Supporting Information

Hadorn et al. 10.1073/pnas.1214386109

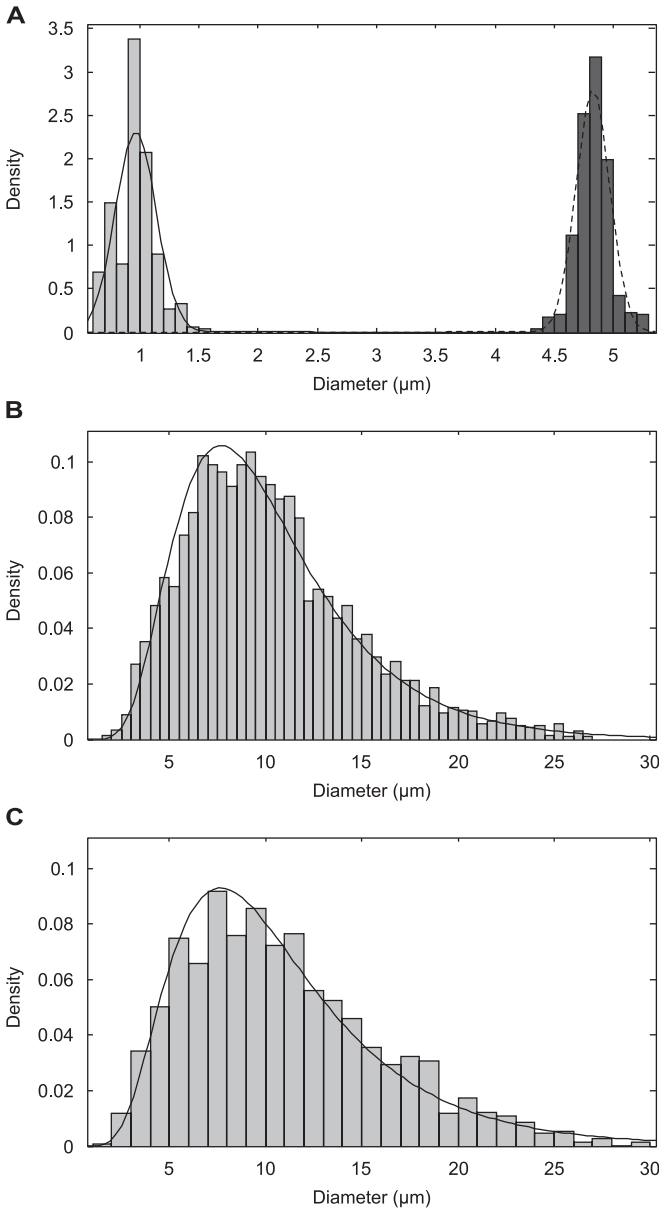

Fig. S1. Size distribution. Histograms for control samples containing polystyrene and silica beads and emulsion droplet (ED) populations 3 and 3′. (*A*) In two independent control experiments, the custom Matlab algorithm was found to have good accuracy in determining particle sizes, as evident by the calculated particle means of 0.97 ± 0.17 μm (SD) for the polystyrene beads (light gray) with a 1.0-μm diameter (according to the supplier) and 4.83 ± 0.14 μm (SD) for the silica beads (dark gray) with a 5.0-μm diameter (according to the supplier). Lines represent individual Gaussian fits. (*B* and *C*) The same Matlab algorithm revealed the mean diameter of 10.29 ± 4.47 μm (SD) for ED3 (*B*) and 10.97 ± 5.14 μm (SD) for ED3′ (*C*). Lines represent log-normal fits.

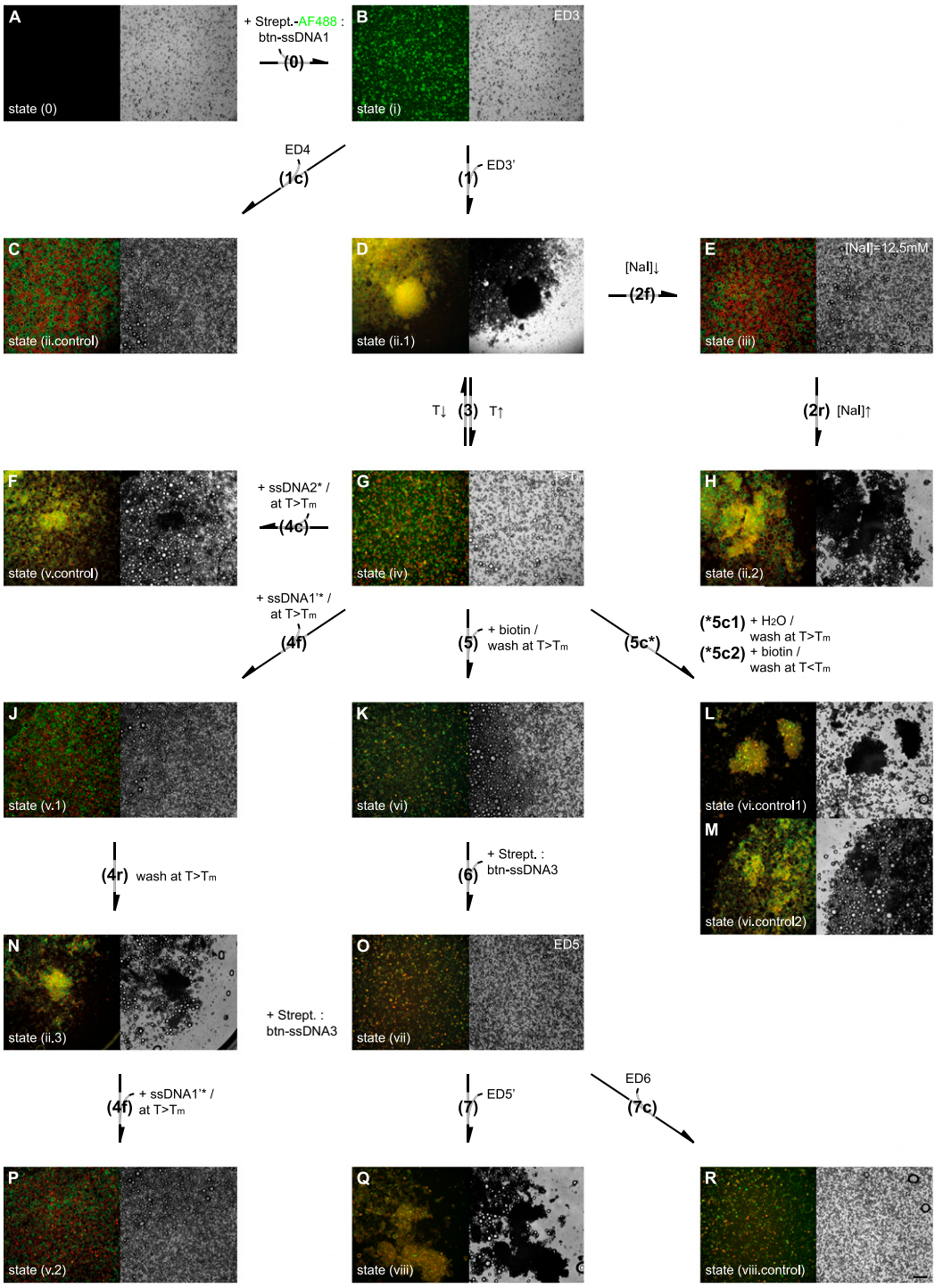

Fig. S2. Specificity and reversibility of the self-assembly process and recyclability of emulsion droplets. Extended scheme of all steps (Arabic numerals) and states (Roman numeral). (*A*–*R*) For all states, illustrated by representative samples shown as both fluorescence and transmission micrographs, the temperature was at room temperature (i.e., below the melting temperature of the dsDNAs), and the sodium iodide concentration was 25 mM unless stated otherwise. The labeling of the states and steps corresponds to the labeling scheme used in Fig. 3*A*. Extensions compared to Fig. 3*A* are state 0, step 0, and all control experiments labeled with "c" or "control" (e.g., step 1c, state ii.control). The forward (*f*) and reverse (*r*) reactions depicted in Fig. 3*A* as arrows pointing in both ways are depicted individually for most steps (e.g., step 2f). Biotinylated emulsion droplets (EDs) before (*A*) after (*B*) surface functionalization with biotinylated single stranded DNA oligonucleotides of sequence 1 (btn-ssDNA1) anchored to the biotinylated emulsion droplet surface by an fluorescently active streptavidin Alexa Fluor conjugate (Strept.-AF488). Binary mixtures of ED populations functionalized with (*C*) noncomplementary and (*D*) complementary btn-ssDNA oligonucleotides. (*E*) Disassembled state after decreasing the electrolyte concentration; (*H*) reassembled state after increasing the electrolyte concentration. (*G*) Disassembled state after increasing the temperature. (*J*) Preserved disassembled state after addition of competing non-biotinylated ssDNA (ssDNA1′*) complementary to btn-ssDNA1. (*F*) The disassembled state was not preserved by the addition of noncomplementary non-biotinylated ssDNA (ssDNA2′*). (*N*) Reassembled state after removal of competing ssDNA1′*. (*P*) Disassembled state after second addition of competing ssDNA1′*. EDs after (*K*) surface reset and (*O*) refunctionalization. (*L*) Neither addition of water (*M*) nor washing at room temperature successfully reset the surface. Binary mixtures of ED populations functionalized with (*Q*) complementary and (*R*) noncomplementary btn-ssDNA oligonucleotides. (Scale bar: 250 μm.)

A

ED1 btn-ssDNA1-AF488
ED-btn : Strept.-AF532 : btn-TGTACGTCACAACTA-AF488

ED2 btn-ssDNA2
ED-btn : Strept.-AF350 : btn-TGGAGGGCTCTTTCT-3'

ED3 btn-ssDNA1
ED-btn : Strept.-AF488 : btn-TGTACGTCACAACTA-3'

ED3' btn-ssDNA1'
ED-btn : Strept.-AF532 : btn-TAGTTGTGACGTACA-3'

ED4 btn-ssDNA2
ED-btn : Strept.-AF532 : btn-TGGAGGGCTCTTTCT-3'

ED5 btn-ssDNA3
ED-btn : Strept. : btn-TCGTGTGTAATCTTT-3'

ED5' btn-ssDNA3'
ED-btn : Strept. : btn-AAAGATTACACACGA-3'

ED6 btn-ssDNA4
ED-btn : Strept. : btn-CATCCATGGTGGAGG-3'

B **Stability of Surface Functionalization** T < Tm, [NaI] = 25 mM

ED1 btn-ssDNA1-AF488
ED-btn : Strept.-AF532 : btn-TGTACGTCACAACTA-AF488

3'-TCTTTCTCGGGAGGT-btn : Strept.-AF350 : btn-ED
btn-ssDNA2 ED2

C

state (0) T < Tm, [NaI] = 25 mM
ED-btn

state (i) T < Tm, [NaI] = 25 mM
ED3 btn-ssDNA1
ED-btn : Strept.-AF488 : btn-TGTACGTCACAACTA-3'

state (ii.1-3) T < Tm, [NaI] = 25 mM
ED3 btn-ssDNA1
ED-btn : Strept.-AF488 : btn-TGTACGTCACAACTA-3'
3'-ACATGCAGTGTTGAT-btn : Strept.-AF532 : btn-ED
btn-ssDNA1' ED3'

state (ii.control) T < Tm, [NaI] = 25 mM
ED3 btn-ssDNA1
ED-btn : Strept.-AF488 : btn-TGTACGTCACAACTA-3'
3'-TCTTTCTCGGGAGGT-btn : Strept.-AF532 : btn-ED
btn-ssDNA2 ED4

state (iii) T < Tm, **[NaI] = 12.5 mM**
ED3 btn-ssDNA1
ED-btn : Strept.-AF488 : btn-TGTACGTCACAACTA-3'
3'-ACATGCAGTGTTGAT-btn : Strept.-AF532 : btn-ED
btn-ssDNA1' ED3'

state (iv) **T > Tm**, [NaI] = 25 mM
ED3 btn-ssDNA1
ED-btn : Strept.-AF488 : btn-TGTACGTCACAACTA-3'
3'-ACATGCAGTGTTGAT-btn : Strept.-AF532 : btn-ED
btn-ssDNA1' ED3'

state (v.1,2) T < Tm, [NaI] = 25 mM
ED3 btn-ssDNA1
ED-btn : Strept.-AF488 : btn-TGTACGTCACAACTA-3'
3'-ACATGCAGTGTTGAT-5'
ssDNA1'*
3'-ACATGCAGTGTTGAT-btn : Strept.-AF532 : btn-ED
btn-ssDNA1' ED3'

state (v.control) T < Tm, [NaI] = 25 mM
ED3 btn-ssDNA1
ED-btn : Strept.-AF488 : btn-TGTACGTCACAACTA-3'
3'-ACATGCAGTGTTGAT-btn : Strept.-AF532 : btn-ED
btn-ssDNA1' ED3'
3'-TCTTTCTCGGGAGGT-5'
ssDNA2*

state (vi) T < Tm, [NaI] = 25 mM
ED-btn

state (vi.control) T < Tm, [NaI] = 25 mM
ED3 btn-ssDNA1
ED-btn : Strept.-AF488 : btn-TGTACGTCACAACTA-3'
3'-ACATGCAGTGTTGAT-btn : Strept.-AF532 : btn-ED
btn-ssDNA1' ED3'

state (vii) T < Tm, [NaI] = 25 mM
ED5 btn-ssDNA3
ED-btn : Strept. : btn-TCGTGTGTAATCTTT-3'

state (viii) T < Tm, [NaI] = 25 mM
ED5 btn-ssDNA3
ED-btn : Strept. : btn-TCGTGTGTAATCTTT-3'
3'-AGCACACATTAGAAA-btn : Strept. : btn-ED
btn-ssDNA3' ED5'

state (viii.control) T < Tm, [NaI] = 25 mM
ED5 btn-ssDNA3
ED-btn : Strept. : btn-TCGTGTGTAATCTTT-3'
3'-GGAGGTGGTACCTAC-btn : Strept. : btn-ED
btn-ssDNA4 ED6

Fig. S3. Surface functionalization, sequences of the ssDNA oligonucleotides, and DNA hybridization schemes. (*A*) Fluorescently labeled streptavidin Alexa Fluor (AF) 350, 488, and 532 conjugates were used to functionalize the emulsion droplet (ED) populations with biotinylated single-stranded DNA oligonucleotides (btn-ssDNA) of different sequences. (*B*) In the experiment to analyze the stability of the surface functionalization, a binary mixture of ED populations functionalized with noncomplementary btn-ssDNA oligonucleotides (ED1:ED2) was used. In addition to the fluorescently labeled streptavidin used throughout the subsequent experiments, AF488-labeled btn-ssDNA oligonucleotides were used. (*C*) For all states of the experiments, to assess the specificity and reversibility of the assembly process and recyclability of the EDs the temperature was at room temperature (i.e., below the melting temperature of the dsDNAs) and the sodium iodide concentration was 25 mM unless stated otherwise.