of target DNA on the s15-PS(1.3) in detail, we quantified the hybridized target DNA by using 5’-terminal FAM-labeled DNA (5’-FAM target DNA, Table S1 in Supporting Information). The number of duplexes formed on the s15-PS(1.3) surface ($N_{\text{duplex}}$) and the hybridization efficiency ($E_H$) that corresponds
to the percentage of probe DNA hybridized with the 5’-FAM target DNA at each condition were determined and are summarized in Table 3.

For the hybridization with the 5’-FAM 15-comp, the $E_H$ values were low (approx. 3.0% to 4.7%) compared to those for the typical gold nanoparticle surfaces (approx. 30% to 50%)\(^6\) and the flat gold surface (approx. 33%)\(^3\) at similar NaCl concentrations. Moreover, unexpectedly, the $E_H$ value was hardly affected by the NaCl concentration. We also assessed the hybridization of 15-comp by using the 5’-terminal Cy3-labeled one (5’-Cy3 15-comp, Table S1 in Supporting Information). The $N_{\text{duplex}}$ and $E_H$ values were determined to be slightly higher than those for the 5’-FAM-labeled one, but the hybridization degree was significantly low regardless of the NaCl concentration (Table S2 in Supporting Information). The low $E_H$ values observed herein were probably due to the steric hindrance caused by the sterically crowded environment of the s15-PS(1.3) surface and the electrostatic repulsive interactions caused by the unreacted carboxylate groups on the s15-PS(1.3) surface. Furthermore, we confirmed that there was no significant difference between the 15-comp and other target DNAs in terms of the $E_H$ value at 750 mM NaCl where a distinct difference in the dispersion behavior was observed.

Taken together, our findings demonstrate that the distinct difference in the dispersion behavior among four target DNA-hybridized s15-PS(1.3) particles under high-NaCl conditions arose from the terminal sequence of the duplexes partially formed on the PS surface. In other words, these results mean that the densely oligo-DNA-immobilized particles $\geq$1 μm diameter also have high susceptibility to structural differences at the duplex terminal in their dispersion property, as do nanometer-sized particles such as DNA-conjugated polymer micelles\(^4,9-12\) and DNA-functionalized gold nanomaterials.\(^5-8,14\)
The contribution of NaCl to the terminal sequence-specific non-crosslinking aggregation

Since the base sequence of 15-comp cannot crosslink s15-PS(1.3) particles through interparticle hybridization, the above aggregation observed for 15-comp-hybridized s15-PS(1.3) particles is a spontaneous non-crosslinking aggregation. To induce the terminal sequence-specific non-crosslinking aggregation, a high concentration of NaCl was required in addition to the formation of a fully-matched duplex on the s15-PS(1.3) surface. However, as shown in Table 3, the increase in the NaCl concentration had a negligible effect on the duplex formation on the s15-PS(1.3) surface. One possible interpretation for such terminal sequence-specific dispersion behavior of the target DNA-hybridized s15-PS(1.3) particles is that they may have a different surface charge under the high NaCl conditions. We therefore examined the surface charge of the target DNA-hybridized s15-PS(1.3) particles by conducting zeta potential measurements.

As shown in Fig.5a, the zeta potential of the 15-comp-hybridized s15-PS(1.3) gradually reduced with an increase in the NaCl concentration, and no drastic change was observed around the NaCl concentration of 300 mM (at which the non-crosslinking aggregation occurred). There were no significant differences in zeta potentials among the four target DNA-hybridized s15-PS(1.3) particles at the identical high NaCl concentration ([NaCl] = 750 mM, Fig.5b). From these results, we concluded that the surface charge is not responsible for the terminal sequence-specificity in dispersion behavior of the target DNA-hybridized s15-PS(1.3) particles under high-NaCl conditions.

At present, the essential contribution of the high concentration of NaCl to the
terminal sequence-specific non-crosslinking aggregation observed at 15-comp-hybridized s15-PS(1.3) particles is unclear except for the reduction of electrostatic repulsion. Considering that fully-matched duplexes partially formed on the s15-PS(1.3) surface were also necessary for the non-crosslinking aggregation, we anticipate that the high concentration of NaCl would promote the end-to-end interaction between pairs of fully-matched duplexes (i.e., blunt-end stacking$^{37,38}$) on the individual s15-PS(1.3) particles, leading to the terminal sequence-specific aggregation in a non-crosslinking manner. Indeed, we recently observed the appearance of such end-to-end interaction between the DNA strands immobilized on the gold surface.$^{39}$ Direct measurements of interparticle forces in the dispersing state$^{40}$ might provide useful information to understand the underlying molecular mechanisms of the above terminal sequence-specificity in the dispersion behavior. PS particles with a diameter of approx. 1 to 3 µm are suitable to directly analyze the interparticle force in dispersion with the optical tweezers technique. Further studies of this topic are currently underway.

Conclusions
We developed an effective and versatile method to immobilize 5’-amino-terminated oligo-DNA on the carboxylate PS surface via amide bond formation. This method can be easily applied for DNA-functionalization of various carboxylate-modified particles including the fluorospheres and Q-dots. Using this method, we successfully prepared various sizes of ssDNA-PS particles densely covered with oligo-DNA strands. For the ssDNA-PS particles $\geq$1 µm diameter, we observed that only 3%–5% of probe DNA on the PS surface formed a duplex with the complementary strands. Nevertheless, we confirmed that the resulting particles show a distinct duplex terminal dependency in their dispersion behavior at neutral and high-salt conditions, similar to that reported for
various DNA-functionalized nanoparticles. Considering the negligible difference in the surface charges of the particles, we speculate that the distinct difference in the dispersion behavior is due to a terminal-specific interaction between the duplexes partially formed on the individual particles. To our knowledge, this is the first example that demonstrates high terminal sequence susceptibility of micrometer-scale DNA-functionalized particles in their dispersion behavior.

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Supporting Information
The melting curves of DNA duplexes, base sequences of the fluorophore-labeled oligo-DNA strands, and hybridization degree of complementary target DNA onto s15-PS(1.3) surface ($N_{\text{duplex}}$ and $E_H$ values) estimated for the 5’-terminal Cy3-labeled one, are available free of charge on the Web at http://www.jasc.or.jp/analsci/.
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Table 1  Base sequences of the oligo-DNA strands

| Code  | Sequence (5’ to 3’)                     |
|-------|----------------------------------------|
| Probe DNA |                                             |
| S9    | aAminoC₆–GCC ACC AGC                    |
| S15   | aAminoC₆–TAG GCC ACC AGC TCC             |
| S30   | aAminoC₆–TTC CAT ATT CTT CGT TAG GCC ACC AGC TCC |
| Target DNA |                                           |
| 15-comp | GGA GCT GGT GGC CTA                     |
| 15-sub-C | CGA GCT GGT GGC CTA                     |
| 15-sub-A | AGA GCT GGT GGC CTA                   |
| 15-sub-T | TGA GCT GGT GGC CTA                     |

a. AminoC₆: aminohexyl terminal (H₂N–(CH₂)₆–); The underlined bases make unpaired terminals after the duplex formation with 15-nt probe DNA (s15).

Table 2  Characterization of ssDNA-PS

| Code    | Probe DNA | PS size⁴ | Nprobe | Sprobe | Dprobe |
|---------|-----------|----------|--------|--------|--------|
|         | (length)  | (µm)     | (particles)³ | (nm²/strand)³ | (strand/µm²)³ |
| s15-PS(0.45) | s15 (15 nt) | 0.45 | 5.4(±0.5) × 10⁴ | 12±1.2 | 8.5(±0.8) × 10⁴ |
| s15-PS(1.0) | s15 (15 nt) | 1.0  | 2.4(±0.2) × 10⁵ | 13±1.2 | 7.6(±0.6) × 10⁴ |
| s15-PS(1.3) | s15 (15 nt) | 1.3  | 6.5(±1.0) × 10⁵ | 8.3±1.2 | 1.2(±0.2) × 10⁵ |
| s9-PS(1.3) | s9 (9 nt)  | 1.3  | 9.8(±1.8) × 10⁵ | 5.6±1.1 | 1.8(±0.3) × 10⁵ |
| s30-PS(1.3) | s30 (30 nt) | 1.3  | 5.2(±0.3) × 10⁵ | 10±0.6 | 9.8(±0.5) × 10⁴ |

a. diameter; b. Nprobe: Number of probe DNA on the PS surface; c. Sprobe: Molecular footprint of probe DNA on the PS surface; d. Dprobe: Areal density of probe DNA per square micrometer; e.
Table 3  Hybridization of target DNA on the s15-PS(1.3) surface

| Target DNA  | NaCl (mM) | N\textsubscript{Duplex} (/particle) | E\textsubscript{H} (%) |
|-------------|-----------|-----------------------------------|-----------------------|
| 15-comp     | 150       | 2.1(±0.4) × 10\textsuperscript{4}  | 3.2±0.6               |
|             | 300       | 2.1(±0.5) × 10\textsuperscript{4}  | 3.3±0.7               |
|             | 500       | 3.0(±0.3) × 10\textsuperscript{4}  | 4.7±0.4               |
|             | 750       | 2.0(±0.5) × 10\textsuperscript{4}  | 3.0±0.7               |
| 15-sub-C    | 750       | 2.7(±0.3) × 10\textsuperscript{4}  | 4.1±0.5               |
| 15-sub-A    | 750       | 1.7(±0.2) × 10\textsuperscript{4}  | 2.7±0.4               |
| 15-sub-T    | 750       | 2.7(±0.3) × 10\textsuperscript{4}  | 4.2±0.4               |

a. 5’-Terminal of target DNA was labeled with a FAM-fluorophore; b. N\textsubscript{Duplex}: Number of duplexes on the PS surface; c. E\textsubscript{H}: Hybridization efficiency; d. mean±SD (n = 3).
Figure Captions

Fig. 1 Overview of the terminal sequence-specific dispersion behavior of DNA-functionalized nanoparticles (ssDNA-NP) in a medium with a high-salt concentration.

Fig. 2 Preparation scheme of single-stranded oligo-DNA-immobilized polystyrene latex particles (ssDNA-PS).

Fig. 3 Bright-field microscope images of the s15-PS(1.3) particles (1.4 × 10^8 particles/mL) in the absence (a), and presence of 15-comp (b), 15-sub-C (c), 15-sub-A (d), and 15-sub-T (e) after the 1-hr incubation in 10 mM MES buffer (pH 7.2) containing 750 mM NaCl and 0.01 wt% Tween 20 at 25°C. [target DNA] = 4.0 μM, Scale bar: 20 μm.

Fig. 4 The effect of the NaCl concentration on the mean SF value of the s15-PS(1.3) dispersion in the absence (a) and presence (b) of target DNA in 10 mM MES buffer (pH 7.2) containing 0.01 wt% Tween 20 at 25°C. Conditions: [s15-PS(1.3)] = 1.4 × 10^8 particles/mL; [target DNA] = 4.0 μM. The mean SF value was determined from three microscope images taken at different positions of the sample (mean±SD, n = 3).

Fig. 5 (a) The zeta potential of the 15-comp-hybridized s15-PS(1.3) as a function of the NaCl concentration in 10 mM MES buffer (pH 7.2) at 25°C. (b) Comparison of the zeta potential among the four target DNA-hybridized s15-PS(1.3) particles in 10 mM MES buffer (pH 7.2) containing 750 mM NaCl at 25°C. [s15-PS(1.3)] = 1.4 × 10^8 particles/mL, [target DNA] = 4.0 μM. All of the zeta potentials are represented as the mean±SD (n = 5).
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