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Rapid Communication

CAPABILITY OF DNA-FUELED MOLECULAR MACHINE IN TUNING ASSOCIATION RATE OF DNA-FUNCTIONALIZED GOLD NANOPARTICLES*

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Abstract The capability of our newly developed DNA-machine-driven strategy in tuning the association rate of DNA-AuNPs was compared with that of linker-addition strategy which has potential practical applications in different fields. The new established strategy shows its superiority to the linker-addition strategy in tuning the association rate of DNA-AuNPs in both pre-incubation treatment and non-incubation treatment conditions since its two components (a complex and a catalyst-oligonucleotide) can be individually optimized to make the machine run at an optimal rate. This strategy will provide a more convenient and flexible option in designing an oligonucleotide detection system and building a complex and versatile device.

Keywords: Assembly; DNA; Enhancing; Kinetics; Nanoparticle.

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INTRODUCTION

Ever since the use of oligonucleotides as noncovalent linkers for directing the assembly of DNA-functionalized gold nanoparticles (DNA-AuNPs) was established[1, 2], the strategy has been widely applied to fabricate nanomaterials possessing the hybridized properties of both organic DNA and inorganic colloids[3−15]. DNA-AuNPs have been used for many practical applications in the fields of biodiagnostics[16−21], medicine[22, 23], and biosensors[24−30] due to their distinct optical and catalytic properties. All of these applications require tuning the association rate of DNA-AuNPs *via* controlling the hybridization rate of DNA on a surface. To date, some efforts to accomplish this tuning have been performed, including: designing the dangling ends to consist of a secondary structure[31, 32] or building a short rigid duplex near the target hybridization position[33, 34] that may induce structural changes and base-stacking interactions; tailoring the DNA structure to facilitate the initial binding of targets[35]; optimizing the linker DNA structure to decrease the steric interaction and increase the base stacking energy[36, 37]; and maximizing the hairpin disruption[38, 39] and anchoring the probes on particles with polyadenine[40] to increase the hybridization efficiency. Despite the success in the field, novel strategies still need to be developed to further improve association efficiency.

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RESULTS AND DISCUSSION

Among the methods of accomplishing the assembly of DNA-AuNPs, the ones that have been widely utilized in the past decades involve the addition of linker-oligonucleotides into solution. These linkers possess two blocks that are individually capable of hybridizing with oligonucleotides grafted on the surface of AuNPs. In this strategy, the linker-oligonucleotides play trigger and tune the association reactions of the DNA-AuNPs[1, 36, 37, 40, 41]. Because of the significance of the association rates in deciding the aggregation times of DNA-AuNPs, the purpose of this study was to establish the strategies that are capable of enhancing the efficiency of these reactions. It included the designs and syntheses of five types of oligonucleotides (Table S1 in the Supporting Information). Two types of DNA-AuNPs were first prepared by individually functionalizing the surface of 13 nm diameter AuNPs with one of two oligonucleotides, oligomer-1 or oligomer-2. As to be seen in the following sections, the association reactions of DNA-AuNPs are very sensitive to the manner in which the linker-oligonucleotide was added into the solution. Here, we utilized four strategies to add the linker-oligonucleotides, as depicted in Fig. 1. We termed the first two methods as "linker-addition" strategies (Figs. 1a and 1b). We called the first one "direct-linker-addition" strategy. It has been widely applied in different fields[42–46]. The assembly was accomplished by adding the linker-oligonucleotide directly into the solution containing the mixture of DNA-AuNPs grafted with oligomer-1 and oligomer-2 (Fig. 1a).

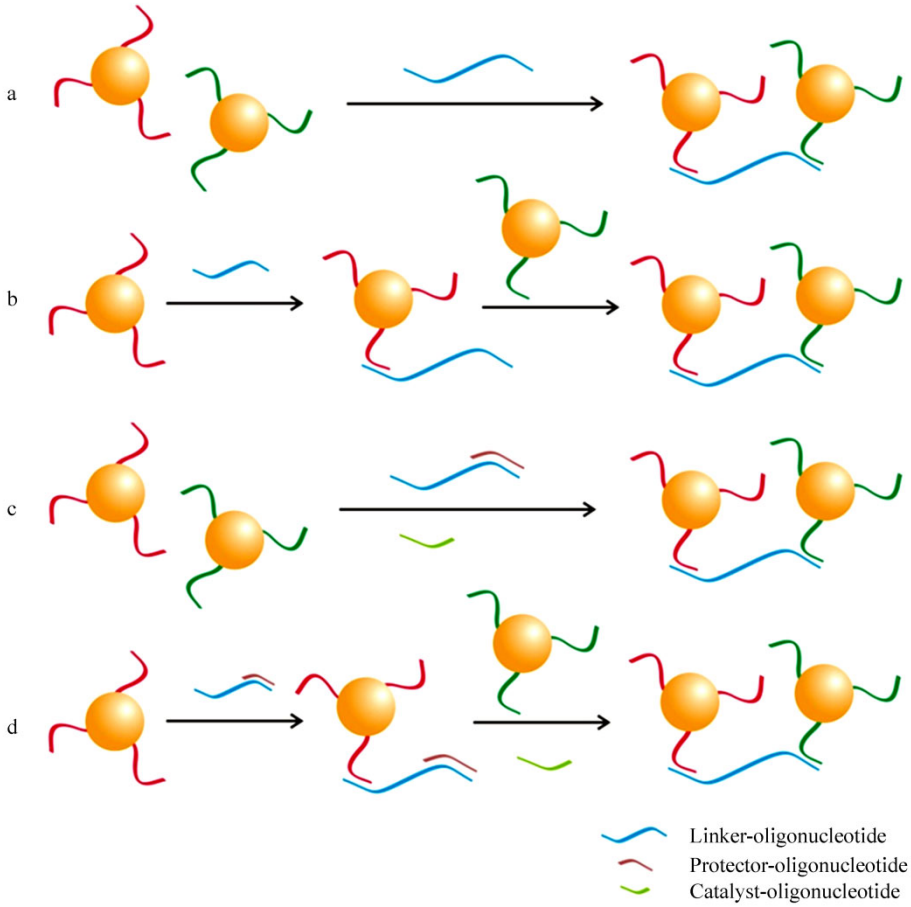

Fig. 1 Different methods of DNA-AuNPs assembly: (a) direct-linker-addition strategy, (b) hybridized-linker-addition strategy, (c) direct-complex-addition strategy and (d) hybridized-complex-addition strategy

For the second method, deemed the "hybridized-linker-addition" strategy, the linker-oligonucleotide was first incubated a short period of time with oligomer-2-functionalized AuNPs before oligomer-1-functionalized AuNPs were added to start the reactions (Fig. 1b). This extra incubation procedure allows a pre-hybridization of linker-oligonucleotide with the oligomer-2 on the surface of AuNPs. To our surprise, the association reactions between the DNA-AuNPs were significantly accelerated within an hour of incubation treatment (Fig. S1). The inserted images in Fig. S1 (right tube) showed a very weak color change after 3 h of reaction for the direct-linker-addition strategy (Fig. 1a), but remarkable color change (left tube in Fig. S1) with the hybridized-linker-addition strategy (Fig. 1b). This acceleration is likely due to the transformation of the strand on the surface of the AuNPs from a flexible ssDNA into a partially rigid dsDNA through the hybridization of linker-oligonucleotides with oligomer-2 on the AuNPs. The similar phenomenon has been previously observed[33, 35, 37]. The existence of the dsDNA segment provides two advantages: first, suppression of entropy arising from the flexibility of the DNA chains; second, prevention of the adsorption of the chains onto the surface of the AuNPs because of the extended conformation of a rigid chain. These two factors are both beneficial to the hybridization reactions of DNA on the surface of AuNPs with other DNA. We observed the dependence of association rates *via* UV-Vis on the molar ratio of [linker]/[AuNPs] (Fig. 2a). We noticed the systems without incubation underwent a slight drop in UV-Vis values after 12 h of reaction (the DLA systems in Fig. 2a), implying a low reaction rate. However, we observed a significant drop (the HLA systems in Fig. 2a) after treatment *via* incubation. This implies a substantial acceleration of the reaction.

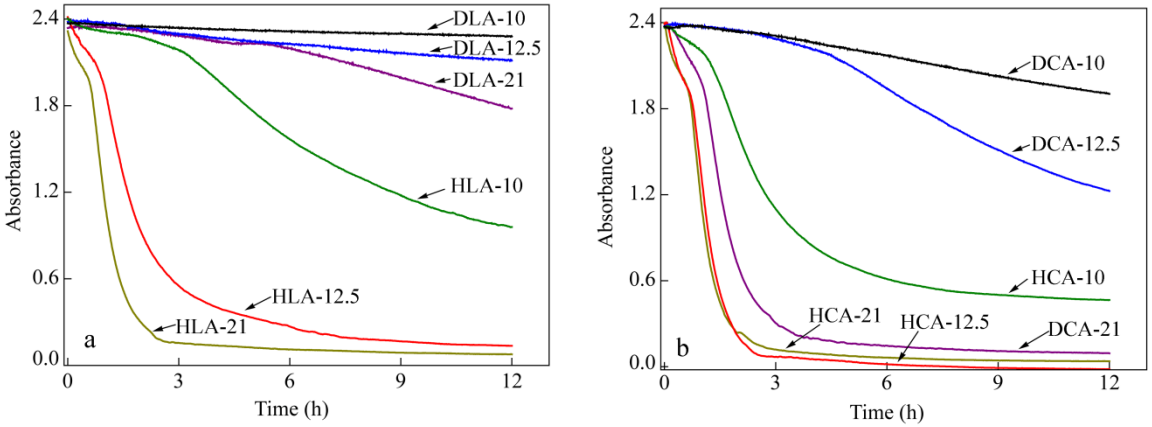

Fig. 2 The kinetics of DNA-AuNPs assembly using (a) linker-addition strategy and (b) DNA-machine-driven strategy at different molar ratios of [linker]/[AuNPs]
DLA, HLA, DCA, and HCA refer to the direct-linker-addition, hybridized-linker-addition, direct-complex-addition, and hybridized-complex-addition strategies, respectively. The numbers of 10, 12.5 and 21 represent [linker]/[AuNPs] = 10, 12.5, and 21, respectively.

In general, detection of low concentrations of oligonucleotide chains with specific sequences is highly desirable in diagnostics. In this linker-addition strategy, the linker-oligonucleotide may be considered the analyte, *i.e.*, the target molecule to be detected. Using this method has resulted in a vital disadvantage arising from two facts. One is that both the association rate and detection sensitivity are simultaneously determined by the amount of linker-oligonucleotide. The other is that the linker-oligonucleotide plays opposite roles in tuning the association rate and deciding detection sensitivity; that is, reducing its amount improves the detection sensitivity but decelerates the association rate. To circumvent this difficulty, our newly developed strategy, called DNA-machine-driven strategy, will be used to regulate the association rate of DNA-AuNPs[41]. In this strategy, the sticky end of the linker-oligonucleotide was first protected *via* hybridization with a short oligonucleotide sequence (protector-oligonucleotide) to generate a complex, then small quantities of catalyst-oligonucleotide were introduced to trigger the running of the DNA-fueled molecular machine *via* a series of toehold-mediated DNA strand displacement reactions. This ultimately resulted in the linking of the two types of

DNA-AuNPs.

As will be evident in the following, the association rates of DNA-AuNPs with the DNA-driven-machine strategy depend significantly on the methods by which the catalyst-oligonucleotide and the complex (prepared from the hybridization of the linker-oligonucleotide and the protector-oligonucleotide) are applied. Herein, we introduce two ways for running the machines. We deemed the first method as the direct-complex-addition strategy (Fig. 1c). Under this strategy, the complex and the catalyst-oligonucleotide are added directly and simultaneously into the solution to start the reactions. The second strategy is called the hybridized-complex-addition strategy (Fig. 1d). Under this strategy, the complex was first incubated with oligomer-2-modified AuNPs for a short period of time (–an hour). Since only half of the block of linker-oligonucleotide is exposed to the solution while the other half is protected, the exposed part is free for hybridization with oligomer-2 on the surface of the AuNPs. Thereafter, the reactions leading to the association of DNA-AuNPs are triggered when the catalyst-oligonucleotide and oligomer-1-modified AuNPs are introduced (Fig. 1d). Again, we observed that incubation treatment resulted in a positive acceleration of the association reaction of DNA-AuNPs (Fig. 2b). This acceleration is particularly pronounced when relatively low molar ratios of [complex]/[AuNPs] are used, specifically at the value of 10 or 12.5 (compare the systems of DCA-10 and HCA-10; systems of DCA-12.5 and HCA-12.5 in Fig. 2b). Interestingly, with the incubation strategy, the reactions at molar ratios of 12.5 and 21 follow identical kinetic behavior (systems of HCA-12.5 and HCA-21 in Fig. 2b), indicating the reaction at the low molar ratio of 12.5 has already reached the maximum rate; adding more complex does not make an additional contribution to the reaction. These observations indicate the incubation strategy does not only enhance the reaction rate, but also allows the system to gain the maximum reaction rate at low complex concentrations. However, under the non-incubation strategy, the reaction at the ratio of 12.5 has only slightly increased in rate (system of DCA-12.5 in Fig. 2b). We also noticed that in the DNA-machine-driven system, although the hybridized-complex-addition strategy has advantages over the direct-complex-addition strategy in tuning the reaction rates, direct-complex-addition strategy can still attain a very high reaction rate (compare the systems of DCA-21 and HCA-21 in Fig. 2b).

As pointed out above, the detection of oligonucleotides at low concentrations is highly desired in some practical applications. In the DNA-machine-driven strategy, the catalyst-oligonucleotide is supposed to be the analyte, the target molecule for detection. Unlike in the linker-addition strategy, where the linker-oligonucleotide is the analyte while also the regulator of the reaction rate, the DNA-machine-driven strategy utilizes two components, a complex and a catalyst-oligonucleotide, to tune the reaction rate. The dependence of the reaction rate on the amount of catalyst-oligonucleotides was investigated at two concentrations of complex; that is, the large [complex]/[AuNPs] ratio of 21 (Fig. 3a) and small ratio of 10 (Fig. 3b). In the high molar ratio case, the amount of catalyst-oligonucleotides in 5 percent of the complex resulted in a sufficiently high reaction rate. However, in the low concentration case, the system with the amount of catalyst-oligonucleotides in 10 to 20 percent of complex exhibited a slow reaction. These results indicate that the responses of the two systems to the amount of catalyst-oligonucleotides are different: fast for the system with higher complex concentration, while sluggish for the one with lower concentration. This feature of the DNA-machine-driven strategy allows optimization of the amount of complexes such that the system is guaranteed to run at an optimal rate. This then allows the association rate to be further tunable *via* the amount of catalyst-oligonucleotides.

Next, we compared the abilities of the linker-addition strategy and the DNA-machine-driven strategy in regulating the association reactions of DNA-AuNPs. Two systems with a lower and a higher molar ratios of [linker or complex]/[AuNPs] were chosen for this comparison. At the lower molar ratio of 10, the DNA-machine-driven strategy exhibited a distinguished capability of enhancing the reactions in the incubation system (Fig. 4a). After analyzing the data, we became interested in the two reaction routes in the incubation systems (Figs. 1b and 1d); *i.e.*, the hybridized-linker-addition and the hybridized-complex-addition strategies. In both routes, a half block of the linker-oligonucleotides was pre-hybridized with the corresponding sequence of oligomer-2 on the AuNPs. We understand that, in the system utilizing hybridized-linker-addition (Fig. 1b), a hybridization reaction between oligomer-1 and another exposed half block of linker-oligonucleotide is

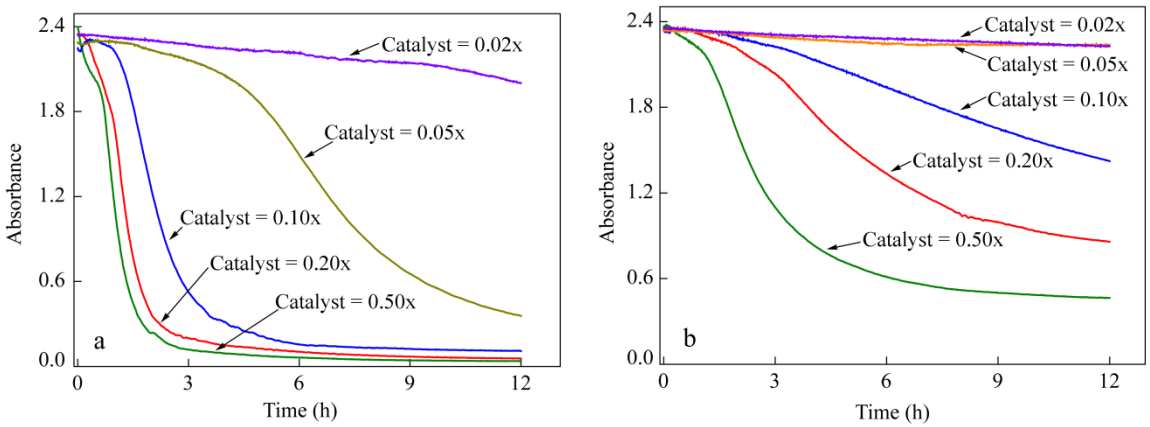

Fig. 3 Dependence of the kinetics of the assembly of DNA-AuNPs using the hybridized-complex-addition strategy on catalyst-oligonucleotide concentrations at different molar ratios of [complex]/[AuNPs]: (a) [complex]/[AuNPs] = 21 and (b) [complex]/[AuNPs] = 10
The amount of catalyst-oligonucleotide changed with various coefficient according to the amount of complex used in different systems.

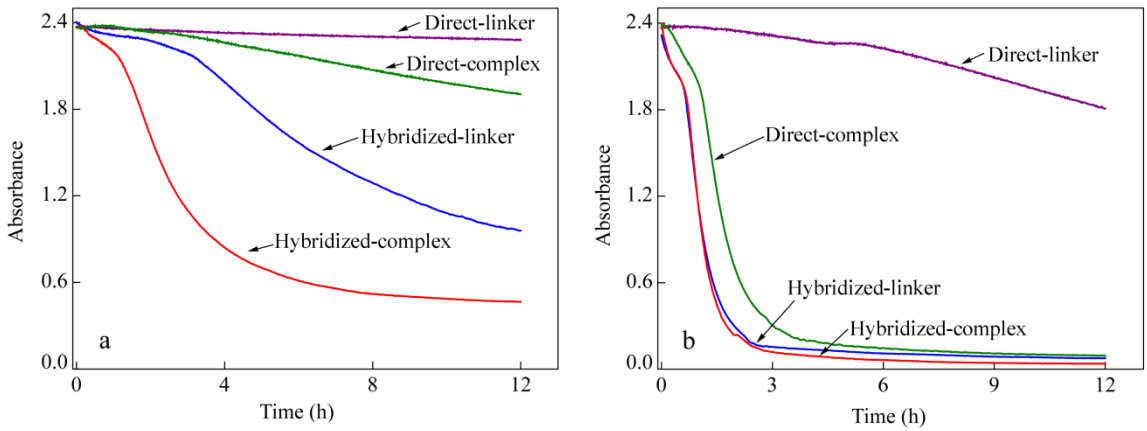

Fig. 4 Comparison of the kinetics for assembly of DNA-AuNPs in linker-addition and DNA-machine-driven strategies at different molar ratios of [linker or complex]/[AuNPs]: (a) [linker or complex]/[AuNPs] = 10 and (b) [linker or complex]/[AuNPs] = 21

immediately triggered upon addition of the oligomer-1-grafted AuNPs into the solution. However, in the system using the hybridized-complex-addition strategy (Fig. 1d), a series of displacement reactions[41, 47] must take place to accomplish the crosslinking of DNA-AuNPs after oligomer-1-grafted AuNPs and catalyst-oligonucleotides are put into the solution. It should be reasonable to assume that relatively longer times are needed for the hybridized-complex-addition strategy because of the additional reaction steps compared to the hybridized-linker-addition strategy, which is only a one step reaction. However, the observed experimental results are beyond our assumption, since the hybridized-complex-addition strategy exhibited a relatively high reaction rate (compare the hybridized-linker and hybridized-complex systems in Fig. 4a). The phenomenon puzzles us because the physical origins behind it are currently unclear. It is difficult to explain based on the stiffness of the DNA chain on the surface of AuNPs, as mentioned above. On the other hand, at high [linker or complex]/[AuNPs] ratio of 21, the reactions in the hybridized-linker-addition and hybridized-complex-addition systems show similar kinetic behaviors (hybridized-linker and hybridized-complex systems in Fig. 4b). It seems there is a maximum reaction rate in this system that is attainable using the incubation strategy at an optimal amount of linker-oligonucleotides, regardless of whether the hybridized-linker-addition or the hybridized-complex-addition strategy is employed. However, the remarkable difference exists in the non-incubation cases. The direct-

complex-addition strategy has shown its ability to attain a high reaction rate, while the direct-linker-addition strategy can only achieve a low reaction rate (the direct-linker and direct-complex systems in Fig. 4b).

CONCLUSIONS

In summary, the methods of tuning the association rate of DNA-AuNPs have been developed in terms of the linker-addition or DNA-machine-driven strategies. In the linker-addition strategy, the association rate of DNA-AuNPs has been accelerated tremendously by pre-incubating the linker-oligonucleotides with one type of DNA-AuNP before adding the second type of DNA-AuNP. Our newly developed DNA-machine-driven strategy is superior to the linker-addition strategy in tuning the association rate of DNA-AuNPs since its two components (a complex and a catalyst-oligonucleotide) can be individually optimized to make the machine run at an optimal rate. Thus, this new established strategy provides a more convenient and flexible option in designing an oligonucleotide detection system and building a complex and versatile device.

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