Electronic Supplementary Material (ESI) for Soft Matter.

Supplemental material for "A simple method to reprogram the binding specificity of DNA-coated colloids that crystallize"

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1 DNA Sequences

Here we list the DNA sequences (Integrated DNA Technologies) that we used for this work. The sequences are color coded to match Figure 1: the input domain is in black and bold, output A and its sequence complement in red, output B and its sequence complement in blue, and output C and its sequence complement in purple.

	Name	Sequence	Figures
1	*I*	/5DBCON/ (T)40 TAACTTC **ACTTCACTT**	2,3,4,S1,S3
2	*IA*	/5DBCON/ (T)40 TAACTTC **ACTTCACTT** ACCATCCTACC	2,3,4,S1,S3
4	*A'*	/5DBCON/ (T)40 GGATGGT	3,4,S3
5	*B'*	/5DBCON/ (T)40 GAGATG	3,4
7	*HP_I_to_A*	ACCATCCTACC GGGCCTTTTGGCCC GGTAGGATGGT **AAGTGAAGT** /3InvdT/	2,3,4,S1,S3
8	*HP_I_to_B*	ACTCATCTCAA GGGCCTTTTGGCCC TTGAGATGAGT **AAGTGAAGT** /3InvdT/	4
9	*HP_I_to_C*	CCCACACA GGGCCTTTTGGCCC TGTGTGGG **AAGTGAAGT** /3InvdT/	4
10	*HP_I_to_C'*	TGTGTGGG CCCGGTTTTCCGGG CCCACACA **AAGTGAAGT** /3InvdT/	4
11	*A'*_Cy5	/5Cy5/ GGTAGGATGGTAA	2,S1
12	Bio_*I*	/5Biosg/ (T)40 TAACTTC **ACTTCACTT**	S2
13	Bio_*IA*	/5Biosg/ (T)40 TAACTTC **ACTTCACTT** ACCATCCTACC	S2

2 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 that should be taken into account.

2.1 Inherent limitations

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. Third, our method only works well if the DNA on the feed stock particles already have a single-stranded domain of at least 8 bases.

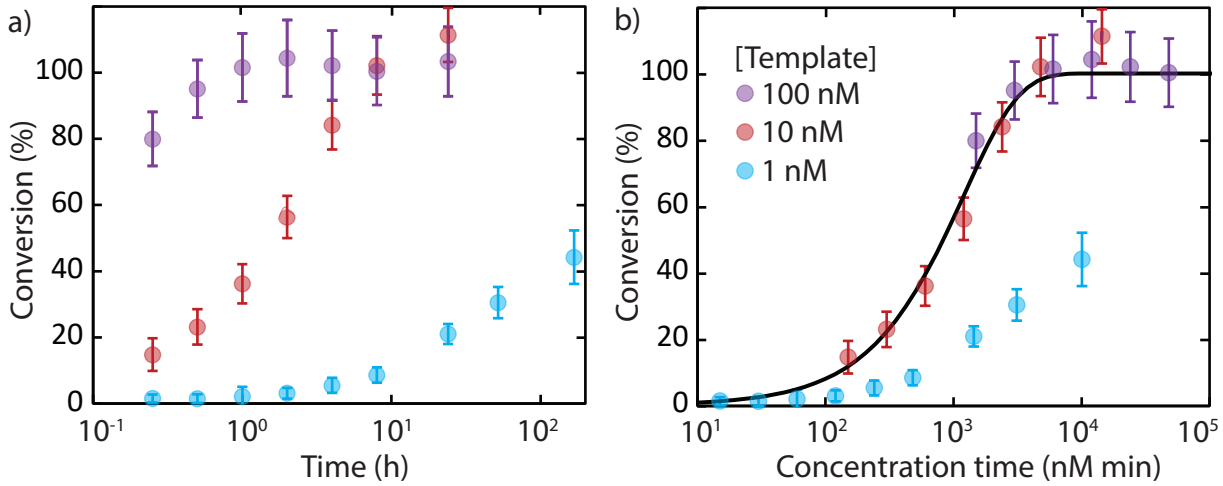
2.2 Template design principles

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.

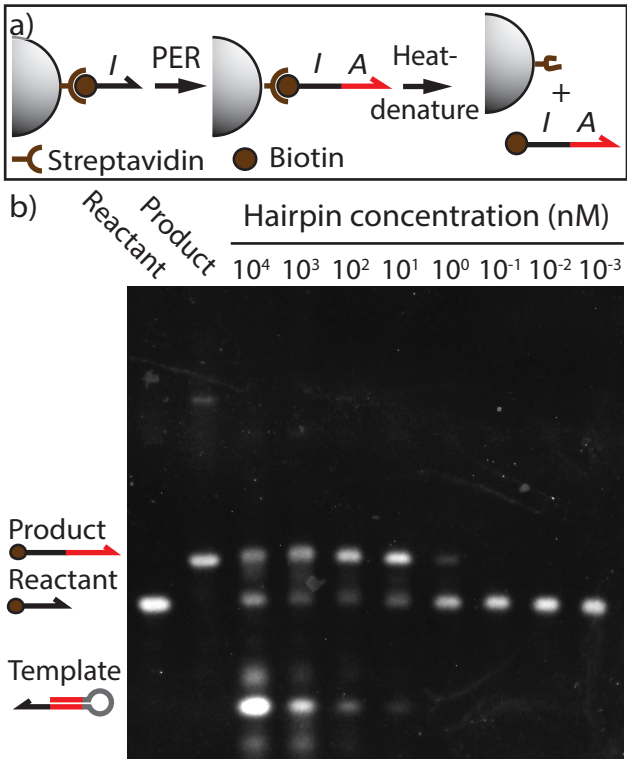
The single-stranded binding domain is complementary to the input sequence and is responsible for hybridization to the input strand. The length of this binding domain determines the rate of the reaction. In our earlier work on PER [2], we found that the reaction halftime is given by $\tau = (\frac{1}{k_2} + \frac{K}{k_f})(\frac{R_0}{C_0} + \frac{1}{KC_0})$, 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 [3]. 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 [1]. 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. We also expect that appending shorter-than-ten-nucleotide domains is not a problem, but have not tested it.

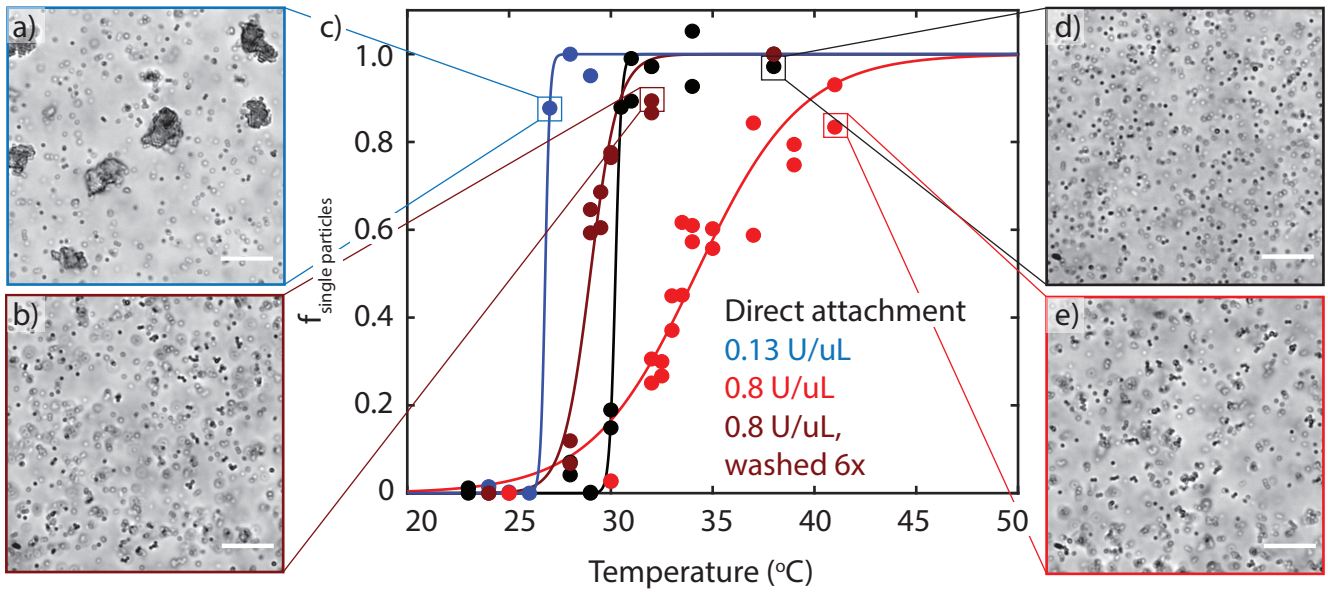
The final design rule considers the DNA stop sequence. The DNA template used for PER requires a stop sequence that the DNA polymerase cannot copy. One useful trick is to append a DNA sequence that contain only 3 out of the 4 nucleotides, which allows you to use a stop sequence that is the fourth nucleotide. 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 or methylated RNA bases as a stop sequence. However, strands containing these non-natural bases are more expensive and time-consuming to produce. Following the design of Kishi *et al.* [1] we cap the 3' end of the hairpin with an inverse dT—essentially a thymine base of which the 3' end is coupled to the 3' end of the hairpin. This inverse dT functions as a stop sequence so that the hairpin itself cannot be extended when it is hybridized to an input strand.



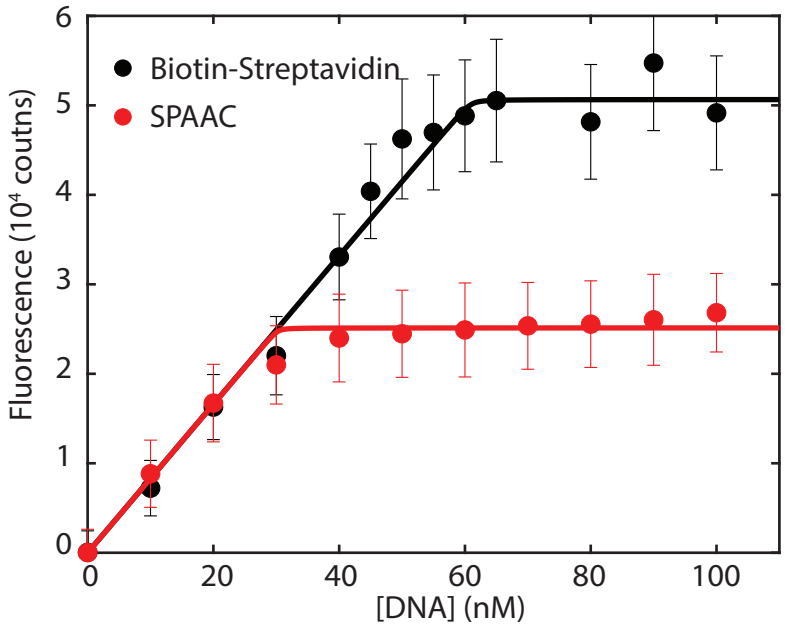
Supplementary Figure 1: a) Conversion of particle-grafted DNA as a function of time due to the Primer Exchange Reaction. The samples contain 100 nM hairpin (purple), 10 nM hairpin (red), and 1 nM hairpin (blue). Where higher catalyst concentrations lead to complete conversion in 1 and 8 hours respectively, the sample with 1 nM hairpin does not reach complete conversion within one week. b) The same data as in (a) plotted as function of a normalized time (time multiplied by hairpin concentration). The higher concentration data collapse onto the same curve, but the low concentration data result in lower than expected conversion. This is probably due to a small amount of template sticking to the reaction tube. If 0.5 nM is removed from the reaction because it sticks to the tube, that hardly affects rates at hairpin concentrations of 10 nM and 100 nM, but it has a large effect on reaction rates when only 1 nM hairpin is present. The black line is a fit of $P/P_0 = 1 - \exp(\tau/t)$. Based on our earlier work [2], we predicted that under the experimental conditions ($R_0 = 36$ nM, $K = 0.5$ nM^{-1}, $k_2 = 2 \times 10^{-3}$s^{-1}, $k_f = 3 \times 10^6$ M^{-1}s^{-1}) $\tau = 446$ min nM. The fit yields $\tau = 1.2 \times 10^3$ min nM, suggesting PER proceeds slightly slower on the surface of colloids than in bulk.



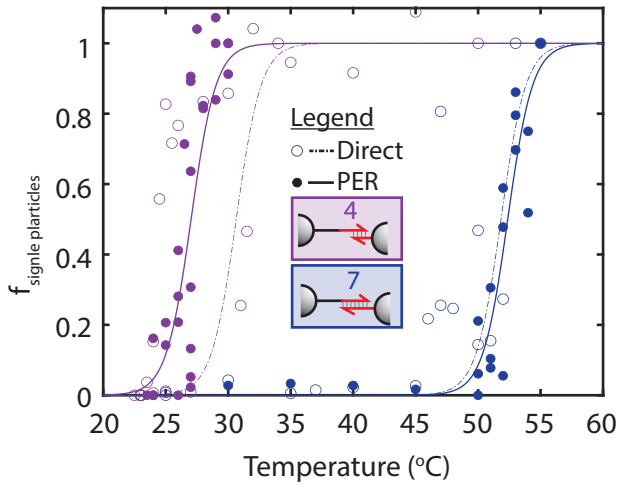
Supplementary Figure 2: Electrophoresis gel of DNA grafted onto particles before and after PER. Samples were prepared by attaching Biotinylated DNA to Streptavidin-coated particles, then performing the Primer Exchange Reaction, and finally removing the DNA from the particles under denaturing conditions. The first two lanes contain samples in which no PER was performed, but reactant and product strands were attached to the particle. The lanes indicate that the strands can be removed from the particles and imaged in an electrophoresis gel. The other lanes show the DNA that results after 2 hours of PER with decreasing template concentrations. Samples with higher template concentrations result in more conversion, but no complete conversion is observed for these particles at any conditions. Notably, in each sample there are only two bands observable: the reactant and the product. No unintended side products are formed at any conditions. In the samples with higher template concentrations, the template band is also visible.



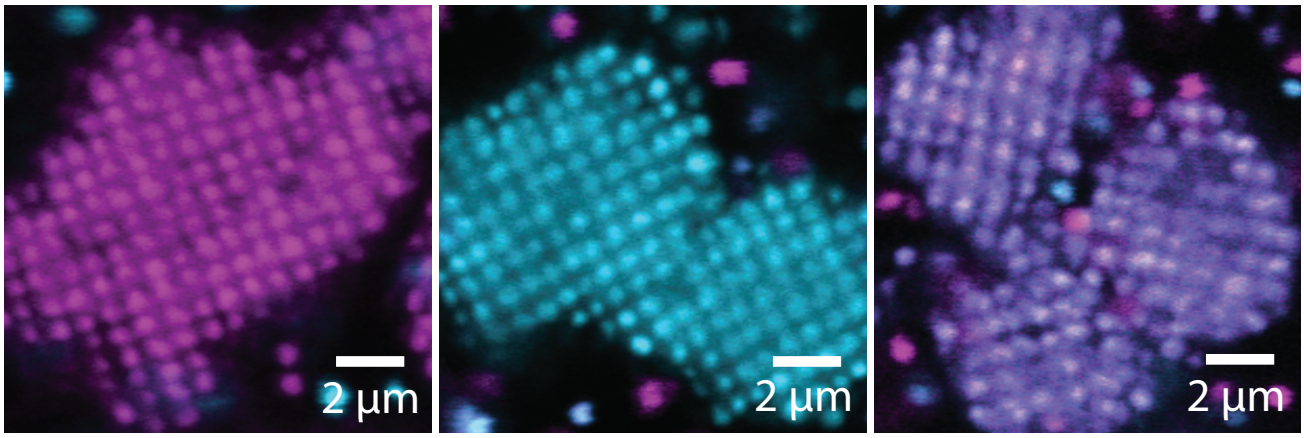
Supplementary Figure 3: Melting curves of DNA-coated particles with their binding particles that have been prepared under various PER conditions. The black line represents reference particles to which the assembly DNA is attached directly via click chemistry. This sample showed the characteristic steep melting curve where all particles are singlets only a few degrees above the melting temperature (iii). The red line represents a sample prepared using PER where we used the DNA polymerase concentration that was originally used in the paper by Kishi *et al.* [1]. We observed non-specific aggregation that persisted well above the melting temperature (iv). Washing the particles repeatedly in water to remove excess polymerase reduced the problem, but small clusters continued to persist and the sample did not crystallize (ii). Preparing samples using a 5-fold reduced DNA polymerase concentration (blue line) resulted in a sharp melting curve again and enabled crystallization (i).



Supplementary Figure 4: The binding capacity of fluorescently labeled strands onto a DNA-coated particle is measured as a proxy for the DNA grafting density. We measured the fluorescent signal of DNA-coated particles mixed with increasing amounts of Hex-labeled complementary DNA. The fluorescent signal linearly increased until it plateaus. The transition to a plateau indicates saturation of the DNA grafted onto the colloids. We fit the data with equation $F = \frac{q}{2Kc_p}\left((c_f K + c_p N K + 1) - \sqrt{(c_f K + c_p N K + 1)^2 - 4K^2 c_f c_p N}\right)$ that follows from equilibrium binding. Here F is the fluorescent signal in count, $q = 0.2$ is a fit parameter that represents the fluorescent signal per fluorescently labeled DNA strand $c_p = 2.6 \pm 0.2 \times 10^{-4}$nM is the particle concentration, c_f is the fluorescently-labeled DNA concentration in nanomolar, $K = 139.5$ nM^{-1} is the equilibrium constant for the hybridization reaction between a fluorescently labeled DNA strand and a DNA strand on the particle, and N is the number of DNA strands per particle. After fitting we find that $N = 2.3 \pm 0.2 \times 10^5$ strands per particle on the DNA-coated particles prepared using Biotion-Streptavidin chemistry and $N = 1.14 \pm 0.05 \times 10^5$ strands per particle on the DNA coated particles prepared using click chemistry.



Supplementary Figure 5: 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 well in the system in which the co-assembling particles have 7 complementary bases (blue) and are within the error in the system in which they have 4 complementary bases (purple).



Supplementary Figure 6: A zoom-in of the crystals in Figure 6 of the main manuscript that indicates that the particles are crystalline.

References

[1] Kishi, J.Y.; Schaus, T.E.; Gopalkrishnan, N.; Xuan, F.; Yin, P. Programmable autonomous synthesis of single-stranded DNA *Nature Chemistry* **2018**, 10, 155-164. doi:10.1038/nchem.2872

[2] Moerman, P.G.; Gavrilov, M.; Ha, T.J.; Schulman, R. Catalytic DNA Polymerization Can Be Expedited by Active Product Release. doi:10.26434/chemrxiv-2022-3k98v

[3] SantaLucia, J. Jr. A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics. *Proc. Natl. Acac. Sci. USA* **1998**, 95, 1460-1465. doi:10.1073/pnas.95.4.1460