Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Transfer of multi-DNA patches by colloidal stamping

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Experimental Procedures

Chemicals

3-(trimethoxysilyl)propyl methacrylate (TPM, catalog number # 440159), 3-chloro-2-hydroxypropyl methacrylate (CHPMA, catalog number # 454923), sodium azide (NaN_3, ≥ 99.5%, catalog number # S2002), (3-Aminopropyl)triethoxysilane (APTS, 99%, catalog number # 440140), sodium dodecyl sulfate (SDS, ≥ 99%, catalog number # 436143), ethylenediaminetetraacetic acid (EDTA, catalog number # E9884), 2,2′-Azobis(2-methylpropionitrile) (AIBN, 98%, catalog number # 441090), sodium chloride (NaCl, catalog number # S9888), potassium chloride (KCl, catalog number # P3911), potassium iodide (KI, catalog number # 221945), tris (hydroxymethyl)aminomethane (catalog number # 252859), sodium hydrogen phosphate (catalog number # 1.06559), potassium dihydrogen phosphate (catalog number # 1.05108), magnesium chloride (catalog number # M8266), hexamethyldisilazane (99.9%, catalog number # 379212), Triton™ X-100 (catalog number # X100), hydrochloric acid (HCl, 37%, catalog number # 320331) and Deuterium oxide (D_2O, catalog number # 151882) were purchased from Sigma-Aldrich (France). Sodium chloride (catalog number # 10123443) and ammonium hydroxide (28-30%, catalog number # 10269800) were purchased from Fisher Scientific (France). Absolute ethanol (catalog number # PC80101.5000), tetrahydrofuran (THF, (catalog number # TE02201000) and dimethylformamide (DMF, catalog number # DI10621000) were purchased from Atlantic Labo (France). All chemicals were used as received without further purification. Solutions were prepared with Milli-Q water (Millipore, 18.2 MΩ/cm). Polystyrene (PS) particles with a diameter = 1.5 µm and 4.8 µm were purchased from Thermo Scientific (catalog number # 5153C) and Magsphere (catalog number # PS005UM), respectively. Block-copolymer (PS-*b*-PEO, Mn 3.8k-*b*-6.5k, catalog number # P10078A-SEO) was purchased from Polymer Source (Canada).

The different sequences used are presented color coded in Table S1. Sequences A and B were obtained from Integrated DNA Technologies (IDT, USA). Both sequences consist of a dibenzocylooctyne-sulfo-N-hydroxysuccinimidyl ester at the 5' end of the strand, followed by a 45-thymine long spacer and finished with the 15-base long recognition sequence A or B. Dibenzocylooctyne (DBCO) is used to graft the DNA strands on the surface of azide functionalized particles by strain promoted azide alkyne cycloaddition (SPAAC). The spacer (45 thymine bases) is used to separate the recognition domain from the surface of the colloid and prevents unspecific interactions, such as undesirable aggregations between colloids. The duplexes formed by A and B with their complementary strands melt respectively at 60.5°C and 62.6 °C, as calculated at 100 mM Na^+ and 1000 nM primer, using Oligocalc[1]. These temperatures are well above room temperature which ensures that the assemblies are stable under the experimental conditions. The sequences T-X-A*, X^*_{565}-B*, Y^*_{488}-B*, $Eject_X$, $Eject_Y$ and $Eject_Z$ were purchased from Eurogentec (Belgium).

[1] http://biotools.nubic.northwestern.edu/OligoCalc.html

Table S1. DNA-sequences used in this study

Name	Domains	Sequences from 5' to 3'	Modification	Purification
A	Spacer-A	TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT CAG CGT GCT CGA ACG	5' DBCO	HPLC
B	Spacer-B	TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT GGC ACT CCG CGG CCG	5' DBCO	HPLC
Ink_{565}	X^*_{565}-B*	AGC TAA CGA GAC GGC GTT CCG CGG CCG CGG AGT GCC	5' Atto565	Reversed-phase HPLC
	T-X-A*	AGT TCT CGG AAC GCC CTC TCG CGT TCG AGC ACG CTG	None	Reversed-phase cartridge gold
$Eject_X$	X*- T*	CGA GAC GGC GTT CCG AGA ACT	None	Reversed-phase cartridge gold
Ink_{488}	Y^*_{488}-B*	CAA GTC CCT TGA CTA TAA TCG CGG CCG CGG AGT GCC	5' Alexa488	Reversed-phase HPLC
	$\overline{T}$-Y-A*	TGA GCC CGA TTA TAG TCA AGG CGT TCG AGC ACG CTG	None	Reversed-phase cartridge gold
$Eject_Y$	Y*- $\overline{T}$*	CCT TGA CTA TAA TCG GGC TCA	None	Reversed-phase cartridge gold
Ink_{647}	Z^*_{647}-B*	AAC CGT CTT GCC AAG AAT CGC CGG CCG CGG AGT GCC	5' Alexa647	Reversed-phase HPLC
	$\overline{\overline{T}}$-Z-A*	CTT CCA GCG ATT CTT GGC AAG CGT TCG AGC ACG CTG	None	Reversed-phase cartridge gold
$Eject_Z$	Z*- $\overline{\overline{T}}$*	CTT GCC AAG AAT CGC TGG AAG	None	Reversed-phase cartridge gold

Preparation of buffer solutions

A concentrated PBS buffer (PBS10X) is prepared by dissolving 1.6 g of NaCl, 0.4 g of KCl, 4.2 g of Na_2HPO_4, and 0.48 g of KH_2PO_4 in 200 mL of ultrapure water. Then, 1 mL of PBS10X is diluted in 9 mL of ultrapure water to prepare PBS1X buffer. (PBS1X, Triton 0.1 % w/v) buffer solution is prepared by diluting 100 µL of Triton 10 % w/v in 10 mL of PBS1X.

Concentrated TE buffer (TE10X) is prepared by dissolving 1.21 g Tris base (tris (hydroxymethyl)aminomethane) and 0.37 g ethylenediaminetetraacetic acid (EDTA) in 100 mL of ultrapure water. The pH is adjusted to 8.1 using HCl 37 %. TE10X is stored in a freezer at -20 °C and we regularly prepare fresh TE1X buffer, by diluting 4 mL of TE10X in 36 ml of ultrapure water. TE1X buffer is stored in a fridge at 4 °C.

Suspending DNA strands in TE buffer

The DNA strands are received dried at the bottom of microtubes, they need to be resuspended in an appropriate buffer for storage and further use. The DNA strands are suspended in TE1X at 100 µM and stored at -20 °C.

Synthesis of azidated TPM particles

In a 50 mL round bottom flask are added a magnetic bar, 20 mL of deionized water, a volume, v, of TPM, and 20 µL of ammonium hydroxide. The mixture is stirred at 400 rpm for 4 h at room temperature. After 4 h, 40 µL of CHPMA are added to the reaction mixture under continuous stirring. After 30 more minutes, 5 mL of an aqueous solution of a 5 wt% aqueous solution of SDS are added to the reaction medium and 10 min later 10 mg of AIBN are added. 20 min after the addition of AIBN, the flask is placed in the oil bath at 80 °C and left stirring at 400 rpm for 4 h. After cooling, the particles are washed with 3 cycles of centrifugation (12000 rpm; 5 min) and redispersed in 20 mL of absolute ethanol.

The obtained chlorinated particles are transferred into DMF by 3 cycles of centrifugation (12000 rpm; 5 min), dispersed in 20 mL of DMF and transferred in a 50 mL round bottom flask together with 100 mg of sodium azide (NaN_3) and trace amount of potassium iodide (KI) as a catalyst. The mixture is heated up to 70 °C and left stirring overnight. The particles are then washed twice (12000 rpm; 5 min) with a mixture of DMF and deionized water (50 vol%) and then two more times (12000 rpm; 5 min) with deionized water only. The azidated TPM particles are redispersed in 20 mL of TE1X buffer supplemented with 0.1% w/v Triton and stored at 4 °C until further use. Figure S1 shows that smooth spherical particles are obtained, with a diameter of 1.10 ± 0.04 µm, 1.60 ± 0.05 µm and 2.0 ± 0.1 µm when v = 180 µL, 200 µL and 500 µL, respectively[2].

[2] The diameter of over 300 particles was measured on TEM images.

Functionalization of PS particles with PS-PEO-N_3 copolymer

In an Eppendorf tube, 90 µL of deionized water, 100 µL of the azidated copolymer (PS-PEO-N_3) at a concentration of 1 mM in deionized water, and 50 µL of PS particles (at 10 wt% in water) are added. The mixture is shaken for 5 seconds with a vortex, and sonicated for another 5 seconds. Then, 160 µL of THF (corresponding to 40 vol% of the final volume) are added to the mixture. The suspension is stirred at 1000 rpm for 30 min at room temperature. Then 800 µL of MilliQ water are added to the mixture and the THF is evaporated by shaking at 400 rpm the open Eppendorf tube at 45 ℃ for 40 min using a thermal shaker. The suspension is then cooled back to room temperature and washed by three cycles of centrifugation (7000 rpm; 3 min), removal of the supernatant and redispersion in 100 µL of MilliQ water in an ultrasonic bath. The final sample is stored at 4 ℃ until further use.

DNA grafting on azidated TPM particles

In an Eppendorf tube, 10 µL of azidated TPM particles with a concentration of 1 wt% are firstly washed three times (2000 rpm; 2 min) with 200 µL of buffer solution (PBS1X, Triton 0.1% w/v). After washing, 10 µL of DNA strands A or B at 100 µM are added into the particle suspension. The reaction mixture is agitated at 55 °C and 1500 rpm using a thermal shaker for 24 h. The particles (at 0.05 wt%) are kept in the fridge at 4 ℃ until further use.

DNA grafting on azidated PS particles

In an Eppendorf tube, 20 µL of azidated PS particles (5 wt%) are washed and redispersed three times (5000 rpm; 4 min) with 100 µL (PBS1X, Triton 0.1% w/v) buffer solution. After washing, 10 µL of DNA strand A or B at 100 µM in TE1X buffer solution are added into the particle suspension. The reaction mixture is agitated at 55 °C and 1000 rpm using a thermal shaker for 24 h. The particles (at 1 wt%) are kept in the fridge at 4 ℃ until further use.

DNA ink assembly and inking of the particles

The DNA Ink_{565} is formed by mixing 5 µL of strand X^*_{565}- B* (100 µM) in 15 µL of TE1X and 5 µL of a buffer solution (Triton 0.5% w/v, 25 mM $MgAc_2$ in TE1X solution) in one Eppendorf tube. In a second Eppendorf, 5 µL of strand T-X-A* (100 µM) are mixed in 15 µL of TE1X and 5 µL of a buffer solution (Triton 0.5% w/v, 25 mM $MgAc_2$ in TE1X solution). Both solutions of complementary strands (X^*_{565}- B* and T-X-A*) are mixed together, the mixture then is heated for 5 min at 85 ℃ before cooling down to 25 ℃ at 1 ℃/min using BIO-RAD T100 Thermal cycler, to ensure the correct assembly of the two complementary strands. The final concentration in DNA Ink_{565} is 10 µM.

For the functionalization of the stamp colloid with the ink, 10 µL of TPM_A at 1 wt%, 90 µL of assembly buffer (TE1X, Triton 0.1 wt%, 10 mM Mg^{2+}), and 10 µL of Ink_{565} (10 µM) are added in an Eppendorf tube. The mixture is left resting for 5 min to make sure that Ink_{565} covers all the surface of the colloid. The particles are washed three times (2 min × 2000 rpm) with 90 µL of the assembly buffer, and finally redispersed in 20 µL of assembly buffer. The final particle concentration is ~0.5 wt%.

Assembly and purification of the clusters

For assembly, the particles are dispersed in a (TE1X, Triton 0.1% w/v, 10 mM Mg^{2+}) buffer solution. The TPM_A~PS_B clusters were purified in a PBS based buffer solution of intermediate density prepared by mixing 6 ml of D_2O (Euriso-top), 1.05 ml of MiliQ water and 2.35 ml of a Mg^{2+} enriched TE buffer (TE1X, Triton 0.1% w/v, 40 mM Mg^{2+}). This aqueous buffer is prepared by mixing 1 ml of TE10X, 100 µL Triton 10% w/v, and 800 µL of $MgAc_2$ at 500 mM and 8.1 ml of MiliQ water. The resulting purification buffer solution contains 60 % D_2O in TE1X, Triton 0.1% w/v and 10 mM Mg^{2+} and has a density of about 1.07 g/cm^3. For purification, 100 µL of purification buffer is placed in an Eppendorf tube and 20 µL of the mixture of clusters is carefully deposited on top. The tube is left on the bench top for 20 to 24 hours at 4 °C. Free PS_B particles are removed carefully from the top, and the clusters are collected very carefully by pipetting ~15 µL at the bottom of the tube and mixing with 15 µL of assembly buffer.

Characterization

For confocal fluorescence microscopy experiments, the imaging chamber is prepared using parafilm. Typically 10 layers of parafilm are punched using a 2 mm puncher to form the chamber. The chambered parafilm is glued on top of a glass coverslip by heating everything up to around 100 °C on a heating plate. For confocal imaging, 4-6 µL of the sample are placed in the chamber, which is sealed using another small piece of parafilm. Depending on the type of observation we applied different treatments on the coverslip. To follow in situ the formation of clusters we used hydrophobic coverslips to minimize the interactions between the colloids and the glass slide. Hydrophobic coverslips are prepared by cleaning commercial coverslips with ethanol followed by a 10 min plasma treatment to deeply clean and activate the glass surface. The coverslips are then exposed to vapors of hexamethyldisilazane overnight in a closed petri dish at room temperature. For detailed imaging with longer exposure time, colloids need to be immobilized to prevent Brownian motion. We chose to prepare amine functionalized coverslips that are sticky for DNA-grafted colloids. For this, commercial coverslips are cleaned with ethanol, acetone, dried and treated with plasma for 10 min. The coverslips are then immersed for 40 min in a 2 wt% APTS solution in acetone at room temperature. The coverslips are finally rinsed three times with acetone and dried before use.

Imaging conditions are as followed:

Objective: Plan-Apo 63x/1.4 Oil immersion

Acquisition parameters for the following dyes on the Leica confocal:

	λ_{ex} (nm)	λ_{em} (nm)
Alexa488	488	498 – 533
Atto546	543	553 – 698

Acquisition parameters for the following dyes on the ZEISS confocal:

	λ_{ex} (nm)	λ_{em} (nm)
Alexa488	488	499 – 548
Atto565	561	574 – 627
Alexa647	639	659 - 720
Transmission	488	300 – 900

Images were analyzed using ImageJ and the Zeiss software Zen Blue 3.3.

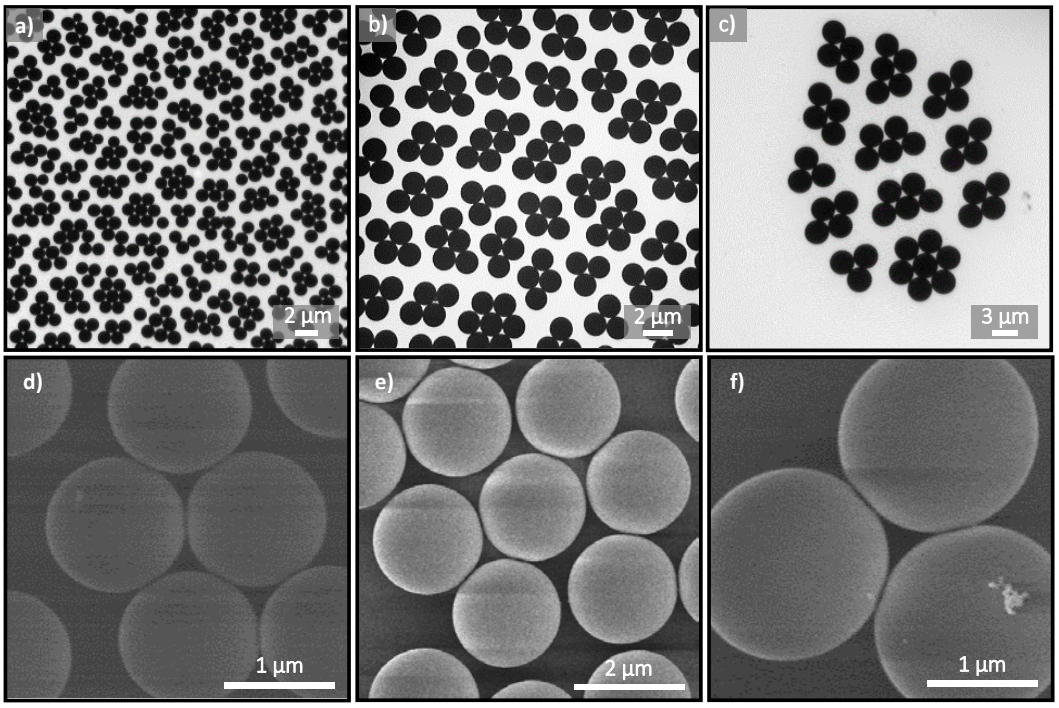


Figure S1. TEM (a-c) and SEM (d-f) images of the azidated TPM particles of different sizes: 1.10 ± 0.04 µm (a,d), 1.60 ± 0.05 µm (b,e) and 2.0 ± 0.1 µm (c-f).

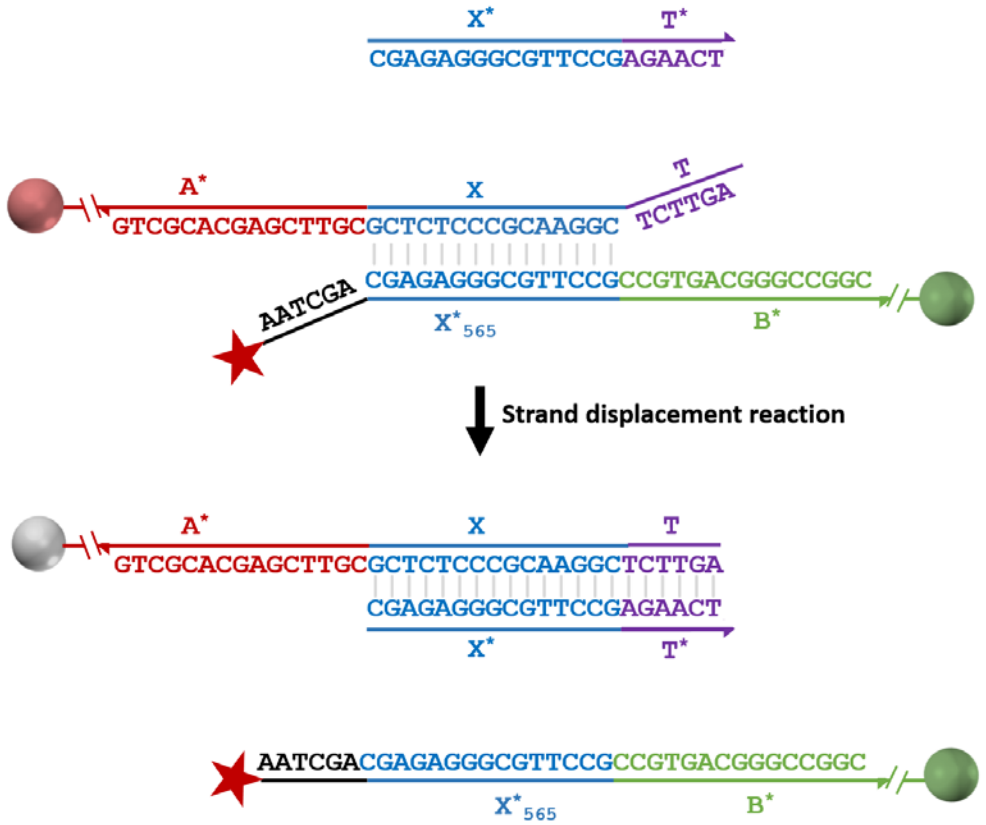


Figure S2. Schematic representation of the strand displacement reaction leading to the transfer of a DNA patch from the stamp particle to the support one.

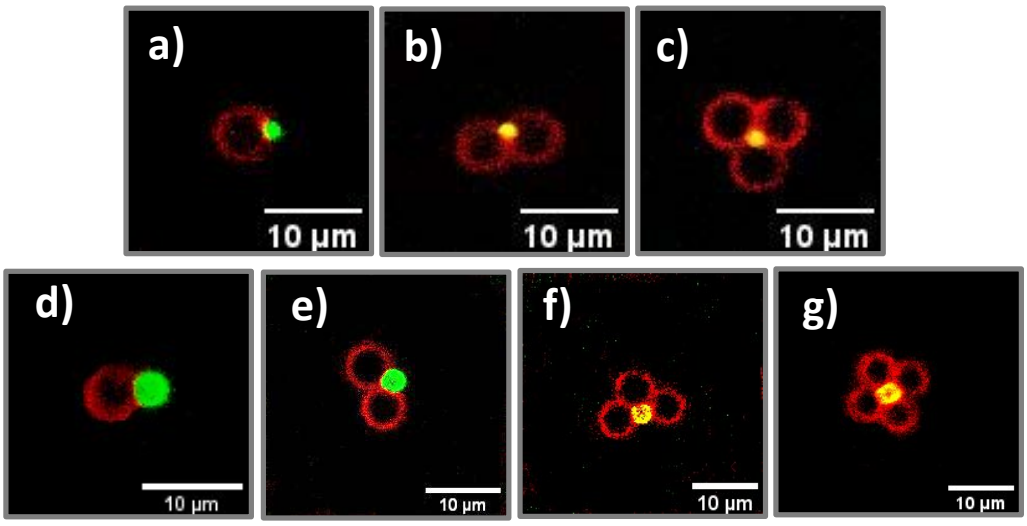


Figure S3. Confocal fluorescence microscopy images of the clusters obtained by incubating TPM_B~Y^*_{488}-B* and PS_A~Ink_{565} in a 1:40 ratio. The diameter of the TPM particles is: a-c) 1.1 µm (α = 4.39), d-g) 2 µm (α = 2.42).

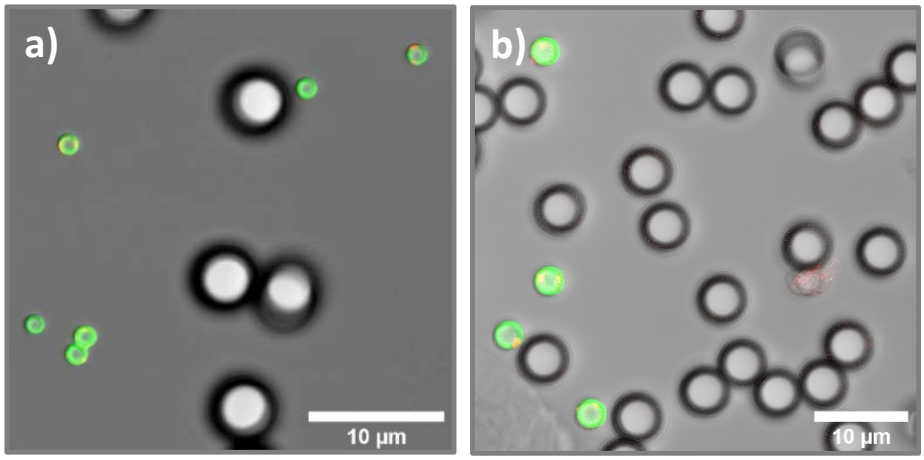


Figure S4. Confocal fluorescence microscopy images (Alexa488, green channel; Atto565, red channel) with transmission microscopy (grey channel) of PS_A~Ink_{565}~TPM_B & Y^*_{488}-B* clusters after strand displacement reaction using $Eject_X$ strand. The diameter of the TPM particles is: a) 1.1 µm (α = 4.39), b) 2 µm (α = 2.42).

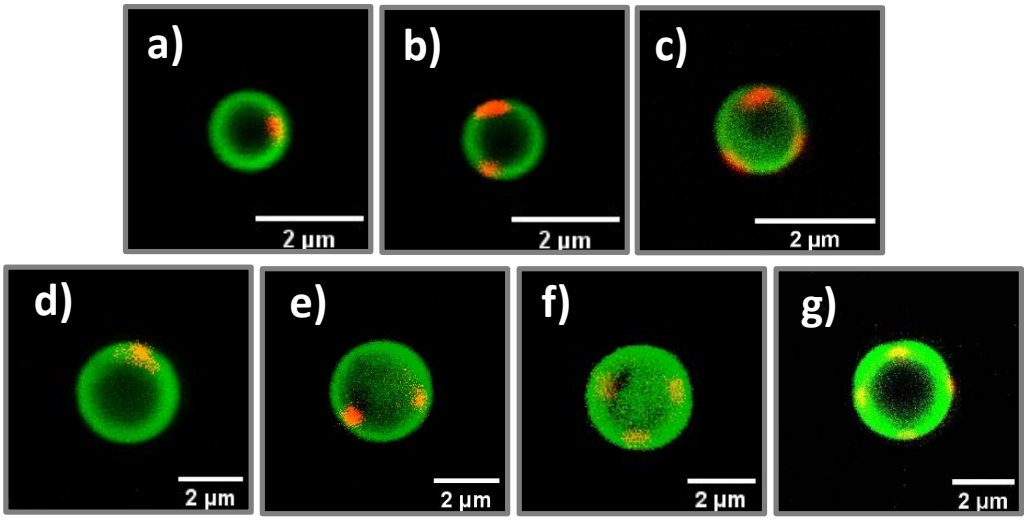


Figure S5. Confocal fluorescence microscopy images (Alexa488, green channel; Atto565, red channel) of patchy TPM particles with an increasing number of patches obtained after strand displacement reaction using $Eject_X$ strand. The diameter of the TPM particles is: a-c) 1.1 µm (α = 4.39), d-g) 2 µm (α = 2.42).

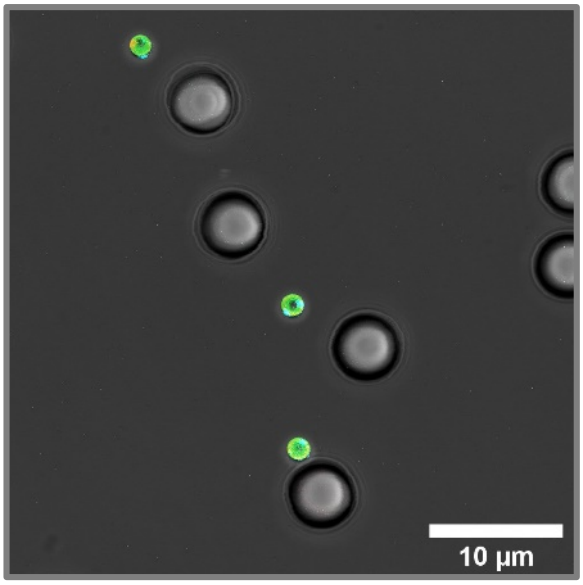
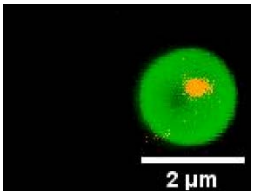
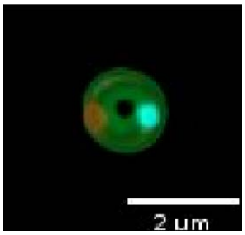


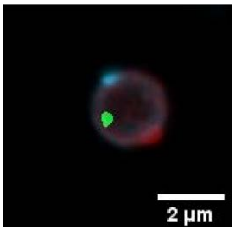
Figure S6. Confocal fluorescence microscopy images (Alexa488, green channel; Atto565, red channel, Alexa647, blue channel) with transmission microscopy (grey channel) of PS_A~Ink_{565}~PS_A~Ink_{647}~TPM_B/Y^*_{488}-B^* clusters after strand displacement reaction using $Eject_X$ and $Eject_Z$ strands. The diameter of the TPM particles is 1.6 µm (α = 3.02).



Movie S1. 3D reconstruction of a PS particle (diameter 1.5 µm) with one red fluorescent patch, composed of Atto565 tagged X^*_{565}-B* strands, obtained after addition of the $Eject_X$ strand, ΔZ = 0.1 µm.



Movie S2. 3D reconstruction of a TPM particle (diameter 1.1 µm) with one red (from Atto565 tagged X^*_{565}-B* strands) and one blue (from Alexa647 tagged Z^*_{647}-B* strands) fluorescent patches obtained after addition of the $Eject_X$ and $Eject_Z$ strands, ΔZ = 0.25 µm.



Movie S3. 3D reconstruction of a TPM particle (diameter 1.6 µm) with one red (from Atto565 tagged X^*_{565}-B* strands), one blue (from Alexa488 tagged Y^*_{488}-B* strands) and one green (from Alexa647 tagged Z^*_{647}-B* strands) fluorescent patches obtained after addition of the $Eject_X$, $Eject_Y$ and $Eject_Z$ strands, ΔZ =0.25 µm.