## SUPPORTING INFORMATION

# Smart Lipids for Programmable Nanomaterials

Matthew P. Thompson‡, Miao-Ping Chien‡, Ti-Hsuan Ku, Anthony M. Rush, and Nathan C. Gianneschi*

*Department of Chemistry and Biochemistry, University of California, San Diego*
*9500 Gilman Drive, La Jolla, CA 92093*

## General methods

All reagents were bought from TCI or Sigma-Aldrich and used without further purification. DNA synthesis reagents and modifiers were bought from Glen Research or AZCO scientific. 3,4-di(octadecyloxy)benzoic acid was synthesized via a standard literature procedure (Tuffin, R. P.; Toyne, K. J.; Goodby, J. W. *J. Mater. Chem.* **1996**, *6*, 1271-1282). All DNA was synthesized on an ABI-391 via standard solid phase synthesis on controlled pore glass supports. Fluorescence measurements were performed on a Zeiss LSM confocal fluorescence microscope. Reaction volumes of 50 µL were used in all fluorescence experiments of this type. HPLC purifications of DNA strands were performed on a Clarity 5u Oligo-RP phenomonex column (150 x 4.60 mm) with a binary gradient using a Hitachi-Elite LaChrom L-2130 pump equipped with UV-Vis detector (Hitachi- Elite LaChrom L-2420). Gradient: (Solvent A: 50mM triethylammonium acetate, pH 7.5; Solvent B: 100% methanol; gradient: 10-45% B from 0-28 minutes, 45-60% B from 28-34 minutes, and 60-70% B from 34-40 minutes, Flow rate: 1 mL/min). To confirm DNA and DNA-surfactant molecular weights, MALDI-TOF mass spectrometry was performed on a ABI MALDI Voyager (equipped with ThermoLaser Science, VSL-337ND) using THAP matrix (2,4,6-trihydroxyacetophenone monohydrate) (18 mg), ammonium citrate (7 mg), acetonitrile:water (1 mL, 1:1). DNA-surfactant and DNA concentrations were determined via UV-Vis on a Hitachi U-2810 spectrophotometer. $D_h$ was determined by DLS on a Nano-ZS90 Malvern Instrument. Zeta potential was also measured on the Nano-ZS90 Malvern instrument. TEM images were acquired on carbon grids (Ted Pella, INC.) with 1% uranyl acetate stain on a FEI Tecnai G2 Sphera at 200 KV. The identities of organic molecules were confirmed by $^{1}H$ (400 MHz) and $^{13}C$ (100 MHz) NMR spectra recorded on a Varian Mercury Plus spectrometer. Mass spectra of organic molecules were obtained at the UCSD Chemistry and Biochemistry Molecular Mass Spectrometry Facility.

## DNA Synthesis

### *Preparation of $DNA_1$*

5’- $NH_2$-TCGCACCCA-3’

A 1 µmol 3’-dA-CPG was utilized as the support. The oligonucleotide was synthesized in the standard manner. A small portion of the synthesized oligonucleotide attached to the CPG was separated and subjected to cleavage and deprotection by ammonium hydroxide overnight. This oligonucleotide was characterizated by MALDI-MS. Mass calcd: 2818.8; Mass obs: 2840.6.

### *Preparation of $DNA_2$*

5’-PEG-PEG-GGAGAGAGACTGGGTGCGA-3’

A 1 µmol 3'-dA-CPG was utilized as the support, with two PEG phosphoramidites (C18-spacer, Glen Research) as the terminus. The oligonucleotide was synthesized in the standard manner leaving the final base protected with a DMT group. Following cleavage and deprotection by ammonium hydroxide overnight, the oligonucleotide was purified by HPLC (retention time = 35 min), treated with acetic acid followed by solvent removal and characterizatized by MALDI-MS. Mass calcd: 6955.2; Mass obs: 6964.1.

### *Preparation of Fluorescein modified ssDNA ($DNA_1$-Fluorescein)*

5’- $NH_2$-TCGCACCCA-3’-Fluorescein

A 1 µmol 3’-Fluorescein-CPG (Glen Research) was utilized as the support, with a 5’-amino modifier (5-member modifier, Glen Research) as the terminus. The oligonucleotide was synthesized in the standard manner. A small portion of the synthesized oligonucleotide attached to the CPG was separated and subjected to cleavage and deprotection by ammonium hydroxide overnight. This oligonucleotide was characterizated by MALDI-MS. Mass calcd: 3417.4; Mass obs: 3440.7.

### *Preparation of Rhodamine modified ssDNA ($DNA_4$-Rhodamine)*

5’- $NH_2$-CTCACGACA-3’-Rhodamine

A 1 µmol 3’-Rhodamine-CPG (Glen Research) was utilized as the support. The oligonucleotide was synthesized in the standard manner. A small portion of the synthesized oligonucleotide attached to the CPG was separated and subjected to cleavage and deprotection by 0.05 M potassium carbonate in methanol overnight. This oligonucleotide was characterizated by MALDI-MS. Mass calcd: 3466.4; Mass obs: 3477.9.

### *Preparation of $DNA_3$*

5’-TCGCACCCAGTCTCTCTCC-3’

A 1 µmol 3’-dC-CPG was utilized as the support. The oligonucleotide was synthesized in the standard manner leaving the final base protected with a DMT group. Following cleavage and deprotection by ammonium hydroxide overnight, the oligonucleotide was purified by HPLC (retention time = 32 min), treated with acetic acid followed by solvent removal and characterization by MALDI-MS. Mass calcd: 5635.7; Mass obs: 5693.1.

### *Preparation of $DNA_5$*

5’-PEG-PEG-AGCGACAGACTGTCGTGAG-3’

A 1 µmol 3'-dG-CPG was utilized as the support, with two PEG phosphoramidites (C18-spacer, Glen Research) as the terminus. The oligonucleotide was synthesized in the standard manner leaving the final base protected with a DMT group. Following cleavage and deprotection by ammonium hydroxide overnight, the oligonucleotide was purified by HPLC (retention time = 36 min), treated with acetic acid followed by solvent removal and characterized by MALDI-MS. Mass calcd: 6566.5; Mass obs: 6533.9.

### *Preparation of $DNA_6$*

5’-CTCACGACAGTCTGTCGCT-3’

A 1 µmol 3’-T-CPG was utilized as the support. The oligonucleotide was synthesized in the standard manner leaving the final base protected with a DMT group. Following cleavage and deprotection by ammonium hydroxide overnight, the oligonucleotide was purified by HPLC (retention time = 30 min), treated with acetic acid followed by solvent removal and characterization by MALDI-MS. Mass calcd: 5739.8; Mass obs: 5712.6.

### *Preparation of $DNA_7$*

5’-GGAGAGAGACTGGGTGCGA-3’

A 1 µmol 3'-dA-CPG was utilized as the support. The oligonucleotide was synthesized in the standard manner leaving the final base protected with a DMT group. Following cleavage

and deprotection by ammonium hydroxide overnight, the oligonucleotide was purified by HPLC (retention time = 30 min), treated with acetic acid followed by solvent removal and characterizatized by MALDI-MS. Mass calcd: 5982.9; Mass obs: 5941.6.

### DNA conjugation to 3,4-di(octadecyloxy)benzoic acid, particle formation and DNA-lipid characterization.

3,4-di(octadecyloxy)benzoic acid (100 μmol, 66 mg) was partially dissolved in 0.5 mL of DMSO, followed by addition of 0.2 mL of N,N-diisopropylethylamine and HBTU (100 μmol, 40 mg). The resulting solution was a light yellow, partially dissolved, soapy suspension. This solution was added via two 1 mL syringes inserted either side of the capsule containing the CPG-bound-DNA. The solution was pushed back and forth across the beads between the syringes several times, wrapped in parafilm to seal, and then left at room temperature for 24 hours. The beads were then washed twice with 2 mL DMSO and twice with 2 mL of acetonitrile. The resulting CPG beads were dried with a stream of nitrogen, removed from the capsule and then treated with 1 mL of ammonium hydroxide for 18 hours. At this time, the CPG was filtered away from the ammonium hydroxide solution. The solution was diluted with 1 mL Tris buffer (50 mM, pH 7.4) and placed in 3500 g/mol molecular weight cut off dialysis tubing and dialyzed against 2 L of buffer for 24 hours. The buffer was changed, and the solution transferred to 20,000 g/mol molecular weight cut off tubing and dialyzed again for 48 hours. The DNA-surfactants were then characterized by directly analyzing the vesicle samples by MALDI-MS. No free DNA was observed by MALDI following dialysis. The following MALDI data was performed using a 1:1 THAP:DHB (2,5-dihydroxybenzoic acid) matrix. Matrix formulations: THAP matrix - (2,4,6-trihydroxyacetophenone monohydrate) (18 mg), ammonium citrate (7 mg), acetonitrile:water (1 mL, 1:1). DHB matrix (15.4 mg/mL, methanol/water 1:1).

**$DNA_1$-lipid**: Mass calcd: 3461.1; Mass obs: 3477.1.

**Rhodamine-$DNA_4$-lipid**: Mass calcd: 4109.5; Mass obs: 4175.9.

**Fluorescein-$DNA_1$-lipid**: Mass calcd: 4060.4; Mass obs: 4050.6.

### Addition of DNA input strands – General conditions

Experiments were carried out in final volumes of 50 μL, Tris/$MgCl_2$ (50 mM/50mM, pH 7.4) at room temperature. DNA-surfactant concentrations in the final particle solutions were determined by UV-Vis from absorbance of DNA in the DNA-lipid at $\lambda_{260}$ and each solution was adjusted to 1 μM. Each addition of DNA as described in the main text was performed via the addition of 2 μM of each DNA strand.

### Fluorescence Experiments

*General Procedure for conducting fluorescence microscopy experiments.* 15-20 μL of sample was used. DNA-lipid assemblies with structures confirmed by TEM and DLS along with appropriate input ssDNA strands were deposited on glass slides and sealed under a cover slip. The edge of the cover slip was then sealed with nail polish after the sample was air dried.

### Critical Micelle Concentration (CMC) determination (see Figure 1SE below)

A stock solution of pyrene was prepared by adding 1 mg pyrene in 10 mL of 20 mM Tris buffer (pH 7.4) and sonicating for 12 hrs, followed by centrifugation at 12,000 x g for 5 min. The supernatant containing pyrene as the fluorescent probe was utilized for this assay and measured on a plate reader fluorometer, SPECTRAMAX GEMINI EM (Molecular Devices). DNA-lipid concentration was measured via UV absorbance and micelles were accordingly diluted serially in 96-well microplates (black well, black bottom, FIA, Greiner Bio-One). Excitation was done over a 300-360 nm wavelength range and the emission was recorded at 390 nm. The slit width for excitation was fixed at 1 nm. The concentration was plotted on a logarithmic scale and the critical micelle concentration was determined at the intercept of the two tangents drawn where the decreasing surface tension becomes constant.

### Zeta potential measurement with variable $MgCl_2$ concentration (see Figure 1SA below)

DNA-lipid at 1 μM concentration was added to 20 mM Tris buffer (pH 7.4) with variable $MgCl_2$ concentration (0, 5, 7.5, and 10 mM). The zeta potential of these samples with variable $MgCl_2$ concentrations was then measured by a Nano-ZS90 Malvern instrument. Zeta potential was not performed in the $MgCl_2$ concentration above 10 mM.

### Hydrodynamic diameter measurement for variable $MgCl_2$ concentration (see Figure 1SC below)

DNA-lipid at 1 μM concentration was added to 20 mM Tris buffer (pH 7.4) with variable $MgCl_2$ concentration (0, 25, 50, 100, and 250 mM). The hydrodynamic diameter ($D_h$) of these samples with variable $MgCl_2$ concentrations was then measured with a Nano-ZS90 Malvern instrument.

### Melting curve of DNA-lipid/$DNA_2$ duplex (see Figure 1SD below)

6 μM $DNA_2$ was added with 6 μM DNA-lipid and allowed to hybridize for 30 min before performing the melting curve experiment. This assay was performed in Tris/$MgCl_2$ buffer (20 mM/200 mM, pH 7.4). The sample was heated to 60 °C and cooled gradually, over which the UV-Vis absorbance at $\lambda_{260}$ was measured at different temperature points as shown in the plot shown below.

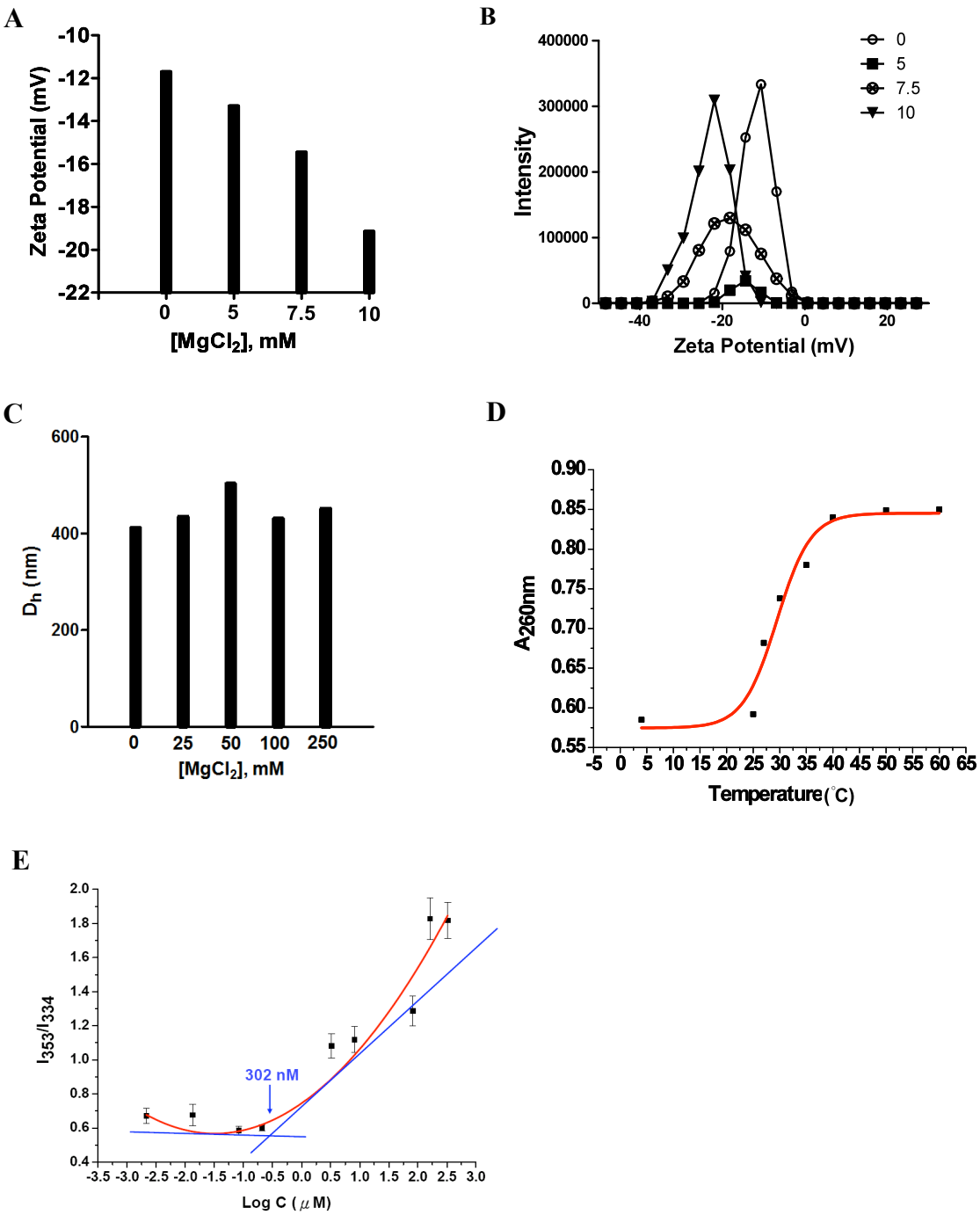



***Figure 1S.*** Stability of the DNA-lipid aggregates under a variety of conditions. (A & B) Zeta potential over various $MgCl_2$ concentrations (0, 5, 7,5, and 10 mM $MgCl_2$). (C) Plot of hydrodynamic diameter ($D_h$) versus $MgCl_2$ concentration. (D) Melting curve for DNA /$DNA_2$ duplex. (E) Plot of the ratio of fluorescent intensity at 353 and 334 nm of pyrene versus the concentration of the DNA-lipid for determination of critical micelle concentration.

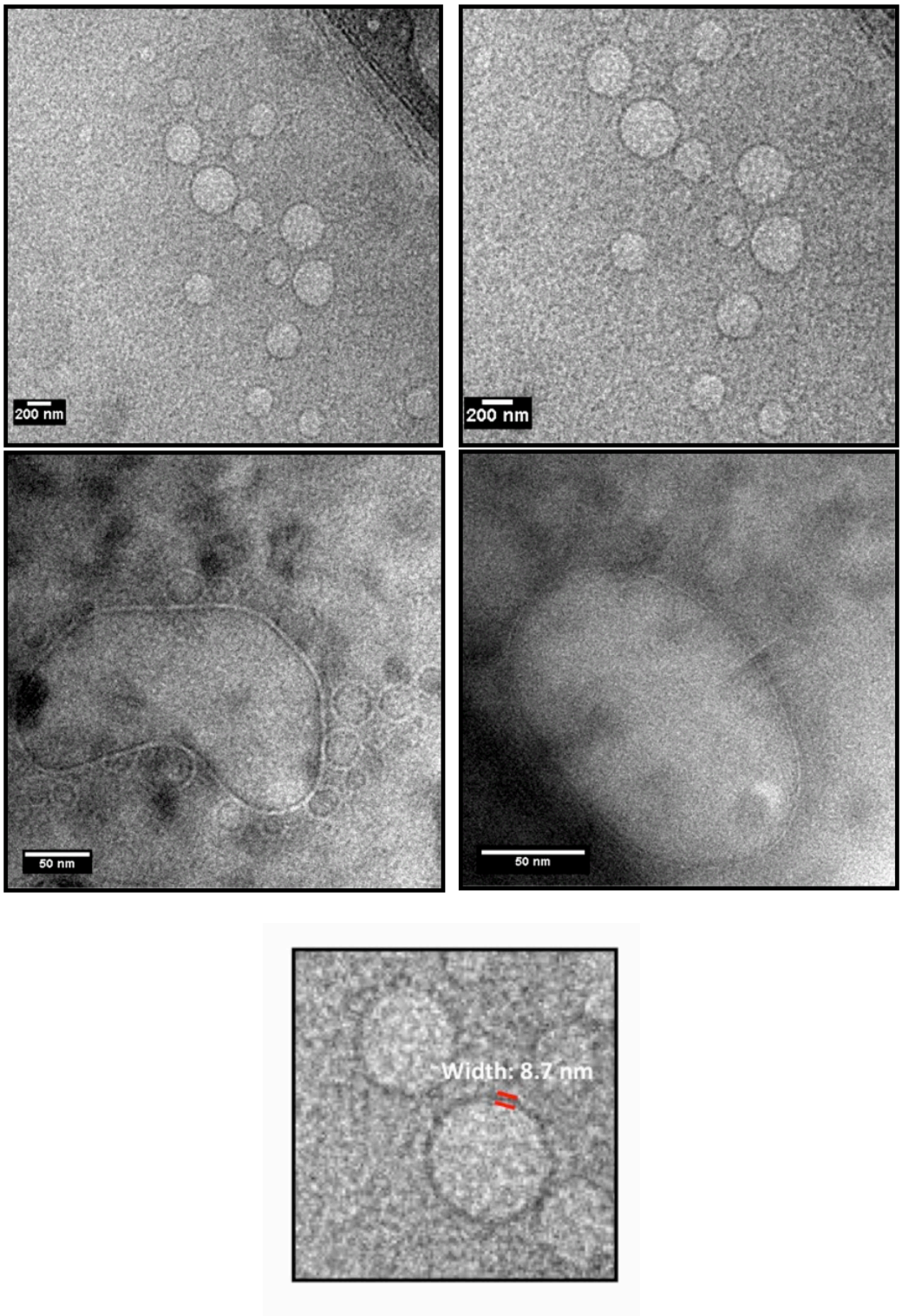
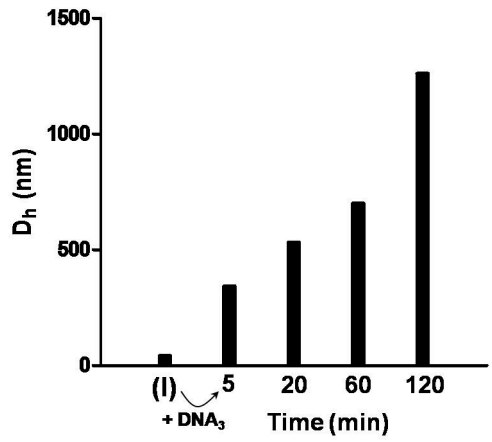
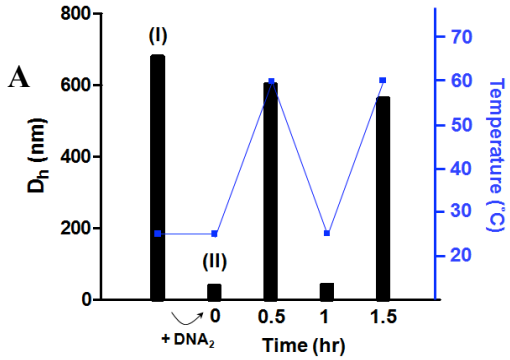



***Figure 2S.*** Various cryo-TEM images of vesicular structures formed from DNA-programmed lipids at different magnifications.

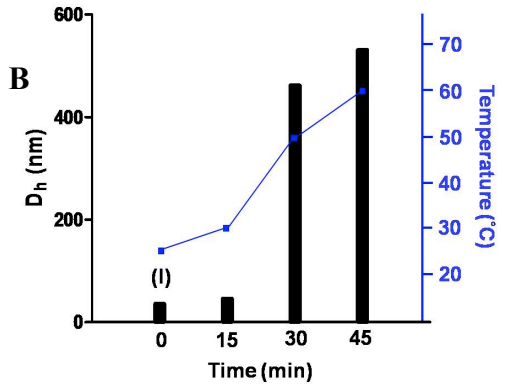



**(I): vesicle + $DNA_2$**

***Figure 3S.*** Plot of hydrodynamic diameter ($D_h$) versus time (min) of aggregates formed after addition of $DNA_3$ to a micelle with $DNA_1$-lipid/$DNA_2$ duplex.




**(I): initial vesicle**
**(II): vesicle + $DNA_2$**




**(I): vesicle + $DNA_2$**

***Figure 4S.*** Hydrodynamic diameter ($D_h$) versus temperature. (A) Size change induced by temperature as a result of DNA duplex formation and melting. (B) Size change resulting from temperature increase.

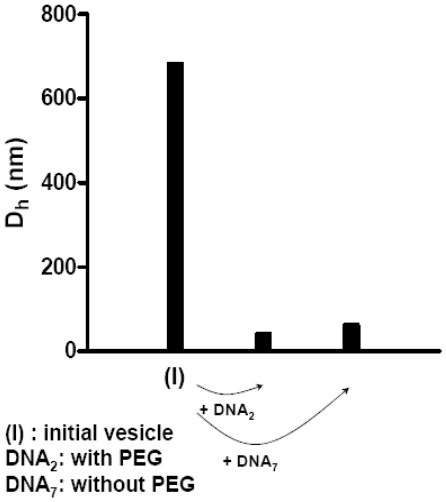



***Figure 5S.*** DLS data of the phase shift induced by DNA sequences with a PEG linker ($DNA_2$) and without a PEG linker ($DNA_7$).

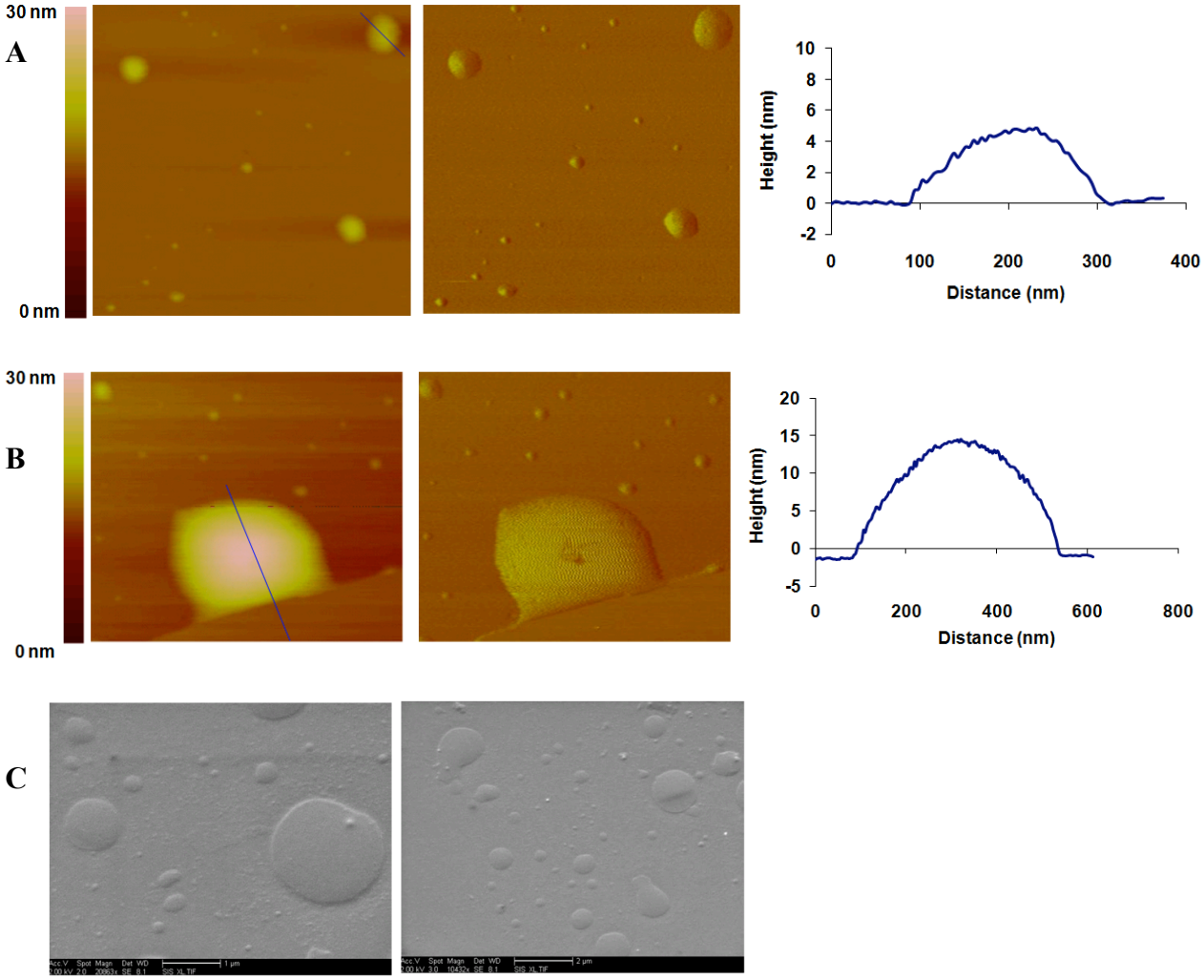



***Figure 6S.*** Characterization of vesicular structures by AFM and SEM. (A & B): AFM image showing vesicular structures. RIGHT: Height profiles from the AFM images with the cross-section shown as a blue line. (C): SEM image of the same material as shown in Main text.

**Switching Cycles**

Figure 7S serves as a supplement to Figure 4G in the main text showing representative TEM data of the switching between vesicle-sphere-vesicle.

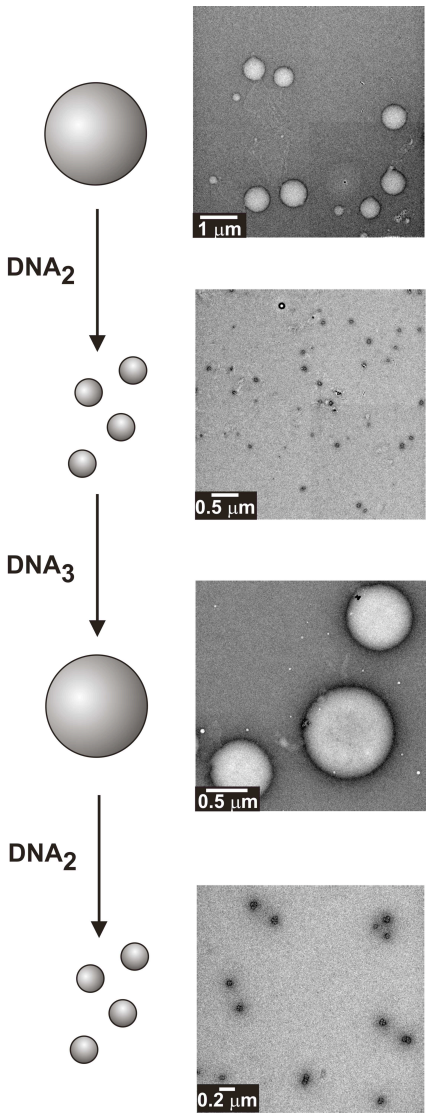



***Figure 7S.*** TEM data to supplement Figure 4G. In addition, data is purposely shown at a variety of magnifications to supplement the figures in the main text.

**Fluorescently labeled surfactant particles**

TEM data are shown here for the green and red particles used in the fluorescence microscopy study shown in Figure 4 of the main text. In addition, the DLS data for vesicles is shown in the far right panel of Figure 8S here. This DLS data shows the same approx. 500 nm sized aggregates observed by TEM and optical microscopy.

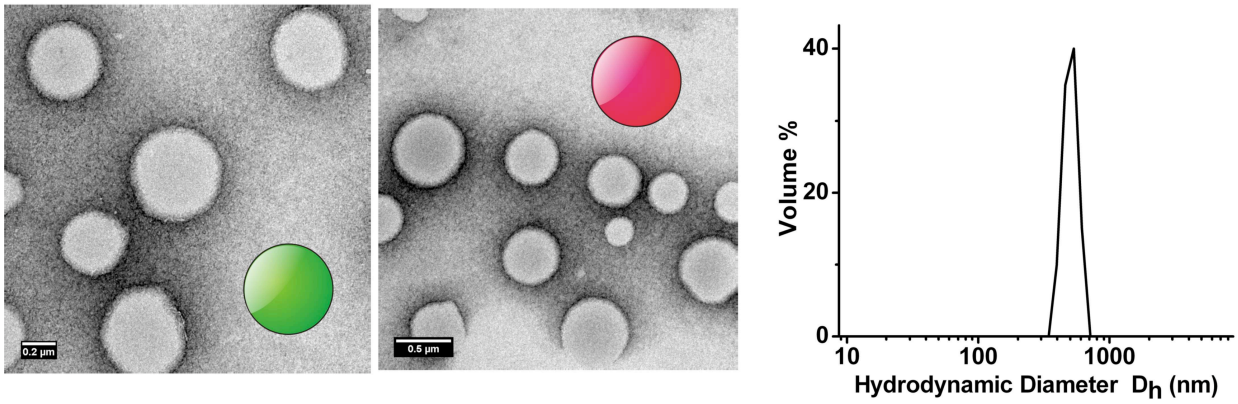



***Figure 8S.*** TEM data to supplement Figure 4. These data were performed on fluorescein labeled DNA-surfactant particles (LEFT PANEL) and rhodamine labeled DNA-surfactant particles (CENTER PANEL). RIGHT PANEL: DLS data for vesicles.

| Experiment | Green Vesicles | Red Vesicles | $DNA_2$ | $DNA_5$ | $DNA_3$ | $DNA_6$ |
|---|---|---|---|---|---|---|
| 1 | + | | | | | |
| 2 | | + | | | | |
| 3 | + | | + | | | |
| 4 | | + | | + | | |
| 5 | + | + | + | | | |
| 6 | + | + | | + | | |
| 7 | + | + | + | + | | |
| 8 | + | | + | | + | |
| 9 | | + | | + | | + |
| 10 | + | + | + | + | + | + |

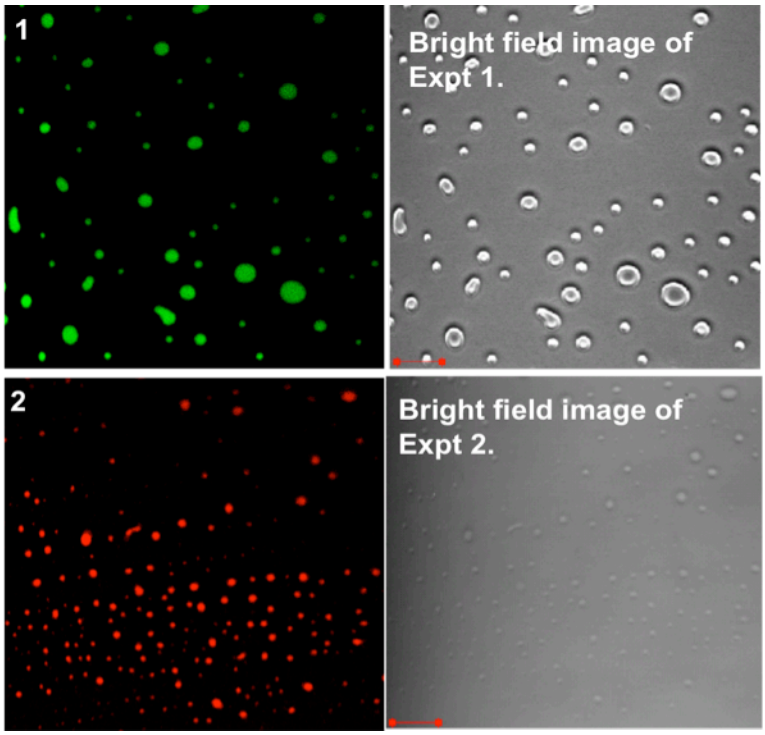
***Table 1S***. This table serves as a key for the fluorescence experiments that follow. All of which are supplements to the data shown in Figure 4 of the main text. Plus signs in the columns indicate what was added to the mixture.

NOTE: ALL DATA THAT FOLLOW CORRESPOND TO THIS TABLE AND INCLUDE DATA SHOWN IN FIGURE 4-MAIN TEXT. RED BOX IN EACH TABLE CORRESPONDS TO THE FLUORESCENCE DATA INDICATED IN THE PANELS TO THE RIGHT OF EACH TABLE. FIGURE 4 OF THE MAIN TEXT SHOULD BE CONSULTED FOR THE CARTOON DESCRIBING THE PARTICLE SIZE CHANGES THAT CORRESPOND TO THE VARIOUS DNA INPUT ADDITIONS.

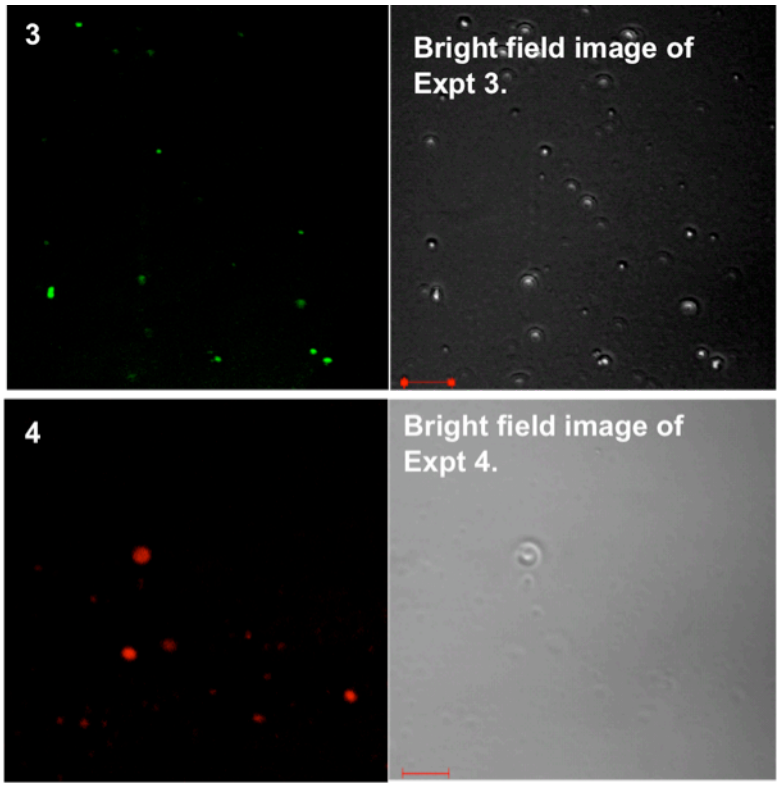
## Supporting Information

NOTE: Where multiple input DNA strands are added to particles, the incubation times are 12 hrs for the initial addition, and 5 hrs for the second prior to measurements. This process allowed the formation of each duplex in turn. e.g. **$DNA_2$** was added to vesicles and allowed to mix for 12 hours, followed by addition of **$DNA_3$** which was allowed 5 hours of mixing prior to fluorescence measurements.

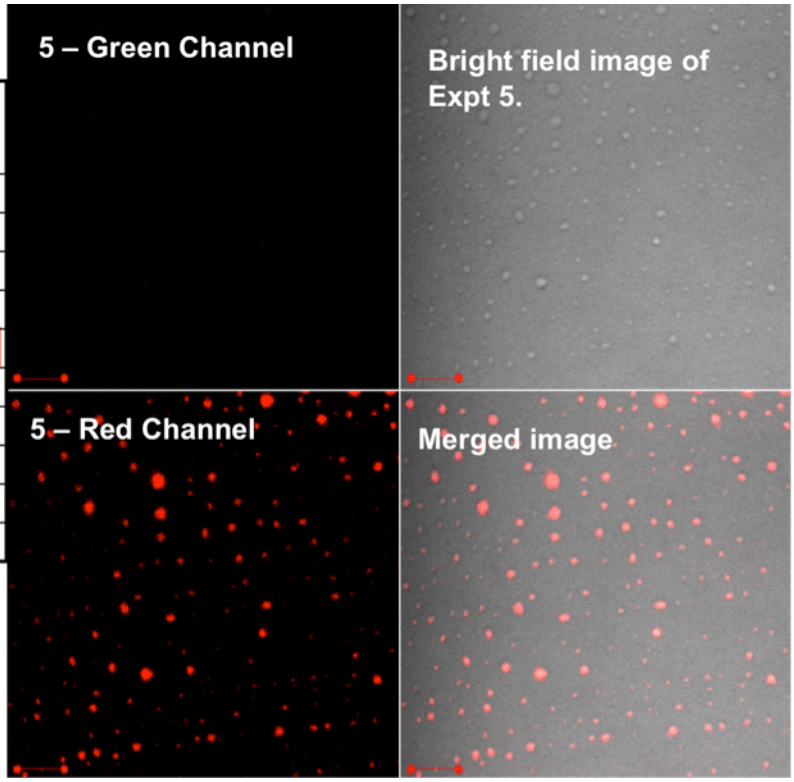
| | G Ves | R Ves | DNA$_2$ | DNA$_5$ | DNA$_3$ | DNA$_6$ |
|---|---|---|---|---|---|---|
| 1 | + | | | | | |
| 2 | | + | | | | |
| 3 | + | | + | | | |
| 4 | | + | | + | | |
| 5 | + | + | + | | | |
| 6 | + | + | | + | | |
| 7 | + | + | + | + | | |
| 8 | + | | + | | + | |
| 9 | | + | | + | | + |
| 10 | + | + | + | + | + | + |



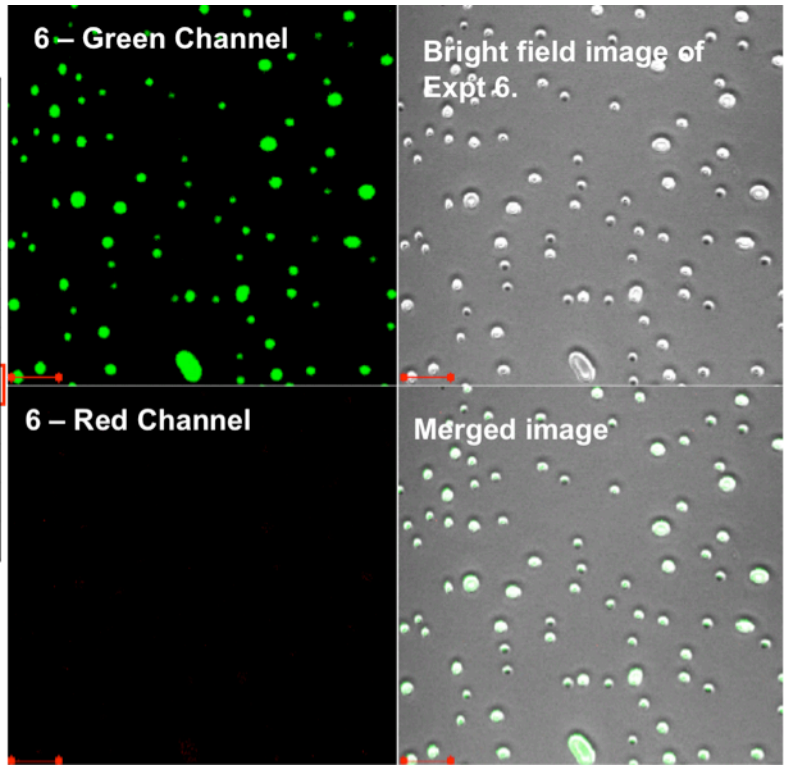

| | G Ves | R Ves | DNA$_2$ | DNA$_5$ | DNA$_3$ | DNA$_6$ |
|---|---|---|---|---|---|---|
| 1 | + | | | | | |
| 2 | | + | | | | |
| 3 | + | | + | | | |
| 4 | | + | | + | | |
| 5 | + | + | + | | | |
| 6 | + | + | | + | | |
| 7 | + | + | + | + | | |
| 8 | + | | + | | + | |
| 9 | | + | | + | | + |
| 10 | + | + | + | + | + | + |



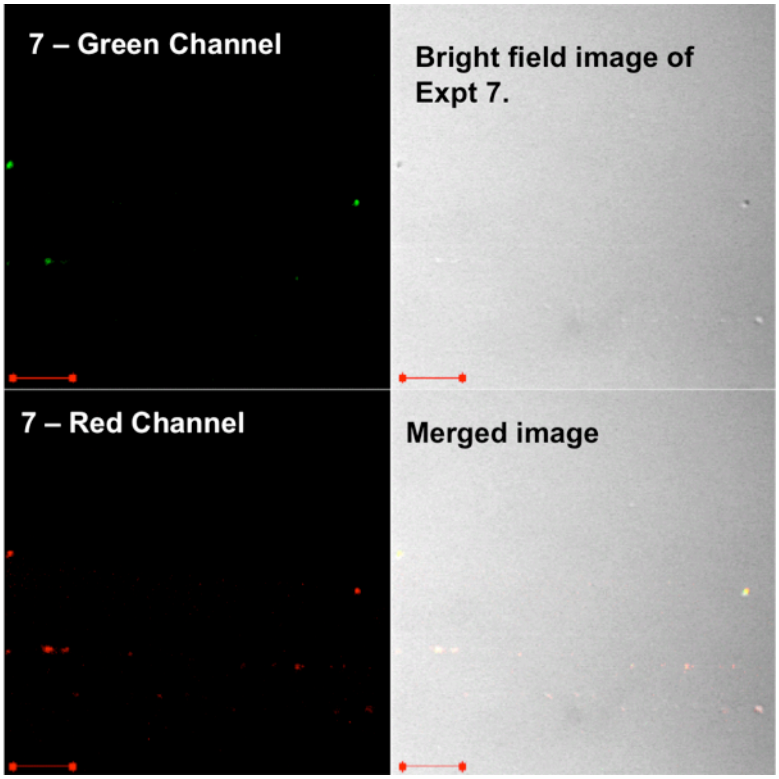
| | G Ves | R Ves | DNA$_2$ | DNA$_5$ | DNA$_3$ | DNA$_6$ |
|---|---|---|---|---|---|---|
| 1 | + | | | | | |
| 2 | | + | | | | |
| 3 | + | | + | | | |
| 4 | | + | | + | | |
| 5 | + | + | + | | | |
| 6 | + | + | | + | | |
| 7 | + | + | + | + | | |
| 8 | + | | + | | + | |
| 9 | | + | | + | | + |
| 10 | + | + | + | + | + | + |



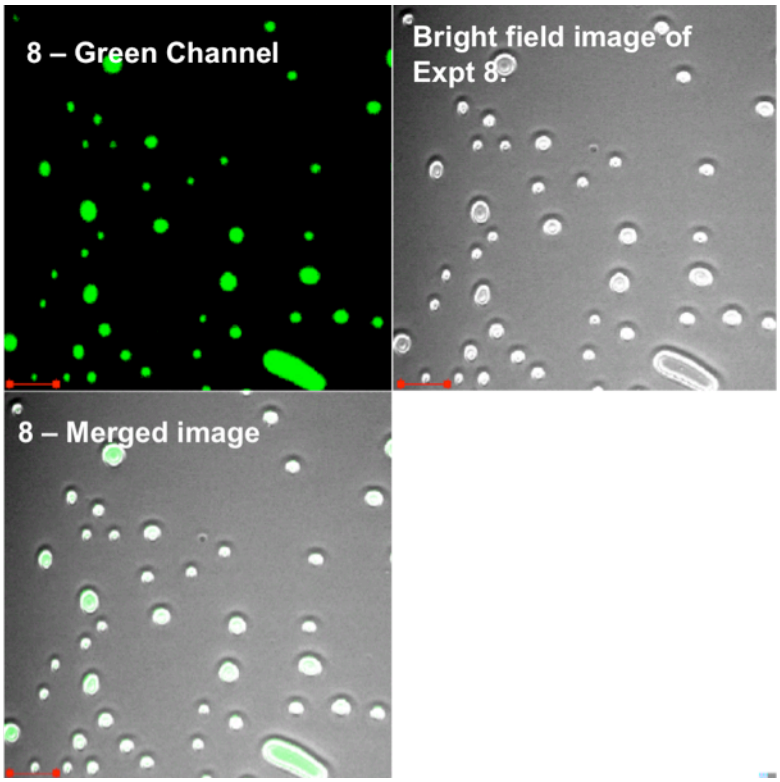

| | G Ves | R Ves | DNA$_2$ | DNA$_5$ | DNA$_3$ | DNA$_6$ |
|---|---|---|---|---|---|---|
| 1 | + | | | | | |
| 2 | | + | | | | |
| 3 | + | | + | | | |
| 4 | | + | | + | | |
| 5 | + | + | + | | | |
| 6 | + | + | | + | | |
| 7 | + | + | + | + | | |
| 8 | + | | + | | + | |
| 9 | | + | | + | | + |
| 10 | + | + | + | + | + | + |



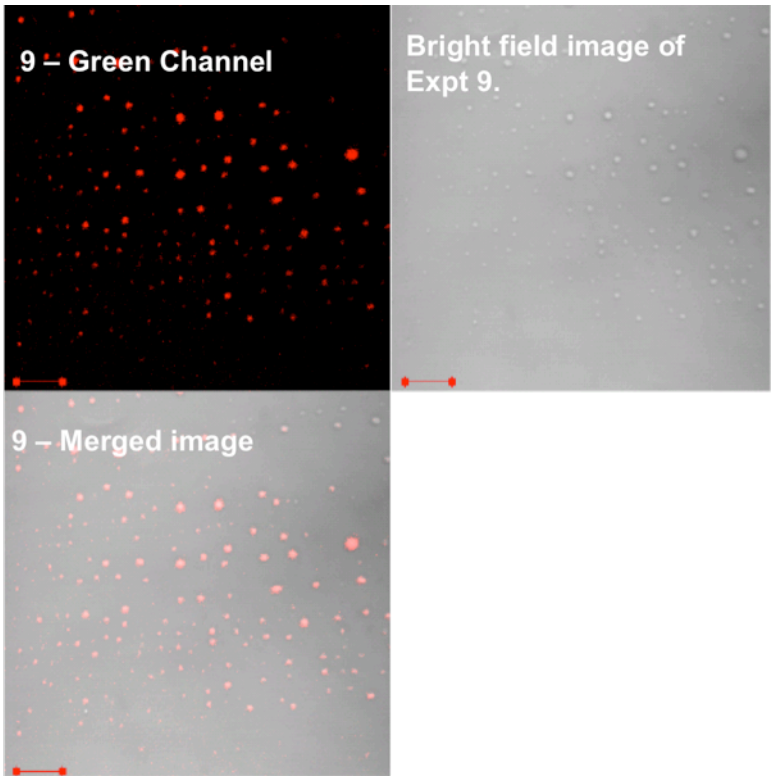
| | GVes | RVes | DNA$_2$ | DNA$_5$ | DNA$_3$ | DNA$_6$ |
|---|---|---|---|---|---|---|
| 1 | + | | | | | |
| 2 | | + | | | | |
| 3 | + | | + | | | |
| 4 | | + | | + | | |
| 5 | + | + | + | | | |
| 6 | + | + | | + | | |
| 7 | + | + | + | + | | |
| 8 | + | | + | | + | |
| 9 | | + | | + | | + |
| 10 | + | + | + | + | + | + |




| | GVes | RVes | DNA$_2$ | DNA$_5$ | DNA$_3$ | DNA$_6$ |
|---|---|---|---|---|---|---|
| 1 | + | | | | | |
| 2 | | + | | | | |
| 3 | + | | + | | | |
| 4 | | + | | + | | |
| 5 | + | + | + | | | |
| 6 | + | + | | + | | |
| 7 | + | + | + | + | | |
| 8 | + | | + | | + | |
| 9 | | + | | + | | + |
| 10 | + | + | + | + | + | + |



| | GVes | RVes | DNA$_2$ | DNA$_5$ | DNA$_3$ | DNA$_6$ |
|---|---|---|---|---|---|---|
| 1 | + | | | | | |
| 2 | | + | | | | |
| 3 | + | | + | | | |
| 4 | | + | | + | | |
| 5 | + | + | + | | | |
| 6 | + | + | | + | | |
| 7 | + | + | + | + | | |
| 8 | + | | + | | + | |
| 9 | | + | | + | | + |
| 10 | + | + | + | + | + | + |




| | GVes | RVes | DNA$_2$ | DNA$_5$ | DNA$_3$ | DNA$_6$ |
|---|---|---|---|---|---|---|
| 1 | + | | | | | |
| 2 | | + | | | | |
| 3 | + | | + | | | |
| 4 | | + | | + | | |
| 5 | + | + | + | | | |
| 6 | + | + | | + | | |
| 7 | + | + | + | + | | |
| 8 | + | | + | | + | |
| 9 | | + | | + | | + |
| 10 | + | + | + | + | + | + |