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DNA-functionalized polystyrene particles and their controlled self-assembly

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The DNA-functionalized polystyrene colloids with the regards to the thermodynamic information are investigated here. This system is built on micro-sized polymer bids with different lengths of DNA spacer grafted on the surface of the particles. Two types of DNA linkers were designed to create a bridge between complementary DNA-functionalized particles, to better understand the effect of DNA sequence on an annealing temperature (T_m) of the aggregation. The disassembly of polymeric scaffold based on DNA melting is in a good agreement with that of calculation from the thermodynamic information. However, the length and the topology of DNA spacer are important for the experimental melting point due to the effect of directionality of double-stranded DNA between DNA-grafted particles and DNA linker.

Introduction

Nowadays, DNA based materials is a very interesting field to investigate self-assembly structures, which can be exploited into a new material, for example, crystallization of colloidal nanoparticles, etc.[1-10] DNA-functionalized particles have become the basis for an increased number of diagnostic applications.[11-15] In these systems, the noncovalent network is held together based on a DNA target sequence and the DNA functionalized particle. This aggregation process is reversible upon the temperature change depending on the DNA sequences, which dominate the melting temperature (T_m) for the system. The aggregation and melting of these assemblies are influenced not only by DNA sequences but also DNA length,[16] particle size,[17] interparticle distance,[18] DNA concentration,[19] etc.

Nanotechnology is a discipline that relates to the design, synthesis, characterization, and the application *via* the structure of nanomaterials.[20-23] DNA nanoparticle assemblies have been proposed for use in DNA detection in medical research, diagnosis of genetic disease, biodefense as well as an alternative technology in DNA microarrays (multiplex gene analysis)[24] and single-molecule sequencing.[25] This technology relies on differentiation in DNA hybridization efficiency. In that system, single stranded DNA molecules are grafted on gold surface *via* an alkanethiol linker. Introducing a fully complementary DNA probe results in double helix formation and intercalation of an optically active staining agent.

In parallel, DNA-functionalized polymer colloid has been developed in macro-scale which can be easily detected by an optical microscopy.[14,26-27] In this system, single stranded DNA, functionalized with an amide group, are deposited on the polystyrene colloid surface. The self-assembly between the particles can be controlled by the addition of a complementary DNA linker. This type of system, which is based on multiple hydrogen bond interactions can be developed into controlled ordered structures. This method of using specific interactions between microsphere particles is different from that of nanoparticle in term of nucleation, growth, annealing, and thermodynamic stability.

Due to the fact that in both, nanoparticle and microsphere systems, DNA fragments are grafted on the surface and therefore the aggregation process depends on the DNA target concentration and inevitably on the DNA melting temperature (T_m) for the system.[19] Thus, the equilibrium binding constant between the target particle can be a fundamental limiting factor for a high sensitivity detection system that does not involve target amplification. In the controlled DNA melting process, the double helical DNA structure is separated into two single strands by the cooperatively effect between nucleobases.[28] The length of the strands and the salt concentration are influential for the melting temperature. The thermodynamic studies of binding properties for DNA-functionalized gold nanoparticles are well-presented.[29-30] However, there are limited reports for thermodynamic studies of the DNA microsphere system. In the present work, the DNA-functionalized polystyrene microsphere particles are prepared. Complementary DNA linker is induced to facilitate the self-assembly between DNA-functionalized particles, based on the specific molecular recognition. Here, we report the thermodynamic investigation on different lengths of DNA molecule grafted on polystyrene particles and different sequences of DNA linkers for controlled microsphere aggregation.

Results and discussion

Thermodynamic properties of DNA sequences and lengths dependent melting studies are investigated here. Polystyrene particles (1 μm) are functionalized with the 5'-amino-derivative DNA through PEG spacers using the polymer

swelling/deswelling method (Fig. 1). The mechanism of DNA functionalized colloid microspheres is well-described in our previous work. Essentially, DNA is covalently attached on the polystyrene particle *via* amide bond with poly(ethylene glycol)-poly(propylene glycol)-poly(ethylene glycol) (PEG-PPG-PEG) triblock-copolymer surfactant.[13] To obtain comparable melting results at an equilibrium state, similar experiments are performed in the same condition under triethylammonium acetate buffer (TEAA) for 24 h.

This system is based on a one-size particle component, sketched in Fig. 2, where every colloids have the DNA-mediated attraction to each other. We also design the DNA linker (Table 1) that is complementary with DNA-functionalized polystyrene particle. Thus, the linkers allow particles to physical crosslink by hydrogen bonding based on Watson-Click pairing between two DNA-grafted particles.

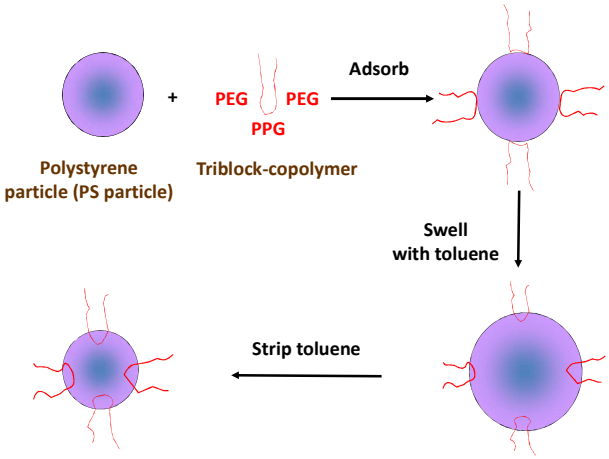

Fig. 1 Polymer swelling/deswelling procedure: polystyrene particles were mixed with the PEG-PPG-PEG triblock-copolymer. The surfactant penetrates the surface of the particle in toluene solvent. After deswelling (toluene strip at 98°C) surfactants became permanently anchored to the surface of the bids.

Table 1 Designed DNA sequences used for DNA-grafted both: particle 1 with DNA linker 11 and particle 2 with DNA linker 12.

Name	Sequences (5' to 3')
DNA-1A (short)	NH_2 - ACTTAACTACAGCATTATCAGTCTCCGAGGCCAACTTGAAATCTCT
DNA-1B (medium)	NH_2 - ACTTAACTACAGCATTATCAGTCTCCGAGGCCCATTGATTCAACTTGAAATCTCT
DNA-1C (long)	NH_2 - ACTTAACTACAGCATTATCAGTCTCCGAGGCCCATTGATTCACACACGTCTAACTTGAAATCTCT
DNA-2A (short)	NH_2 - ACTTAACTACAGCATTATCAGTCTCCGAGGCCTTGTCTACTCTGTC
DNA-2C (long)	NH_2 - ACTTAACTACAGCATTATCAGTCTCCGAGGCCCATTGATTCACACACGTCTTTGTCTACTCTGTC
DNA-11 (linker)	AGAGATTTCAAGTT-AGAGATTTCAAGTT
DNA-12 (linker)	GACAGAGTAGACAA-AGAGATTTCAAGTT

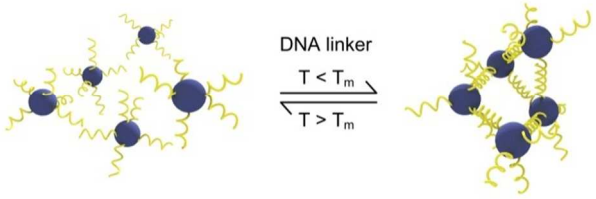

Fig. 2 DNA-functionalized particles mixed with DNA linker form a reversible self-assembly system where scaffold formation depends on the temperature. In the temperature T < T_m aggregated structure is formed but when T > T_m it is dispersed.

The DNA sequences at different lengths (Table 1) can be grafted on the polystyrene particles. Part of DNA sequences (without underline) has been designed as DNA spacer and separating dsDNA from the surface of the particle. Underlined part of DNA sequences is complementary to the DNA linker sequences. The effect of DNA spacer's length for DNA-A, -B and -C (short, medium and long respectively) has a significant effect on the binding properties between polystyrene particles and is a subject of investigation here.

Normally, the DNA spacer is used to stabilize polystyrene particle and also increases its hybridization efficiency by moving the target recognition sequence further from the particle surface.

As it was assessed by the optical microscopy, DNA-grafted polystyrene particles do not assemble without DNA linker. When the DNA-11 linkers are added in the solution, the polystyrene particles aggregate (Fig. 3). Aggregation occurs when polystyrene particles functionalized with single stranded oligonucleotides are mixed with a target of complementary DNA. The hybridization leads to the formation of a polymeric network. However, when the aggregate particles are heated up to 55°C for short (51°C for medium and 47°C for long), the structure dissociates and the particles disperse. When the well-dispersed particles are cooled down to around 30°C, they become aggregated structures again (Fig. 3). This result suggests that the network of DNA-grafted particles is reversible. This result is in good agreement with the previous study.[13]

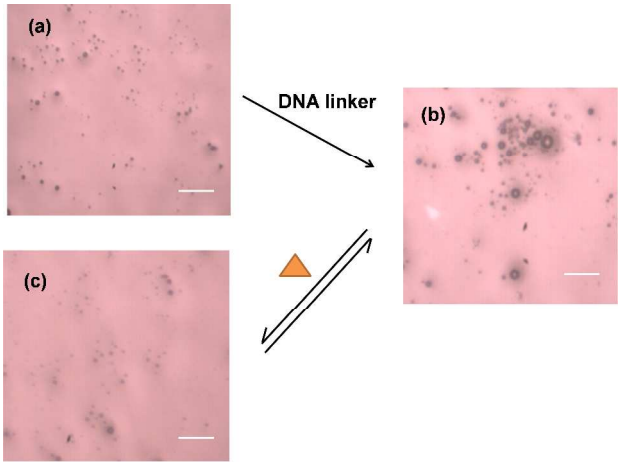

Fig. 3 Optical microscope images of (a) DNA-grafted polystyrene particles, the scale bar is 20 µm. (b) After adding DNA linker particles form aggregated structures. (c) Heating above T_m causes complete redispersion. The system of aggregation and dispersion of particles is reversible.

The AFM results (Fig. 4) confirm the self-assembly of particles in the presence of DNA linker. Aggregated networks were prepared several times to confirm the reproducibility. The particles were assembled in the presence of DNA linker that was complementary to the DNA sequences on the particle. Height image shows expected particle size of approximately 1 µm, as we observed it before,[13] but it is higher in the central part of the spot, which suggest higher density aggregation. Phase images (Fig. 4) present nice topology of the particle's aggregation revealing also remaining buffer solution surrounding the sample's spot.

Melting temperature (T_m) for a free DNA in solution can be calculated using the semi-empirical thermodynamic values based on the constituents of base pairs involved in dsDNA formation. For self-complementary oligonucleotide duplexes, the T_m is calculated from the predicted standard enthalpy ΔH° and standard entropy ΔS° (thermodynamic parameters for base pairs that incorporate nearest-neighbor interactions)[31-32,33-34] and the total oligonucleotide strand concentration *C*, by using this equation:

$$T_m = \Delta H°/(\Delta S° + R \ln C/4)$$

where *R* is the gas constant (1.987 cal K^{-1} mol^{-1}), and *C* is a concentration of oligonucleotide. Based on DNA linker 11 and linker 12 with 28 base pairs from Table 1, thermodynamic data with calculated T_m, based on two independent sources [31-32] and [33-34], is presented in Table 2.

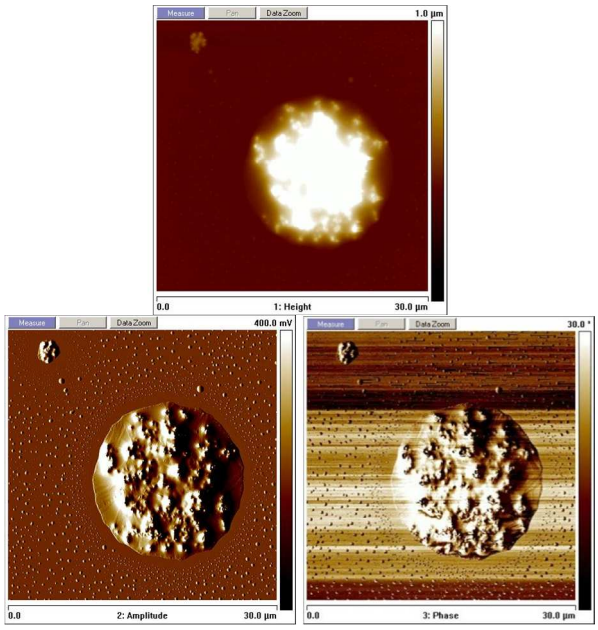

Fig. 4 DNA-functionalized polystyrene particles DNA-1C (long) mixed with DNA-11 linker in 10 mM TEAA buffer. Aggregation was deposited on the mica surface and analysed using AFM. Height and phase images confirm the particle size and higher aggregation arround the central part. Amplitude and phase however, reveal the sample's topology and density of the aggregation.

Table 2 Thermodynamic data and calculated T_m for DNA-1A and DNA-2A with DNA linker 11 and linker 12. [a]Based on the values from reference[31-32], [b]Based on the reference.[33-34]

Thermodynamic data	DNA-1A-11-1A[a]	DNA-1A-11-1A[b]	DNA-1A-12-2A[a]	DNA-1A-12-2A[b]
ΔH° (Kcal/mol)	-205.4	-188.8	-188.6	-123.2
ΔS° (Kcal/mol.K)	-0.562	-0.590	-0.508	-0.370
ΔG° (Kcal/mol)	-38.0	-28.6	-37.0	-23.2
Calculated T_m (°C)	63.0	67.5	65.0	70.5

In this experiment, complementary sequences in DNA grafted on the particle are fixed but the DNA spacer lengths vary. The calculated T_m between DNA-1A and DNA-11 oscillate between 63.0 and 67.5 depends on the calculation method (Table 2). However, we found that the experimental T_m is significantly lower than the calculated T_m (Fig. 5). Moreover, the T_m decreases with the DNA spacer length and for the longest (51nt of spacer) the difference between calculated and measured T_m is more then 20°C depending on the DNA spacer length. This could be explained by the

influence of the DNA spacer length separating two particles, which can cause the sequence defect on double-stranded DNA between DNA-grafted particles and DNA linker.[33] However, it should be possible that the van der Waals interaction becomes dominant for shorter DNA so that the particles bind stronger. Thus, longer DNA spacer length higher deviation between experimental T_m and calculated T_m (Fig. 5).

$$\Delta G° = \Delta H° - T\Delta S°$$

where T is temperature (K) and calculated ΔG° is presented in Table 2. It is well known that the negative values of ΔG° indicate the spontaneous nature of hydrogen bond formation between complementary DNA sequences. The results in Table 2 indicate, as expected, that the ΔG° values in both cases with DNA-11 and DNA-12 are negative, indicating that the self-assembly of DNA should be a spontaneous processes.

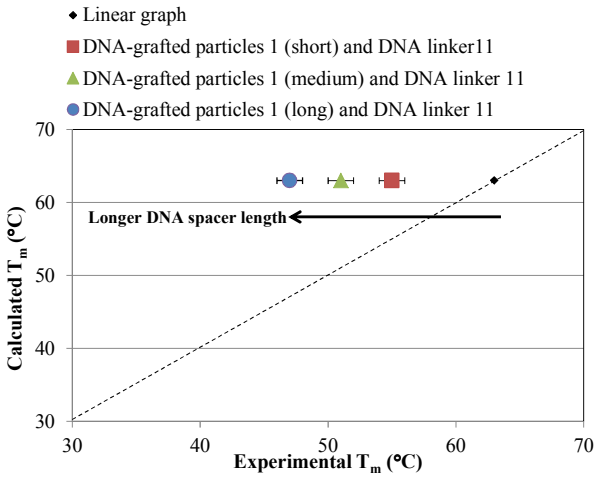

Fig. 5 Graph represented a calculated T_m as a function of experimental T_m. The difference between calculated and experimental T_m increases with the length of DNA spacer, longer DNA spacer higher difference between both T_m. Dash line represents linear relation between these two types of T_m.

The system of two types of DNA-grafted particles (DNA-1 and DNA-2) and DNA linker 12 complementary to both strands have been also investigated here and experimental results are presented in Table 3. Its thermodynamic data and calculated T_m are shown in Table 2. DNA-1 and DNA-2 were grafted on polystyrene particles in separate solutions. Then, two solutions were mixed together keeping final concentration and volume similar to the previous used in self-assembled DNA-1 and linker DNA-11. As we have expected the particles 1 and 2 do not aggregate without a complementary strand. However, when the DNA linker 12 is added to the colloid solution, the aggregation structure forms spontaneously within a few seconds and the structure looks like as Fig 3b. We have also determined that the heating/cooling cycle of this system is at 56°C and 30°C respectively. Similarly as in self-assembled system, the experimental T_m is lower than calculated one with similar difference to the system of DNA-1 and linker 11 at the same DNA spacer length. This confirms the effect of DNA spacer length causes the sequence defect on double-stranded DNA between DNA-grafted particles and DNA linker.

The previous works showed that there is an optimum distance between the gold nanoparticle and the DNA necessary to achieve the maximum enhancement of the binding strength.[19,36]

In the absence of the oligonucleotide spacer, the DNA strands are with close proximity to the particle's surface, reducing the ability of the target sequence to bind to the probe. Introduction of the spacer cause the DNA complementary part to be further from the particle's surface and alleviates steric hindrance, dramatically increasing the binding strength.

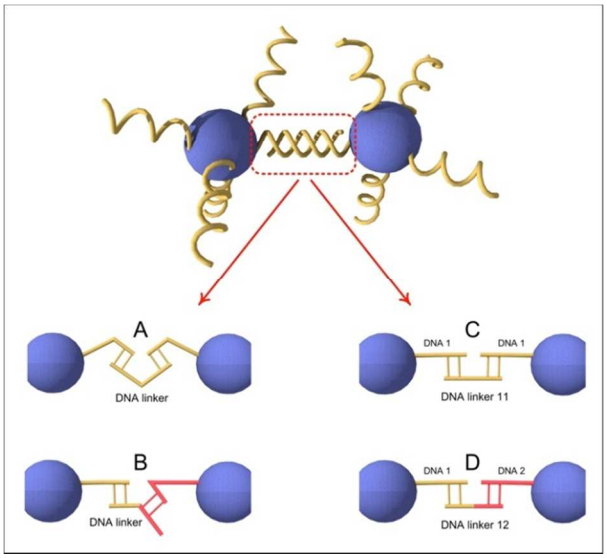

Fig. 6 Schematic representations of DNA-grafted self-assembly between particles, the interaction strength is related to the hydrogen bonding from DNA linker between DNA-grafted particles: (A) symmetry sequence of DNA linker 11 between long DNA spacers, (B) asymmetry sequence of DNA linker 12 between long DNA spacers, (C) symmetry sequence of DNA linker 11 between short DNA spacers, (D) asymmetry sequence of DNA linker 12 between short DNA spacers.

We also found that the system of two particles with DNA-1C, DNA-2C and the DNA-12 linker at longer DNA spacer length presents very similar results as with that of DNA linker 11 (Table 3 and Fig. 6). The experimental T_m is always lower than calculated T_m due to the defect of double-stranded DNA, longer DNA spacer length higher defect on double-stranded DNA (Table 3). So, the difference of experimental and calculated T_m is higher with longer DNA spacer length. However, there is no different significant of results on the gap between calculated T_m and experimental T_m for DNA link 11 and DNA linker 12. It means that the effect of DNA spacer length is the important factor on DNA melting process compared to the effect of DNA linker sequence.

Table 3 Experimental T_m of systems with DNA-11 and DNA-12 linker.

	DNA-1A-11-1A	DNA-1C-11-1C	DNA-1A-12-2A	DNA-2C-12-2C
Experimental T_m (°C)	55.0	47.0	56.0	49.0
Calculated T_m (°C)[31-32]	63.0	63.0	65.0	65.0
Calculated T_m (°C)[33-34]	67.5	67.5	70.5	70.5

Experimental

Materials

The DNA sequences are synthesized by Bioneer, Inc. (Table 1). Triblock copolymer surfactant poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) [Pluronic F108: (PEG)129-(PPG)43-(PEG)129] is obtained from BASF. All other chemicals are obtained from Aldrich and used as received.

Preparation of DNA-functionalized polystyrene particles

The polystyrene particle functionalization methodology is well explained in the previous work.[13] Briefly: polystyrene particles (1 μm) were grafted with the PEG spacers using the polymer swelling/deswelling in toluene. Then, the toluene was removed by steam stripping. After that, the hydroxyl groups of PEG-polystyrene were activated with 4-nitrophenol chloroformate in triethylamine. Finally, amine-modified DNA, in 10 mM triethylamine ammoniumphosphate buffer (TEAA) was added to the PEG-polystyrene particles. Unbounded DNA was removed by rinse with TEAA buffer and centrifugation.

Characterization

Optical microscopy. All suspensions were imaged by means of an optical microscopy with DM IRBE, Leica system, using x10 or x100 objectives and camera image capturing. A copper block with a water thermostat was used to heat up or cool down the sample.

Atomic force microscopy (AFM). Samples for AFM experiments were prepared as for optical microscopy. Generally DNA-functionalized polystyrene particles were mixed with DNA linker in 10 mM TEAA buffer solution and equilibrated for 30 min in room temperature. Samples (50 μL) were deposited on freshly cleaved mica surface (area of 5 cm^2) for 10-15 min, excess of the solution was gently stripped and sample was dried under blow of nitrogen and imaged in air. The AFM analysis was performed using a MultiMode microscope equipped with a Nanoscope IIIA controller (Veeco, Santa Barbara, CA). The AFM images were recorded in tapping mode under ambient conditions using moderate force ratio to avoid contact loss. Experiments were performed using a commercial silicon tip (NSC12) with resonance frequency f = 150–200 kHz and spring constant k = 4–8 N/m. Images were processed and analyzed using NanoScope software. Raw data was leveled by a first and second order of plane fit correction to remove the sample tilt.

Conclusions

We have quantitatively shown the thermodynamic data of DNA-functionalized micro-scale polystyrene particles. We found that the length of DNA spacer plays an important role in the melting temperature (T_m) of experimental system, longer DNA spacer causes higher different values between calculated and experimental T_m. We have also investigated both symmetrical and asymmetrical DNA linkers (11 and 12). The differences between calculated and experimental T_m are at the same level for both types of interactions, self-assembly DNA-1 with DNA-11 and two particle DNA-1,2 with linker 12. However, the experimental T_m still depends on the length of DNA spacer. Therefore, when we design the assembly system based on complementary DNA strands for micron-size particles, we need to consider the length of the spacer as an important factor. More study has to be done to ascertain the role of even longer spacer and its type (hydrophobic/hydrophilic) on the assembly processes. One can also imagine the effect of particle functionalization using DNA multiple sequence on the aggregation.

Acknowledgements

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DNA-functionalized polystyrene particles and their controlled self-assembly

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DNA-grafted particles can assemble together, the interaction strength is related to the hydrogen bonding from DNA linker between DNA-grafted particles. Two types of DNA linker are proposed.