A simple method to reprogram the binding specificity of DNA-coated colloids that crystallize

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Abstract

DNA-coated particles are versatile building blocks for self-assembly, but are difficult, costly, and time-consuming to produce in a way that is compatible with crystallization. For each experiment or application that requires a different DNA sequence, the DNA coating process must be repeated, standing in the way of the widespread use of DNA-coated colloids in research and commercial applications. Here, we introduce a method to convert generic DNA-coated colloids into building blocks with user-specified DNA sequences by appending new DNA domains onto the generic sequence. The reaction is easy and fast, reaching complete conversion in one hour. Most importantly, we show that the assembly of particles produced via our method is indistinguishable from the assembly of particles produced via direct chemical synthesis. Moreover, we show that particles coated with a single generic sequence can be converted into a variety of building blocks with differing specificity by appending different DNA sequences. We expect that our approach will greatly improve the access to DNA-coated particles that can crystallize and pave the way to their commercial application.

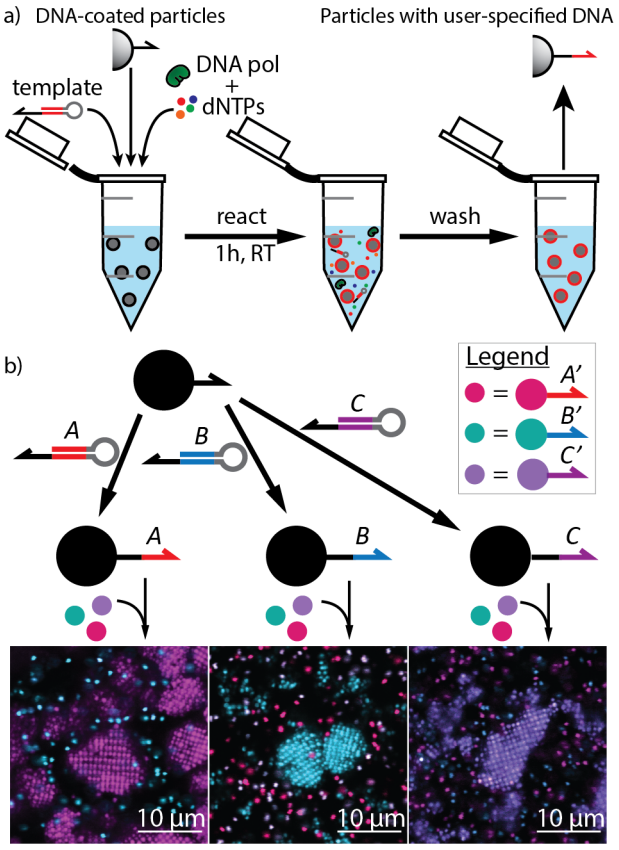

Figure 1: a) Overview of the reaction that extends the DNA on DNA-coated particles. DNA polymerase, a desoxynucleotide triphosphate (dNTP) mixture, and one type of DNA sequence template are mixed in an Eppendorf tube and left at room temperature for 1 hour. Then the particles are separated from the reaction mixture by centrifugation and ready to be used in self-assembly experiments. b) One batch of particles coated with a generic input sequence can be converted into a variety of DNA-coated particles with various binding affinities by adding different catalytic DNA templates. The output particles only bind to their intended targets.

1 Introduction

Owing to the specificity of DNA hybridization, orthogonal interactions can be prescribed between microscopic objects by coating the objects with single-stranded DNA[1, 2, 3]. This use of DNA is an established strategy for producing building blocks that can assemble into a wide variety of microscopic structures, including stick figures [4], crystal lattices [5, 6, 7, 8], flexible bead-chains [9, 10], chiral clusters [11], and even cell aggregates [12]. Because DNA-coated microparticles have sizes comparable to the wavelength of visible light, they

are particularly promising building blocks for the self-assembly of photonic bandgap materials [13, 14, 15], with applications in optical wave guides, lasers, and various light-harvesting technologies. DNA-coated microparticles are also useful as model systems for self-assembly, both in [16] and out of equilibrium [17, 18].

DNA can be grafted onto colloidal particles in various ways, but not all methods produce particles that are compatible with equilibrium assembly of colloidal crystals [19]. When biotin-streptavidin chemistry is used, particles tend to hit-and-stick and become kinetically trapped in fractal-like aggregates, even at temperatures at which the DNA-mediated interactions are reversible [20, 21]. Various attachment strategies based on strain-promoted click chemistry [23, 24, 25] produce DNA-coated colloids that crystallize [26]. However, the click-chemistry-based methods are time-consuming and require specialized knowledge of synthetic chemistry, which stands in the way of the widespread use of DNA-coated colloids. Moreover, they require dibenzocyclooctyne (DBCO)-functionalized DNA, which takes roughly a month to synthesize, so that—even if one has the expertise necessary for this synthesis—the time between the initial idea and the experiment is over a month. Last, once the particles are synthesized, their specificity is fixed. New particles must be synthesized for each experiment or application that requires a unique DNA sequence.

Here we introduce a simple method to synthesize DNA-coated particles with user-prescribed sequences from a single feed stock (Fig. 1a). This method decouples the expensive and time-consuming step of attaching DNA to colloidal particles from the step of tailoring the DNA sequence for its particular purpose, enabling one to convert the sequence of DNA-coated particles for each new experiment, rather than redoing the DNA-coating procedure. Our method uses the Primer Exchange Reaction (PER), introduced by Kishi *et al.* in 2019 [27], to append a user-specified domain to the end of a generic DNA sequence coated on the particles. We show that this reaction reaches complete conversion within 1 hour and that the assembly of the particles synthesized using this method is indistinguishable from the assembly of colloids made via click chemistry. Finally, we show that a single type of generic DNA-coated particles can be converted into a variety of particles with different DNA sequences and binding specificities (Fig. 1b), thereby overcoming some of the key technical bottlenecks to the synthesis of DNA-coated colloids and facilitating their widespread use.

2 Results

We first ask whether or not the primer exchange reaction can be used to append new sequence domains onto DNA-coated colloids. The primer exchange reaction involves

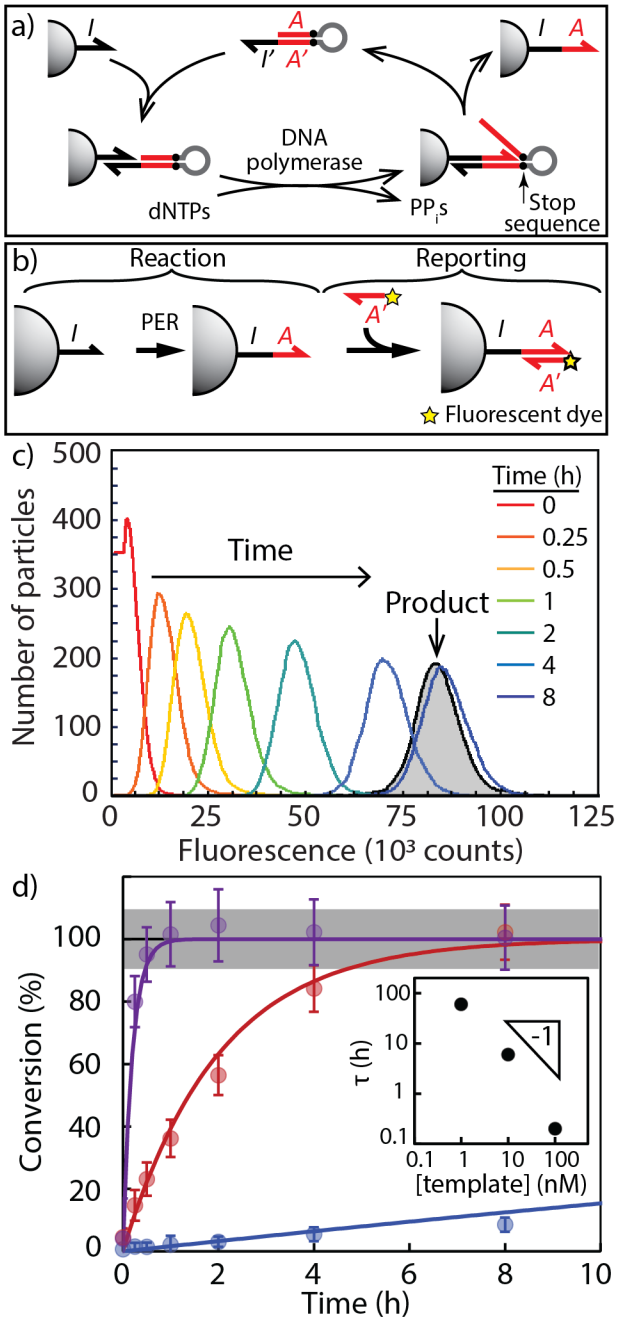

Figure 2: PER can extend the DNA on colloids. a) Schematic of the primer exchange reaction. dNTPs are DNA nucleotides and PP$_i$s are inorganic pyrophosphates. b) Schematic of the reporting reaction used to quantify DNA conversion on particles. c) Distribution of the single-particle fluorescence of DNA-coated particles after varied reaction times, as measured using flow cytometry. Each histogram represents ten thousand particles. The hairpin concentration was 10 nM. d) PER conversion as function of time for 1 nM (blue), 10 nM (red), 100 nM (purple) template. Higher hairpin concentrations lead to faster conversion. Error bars represent the standard deviation of the fluorescence distribution. The particles are 600 nm in diameter. The inset shows the typical reaction time, τ, as a function of template concentration. The inset shows that the typical reaction time τ scales linearly with the inverse template concentration.

the reversible binding of a single-stranded input and a catalytic hairpin. When bound, DNA polymerase copies the hairpin sequence and appends a new domain onto the input strand, producing a longer single-stranded output. The hairpin sequence thus determines the sequence of the output domain (Fig. 2a).

We synthesized colloidal particles coated with the input strand and then initiate the PER reaction in the colloidal suspension. We synthesized 1 µm and 600 nm diameter polystyrene colloids grafted with single-stranded DNA using the click chemistry method developed by Wang *et al.* [23]. The grafted sequence consists of a 40-nucleotide poly-T spacer followed by a 9-nucleotide input domain. We then mixed the colloidal particles at 0.1% (v/v) with 100 nM hairpin strand, 100 µM of each nucleotide triphosphate, and 0.13 U/ µL DNA polymerase, and let the reaction proceed at room temperature for 1 hour. After the reaction, we washed the particles by centrifugation and resuspension. See SI for details of the synthesis and DNA sequences.

2.1 Conversion

We set out to test whether the PER had appended output sequences onto the DNA on the particles and used flow cytometry to quantify the surface density of the appended sequence (Supp. Fig. 4). After the DNA conversion on the particles we added fluorescently labeled strands that are complementary to the output sequences, so that the particle fluoresce only if the output sequence is present (Fig. 2b). Next, we measured the fluorescence of ten thousand individual particles for each experimental condition. We varied the reaction time in order to quantify the rate of conversion. We also varied the catalytic hairpin concentrations to quantify their effect on the reaction rate. Finally, to determine the percent yield of the reaction, we measured the fluorescence of reference particles to which the product DNA was attached directly via click chemistry.

We found that the primer exchange reaction converted one hundred percent of the input sequence with a rate that depends linearly on the hairpin concentration (Fig 2c-d). Figure 2d shows the conversion as a function of the reaction time for three different hairpin concentrations: 1 nM, 10 nM, and 100 nM. We found that the two highest concentrations reach complete conversion with a rate that increases approximately linearly with hairpin concentration. The minimal time to complete conversion was 1 hour with 100 nM hairpin and 8 hours with 10 nM hairpin (Fig. 2c). With 1 nM hairpin, the reaction did not go to completion within 24 hours (Supp. Fig. 1). Fitting the measured conversion as function of time to a single exponential yielded measurements of the half-life that are slightly slower predictions based on the rates of the PER reaction in solution (Supp. Fig. 1) [30], suggesting that the PER rate of DNA grafted onto colloidal surfaces is comparable to that of DNA free in solution.

We confirmed that the primer exchange reaction did not affect the particle-to-particle distribution of the grafting density by examining the width of the fluorescence distribution. The width of the distributions in Figure 2c represents the spread in the DNA grafting density between particles within the same sample at each reaction time. We found that the width of the distribution before and after 100% conversion is comparable (dark blue and gray shaded curve in Fig. 2c), indicating that the variation in DNA density on the particles stems from the DNA grafting step and is not increased during PER, at not beyond the distribution width inherent to the flow cytometry measurement.

To check whether any unintended side products formed, we also performed PER on DNA-grafted particles prepared using Biotin-Streptavidin. The Streptavidin-Biotin bond can be broken by heat denaturing Streptavidin at 95^oC in 50% formamide solution. Using this method, we remove the DNA from the surface of the particles after the primer exchange reaction and analyse the product sequences using gel electrophoresis. This experiment showed that only DNA strands with lengths that correspond to the reactant and the product sequence were present on the particles after the reaction and no significant concentrations of unintended side products were formed (Supp. Fig. 2).

2.2 Self-assembly

Because the purpose of our new synthesis method is to produce DNA-coated colloids that can be used for self-assembly, we tested whether or not particles synthesized via PER crystallized when mixed with complementary particles. To this end, we mixed our PER-synthesized particles with complementary particles that were synthesized directly to have a 7-nucleotide complementary domain—called 'co-assemblers'—and characterized the assemblies that formed near the melting temperature. We compared these assemblies to ones that formed in a mixture of particles coated with the same sequences, but where both particle species were synthesized using only click-chemistry.

Importantly, PER-synthesized particles crystallized when annealed near their melting temperature, similar to the reference particles that were synthesized by only click-chemistry. Figure 3a further shows that random aggregates formed below the melting temperature and that the aggregates melted completely 1 ^oC above the melting temperature. Particles produced via PER and the reference particles displayed the same assembly behavior.

Furthermore, the melting temperature—where 50% of particles are aggregated—of PER particles matched that of particles to which the DNA was directly attached (Figure 3b). Particles whose DNA was only partially converted via PER still aggregated when mixed with particles coated with the complementary DNA, but the melting temperature was lower than for particles that had

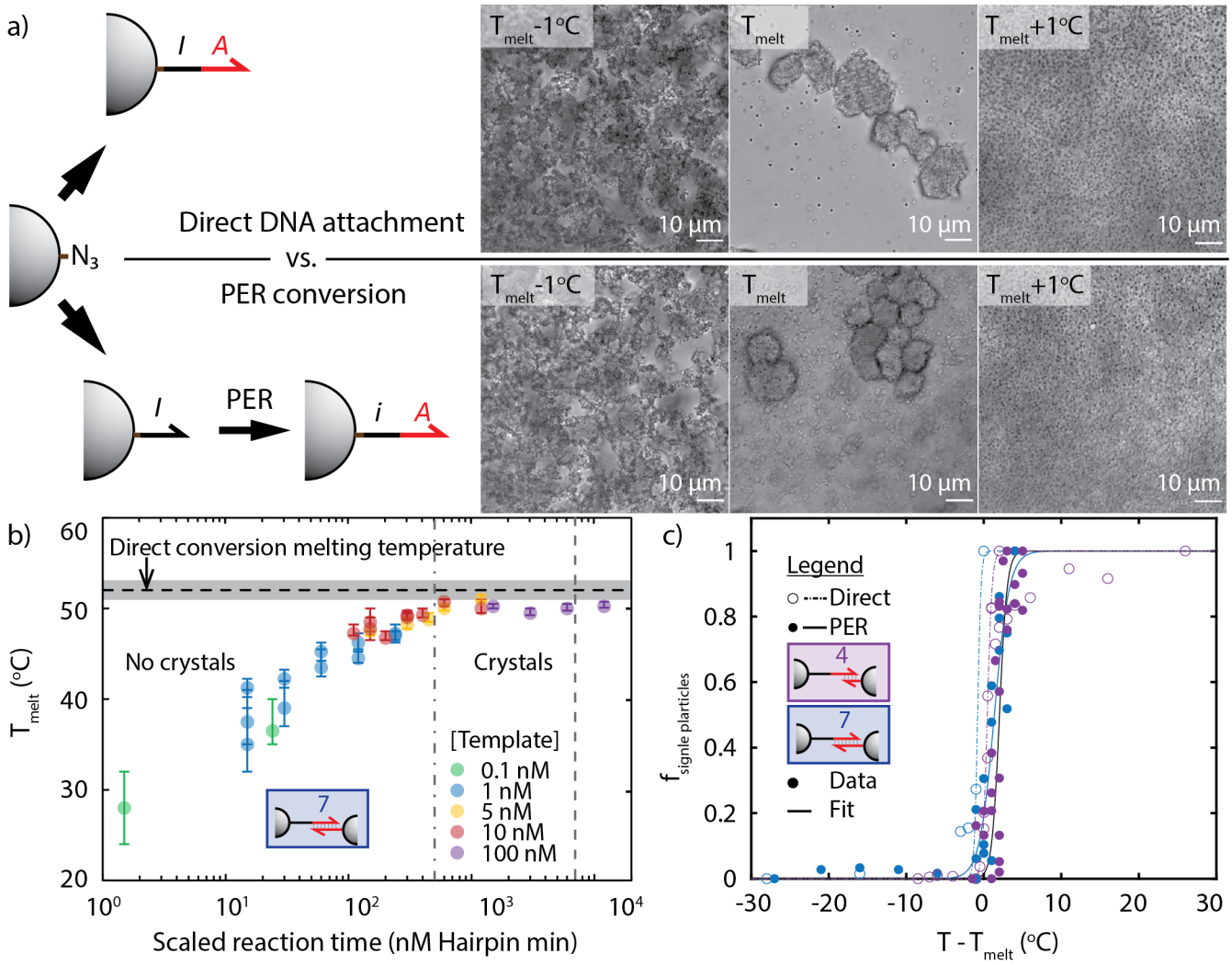

Figure 3: a) The self-assembly of reference particles to which the assembly sequence is attached directly via click chemistry (top row) is compared to colloids whose DNA was converted to the assembly sequence via PER (bottom row). Both the reference and PER particles form random aggregates below the melting temperature, crystallize near the melting temperature, and disassemble above the melting temperature, when mixed with particles coated with the complementary DNA sequence. All particles are 600 nm in diameter. Scale bars are 10 µm. b) The melting temperature as a function of the scaled reaction time (i.e. the product of the primer exchange reaction time and the hairpin concentration). The melting temperature increases with scaled reaction time until it reaches a plateau around the melting temperature of the reference particles (dashed black line) after a scaled reaction time of 5×10^2 nM min. The plateau occurs well below the scaled reaction time where particles reach complete conversion (5×10^3 nM min, dashed gray line). Samples left of the dot-dashed gray line did not crystallize, whereas samples right of the gray dot-dashed line did. c) The fraction of non-aggregated particles as a function of temperature for both the reference particles (hollow circle, dashed line) and particles converted by PER (filled circle, continuous line). The lines are fits to the experimental data. The width of the melting curve is on the order of two degrees, both for the PER particles and the reference particles. The melting curves match both in the system in which the co-assembling particles have 7 complementary bases (blue) and in the system in which they have 4 complementary bases (purple).

been completely converted. Figure 3b shows the melting temperature as a function of the primer exchange reaction time scaled by the hairpin concentration. We found that the melting temperature increased with the scaled reaction time and plateaued at the melting temperature of the reference particles at 5×10^2 nM min. Notably, at this scaled reaction time only 30 % of the DNA on the PER particles was converted. Only at a scaled reaction time of 5×10^3 nM min was the conversion complete (gray dashed line).

We found that partially converted particles can crystallize at the melting temperature, when their conversion is beyond a threshold value of approximately 30 % (gray dot-dashed line), corresponding to the point where the melting temperature matches that of the reference particles. Particles with lower conversions could not be crystallized.

The propensity of PER particles to crystallize depended on the PER conditions. Letting the reaction go for longer than necessary to reach 100% conversion, or using more than 0.13 U/µL Bst DNA polymerase resulted in particles that displayed non-specific aggregation even well above the melting temperature and did not crystallize (Supp. Fig. 3). We think that the non-specific aggregation is due to slow primer-independent or template-independent polymerization reactions [28, 29].

A more stringent test to compare the binding properties of DNA-coated particles than their melting temperature is the melting curve, the fraction of single particles as a function of temperature. Figure 3c shows the melting curves for both PER particles (filled circles) and reference particles (hollow circles) mixed with co-assemblers that have a 4-nucleotide complementary domain (purple) or a 7-nucleotide complementary domain (blue). The temperature was scaled to the melting temperature so that the width of the curves could be compared. The width of the melting curves was on the order of 2 degrees for all combinations and no systematic difference between PER particles and reference particles was observed, suggesting the DNA coatings on both particles are indistinguishable.

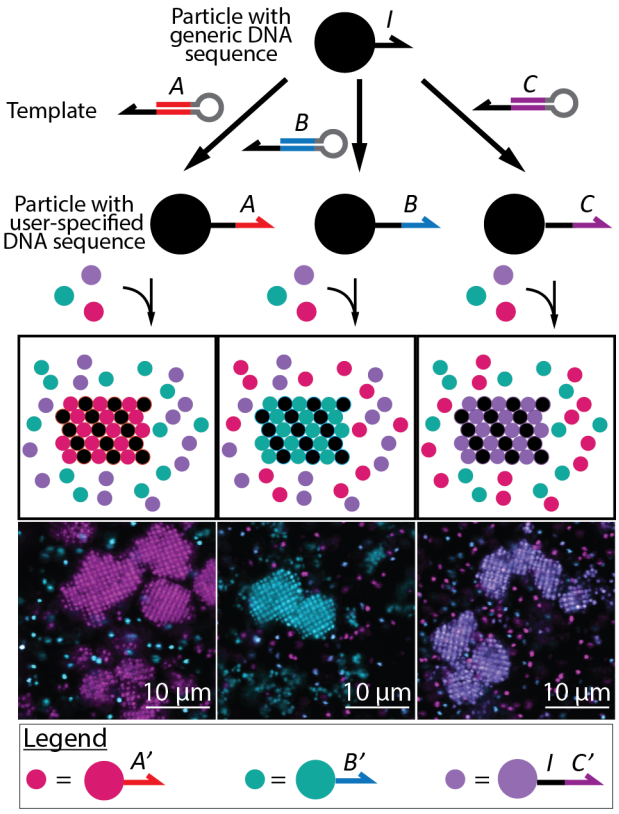

Figure 4: PER converts generic DNA-coated particle into different building blocks for self-assembly. Generic input particles (not fluorescently labeled) are converted into three batches of DNA-coated particles with differing sequence: A, B, and C. Each batch is mixed with three types of fluorescently labeled particles: magenta particles are coated with sequence A', cyan particles are coated with B', and purple particles are coated with C'. The samples are annealed at the melting temperature and imaged at the melting temperature under a confocal microscope. Each type of converted particle aggregated only with their respective complementary particle. Scale bars are 10 µm.

2.3 Reprogramming binding specificity

The key advance enabled by our synthesis method is that a generic DNA sequence grafted to a single batch of particles can be converted into any user-prescribed sequence for a wide variety of assembly experiments. To demonstrate this feature, we show that one type of particle can be converted into three different DNA-coated building blocks that co-crystallize with three types of particles coated with different complementary sequences (Fig. 4). The co-assembler particles were fluorescently labeled with a magenta dye, a cyan dye, or both the magenta and the cyan dye (we show these particles as purple). The particles that underwent the PER reaction were not fluorescently labeled and are not visible in the images. We annealed a suspension of all four particle types at the melting temperature and imaged the resultant crystals using confocal microscopy.

Each of the three species that we prepared crystallized specifically with their complementary species within the mixture. Figure 4 shows representative fluorescence images of the three mixtures at room temperature. By examining the crystals, we can clearly see that the particles converted by PER crystallized only with their intended target; each of the crystals are either fully magenta, cyan, or purple. The unintended targets remained free in solution. These results show that three sets of DNA-coated particles with differing binding specificities could be prepared from a single feed stock.

The co-assembler particles for the purple aggregates were also produced from the initial feed stock using the

Primer Exchange Reaction, showing that crystals form even if both types of DNA-coated particles particles in a binary system were produced using PER.

2.4 Design considerations

While PER has clear advantages in the preparation of DNA-coated colloids, there are also some specific limitations, as well as considerations for designing the templates. First, PER can only extend sequences from the 3' end of DNA. Whenever a 5' sticky end on the DNA is required, our method cannot be used and the particles must be synthesized using click chemistry. Second, PER can not be used to produce grafted DNA molecules with non-natural bases or chemical modifications. The addition of very short or self-complementary sequences is likely fine, but we have not attempted this. We also expect that PER can be used to add multiple different sequences to one particle, where the reaction time and hairpin concentration can be used to control precisely what fraction of the DNA is converted in each step.

To design a new template, it is important to follow three design principles that relate to the three sections of the template: the single-stranded binding domain, the sequence template domain, and the stop sequence. The single-stranded binding domain is complementary to the input sequence and is responsible for hybridization to the input strand. The length of this binding domain determines the rate of the reaction. In our earlier work on PER [30], we found that the reaction halftime is given by $\tau = (\frac{1}{k_2} + \frac{K}{k_f})(\frac{R_0}{C_0} + \frac{1}{KC_0})$, where the polymerization rate $k_2 = 3 \times 10^{-3}$ s^{-1}, the DNA hybridization rate $k_f = 3 \times 10^6$ M^{-1}s^{-1}, the reactant concentration $R_0 \approx 200$ nM, the catalyst concentration C_0 varies, and K is the equilibrium constant for catalyst-reactant binding. K depends on the sequence and length of the binding domain and can be predicted using the well-established parameters of DNA hybridization thermodynamics [31]. The rate is optimal for $K = \left(\frac{k_f}{R_0 k_2}\right)^{0.5}$. At room temperature and for sequences with $0.3 - 0.5$ GC-content, this corresponds to an optimal domain length of 9 nucleotides.

The second design rule considers the template domain, which is the double-stranded domain that contains the sequence of the DNA that will be appended onto the input sequence. Long template strands likely result in slow reaction kinetics, so the addition of domains longer than ten nucleotides should be done in consecutive PER reaction steps (Supp. Fig. 5). Kishi *et al.* showed that multiple PER conversions can be done in a one-pot reaction using multiple hairpins [27]. We anticipate that this same scheme could be used to make DNA-coated colloids with appended domains that are longer than ten nucleotides, but we have not tested it.

The final design rule considers the DNA stop sequence. The DNA template used for PER requires a stop sequence that the DNA polymerase cannot copy. One useful trick is to append a DNA sequence that contains only 3 out of the 4 nucleotides, which allows you to use a stop sequence that is the fourth nucleotide. For example, if one adds a sequence only containing A's, C's, and T's, the stop sequence can be a G-C pair with the G on the non-template strand. In that case, the reaction mixture should not contain any dGTP so that the DNA polymerase stops copying the sequence at the G-C pair. We used this method for the experiments presented in this paper. It is also possible to append sequences containing all four nucleotides by using a non-natural base-pair, such as iso-dC and iso-dG as a stop sequence. However, strands containing these non-natural bases are expensive and time-consuming to produce.

3 Conclusions

We introduced a method to rapidly and easily reprogram the binding specificity of DNA-coated colloids by appending new DNA domains onto the DNA grafted onto colloids. We showed that particles with a generic input sequence can be converted into a set of three assembly building blocks with differing binding specificity using our method. The particles maintain their ability to crystallize after the DNA extension procedure.

We expect our method to be particularly useful for experiments and applications that require many particles with different sequences. Instead of repeating the grafting procedure, one can produce generic DNA-coated particles in bulk and afterwards extend the DNA to contain the desired sequence. This approach is not limited to particles, but could be helpful in other systems where the grafting of DNA onto an object is expensive, time-consuming, or difficult, such as cells [12] and hydrogels [32].

Beyond synthesizing particles with user-prescribed sequences, this work also opens up the possibility to alter the binding properties of particles over time within one experiment, which could be a useful tool in dissipative self-assembly. Time-dependent interactions are increasingly sought after for their ability to create dynamic, reconfigurable, and adaptive structures, but currently only few chemical strategies are available [33, 34]. The primer exchange reaction could be used to initiate sequential assembly stages and freeze objects into kinetically trapped structures by converting the DNA on particles at varying rates, controlled by the hairpin concentration.

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5 Author contributions

P.G.M., R.S., & W.B.R. conceived the experiments. P.G.M., T.E.V., & H.F. performed the experiments and analysis. All authors contributed to writing of the paper.

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