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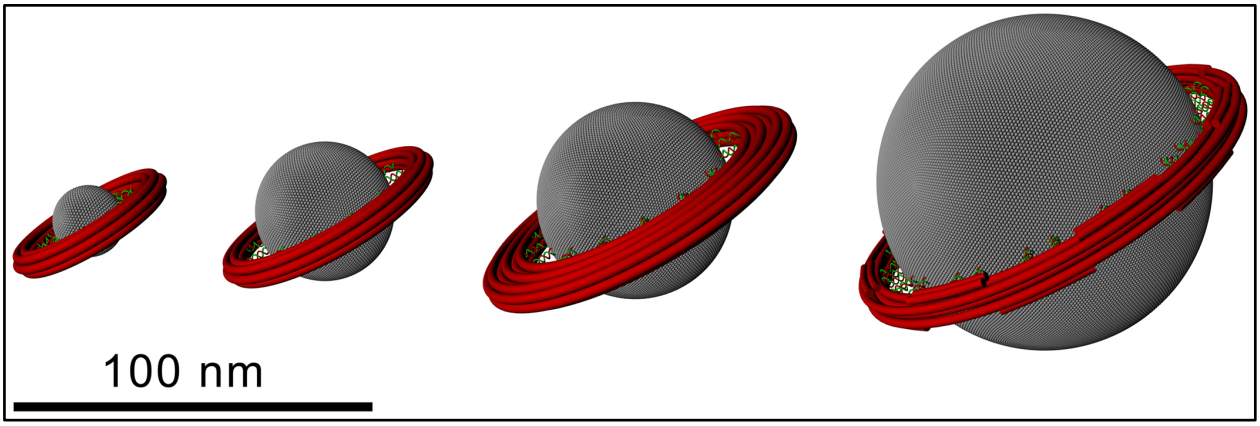
SUPPLEMENTARY INFORMATION

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Self-assembly of size-controlled liposomes on DNA nanotemplates

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One Sentence Summary: Through DNA-nanostructure guided membrane self-assembly, we produced highly monodispersed sub-100 nm vesicles with predefined sizes.



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Materials and Methods

Preparation of lipidated DNA anti-handles

The following DNA strands were synthesized by Integrated DNA Technologies Inc. and further purified *via* denaturing polyacrylamide gel electrophoresis. Strands were dissolved with DI water to reach 1 mM concentration.

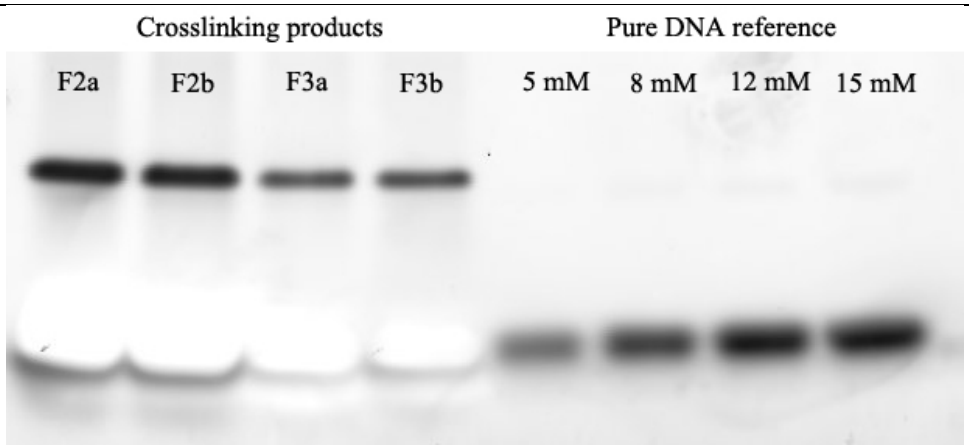
5'-**/5ThioMC6-D/**GTGAGTTGTGGTAGATAATTT-3'
5'-GTGAGTTGTGGTAGATAATTT**/3ThioMC3-D/-3'**

The disulfide bond of the modified DNA was reduced by *tris*(2-carboxyethyl)phosphine (TCEP) in a 50 μL solution containing 400 μM DNA, 25 mM HEPES, 140 mM KCl, 50mM TCEP, pH 7.4. The mixture was incubated for 30 minutes at room temperature.

All the lipids were purchased from Avanti Polar Lipids, Inc. and stored in chloroform. To synthesize lipidated DNA, 0.4 μmole of maleimide lipid (18:1 MPB PE, 20× excess to SH-DNA) was transferred into a glass test tube and blown dry by N_2 gas. The lipid was further dried under vacuum for overnight. A solution containing 50 μL of freshly reduced SH-DNA and 5 μL of 20% Octyl β-D-glucopyranoside (OG) was added to the dried maleimide lipid. This dissolved the lipid film at the bottom of the test tube and initiated the maleimide-thiol crosslinking reaction. After 45 minutes, 45 μL of 1× reaction buffer (25 mM HEPES, 140 mM KCl, pH 7.4) was added to reach 100 μL of total volume. This mixture was transferred into a second glass tube containing 0.6 μmole of pre-dried lipid (99.2% DOPC and 0.8% 18:1 Liss Rhod PE), agitated and further diluted by 200 μL of 1× reaction buffer with 1% OG. The final mixture was injected into a dialysis cassette (7kD molecular weight cut-off, 0.1–0.5 ml capacity, Thermo Scientific) and dialyzed overnight against 2 L of 1× reaction buffer at room temperature.

An isopycnic centrifugation method was used to purify the crosslinking product, which separated the lipidated DNA (incorporated into liposomes and floated up to the top of the gradient) from unconjugated DNA (stayed at the bottom). A three layer gradient was made in a centrifuge tube (5×41 mm, Beckman Coulter Inc.). The bottom layer was made by mixing 150 μL of the recovered solution from dialysis cassette with 150 μL of OptiPrep media (60% iodixanol, Cosmo Bio USA). The middle layer and top layer contained 250 μL of 20% iodixanol in 1× reaction buffer and 50 μL of 1× reaction buffer, respectively. The tube was spun in a Beckman SW-55-Ti rotor at 50 krpm and 4℃ for 5 hours. The contents of the tube were fractionated from top to bottom (52 μL per fraction). The second and third fractions from top (F2 and F3), as well as unconjugated SH-DNA at various concentrations (as references) were loaded onto a 12% polyacrylamide gel, which was run in 1× MES-SDS running buffer (Life Technologies) at 20V/cm for 30 min and stained by SYBR-Gold (Life Technologies). The lipidated DNA concentration was estimated by linear regression. A typical gel image is shown below in Box 1.

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Box 1: A gel image showing the lipidated DNA purified by gradient centrifugation (F2 and F3, each loaded into two lanes) and unconjugated SH-DNA references with known concentrations.

DNA-origami ring design and preparation

The DNA rings were designed by caDNAno (Figure S1). Key design and experiment parameters are summarized in Table S1. All staple strands were purchased from Integrated DNA Technologies Inc. in the format of 96-well plates with their concentrations normalized to 100 μM.

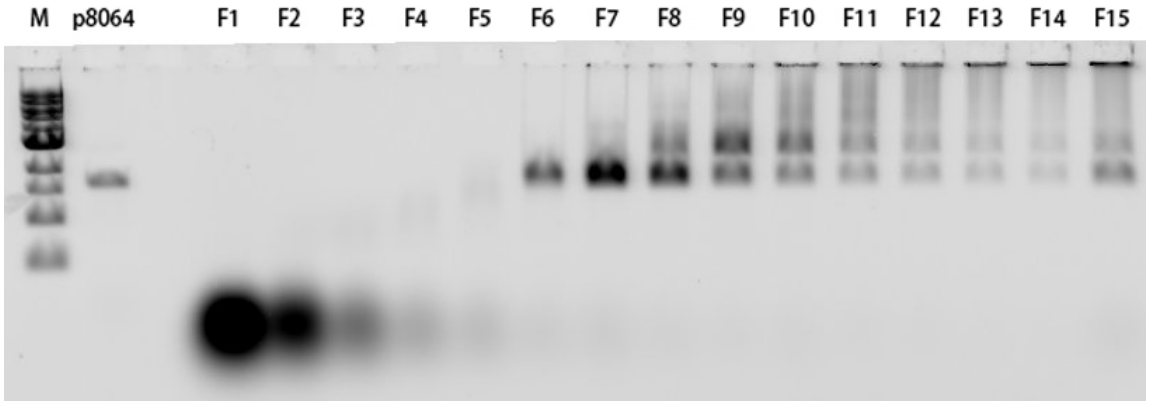
Table S1: Design and experiment parameters for different DNA-origami rings

Ring type	Scaffold strand	Max number of inner and outer handles	Number of helices	Mg^{2+} required for assembly	Centrifugation time and speed
29-nm ring	p7308	16 (inner), 8 (outer)	6	10 mM	90 min @ 50 krpm
46-nm ring	p3024	12 (inner), 12 (outer)	6	10 mM	180 min @ 50 krpm
60-nm ring	p8064	16 (inner), 16 (outer)	12	12 mM	60 min @ 50 krpm
94-nm ring	p7560	31 (inner), 7 (outer)	10	10 mM	40 min @ 50 krpm

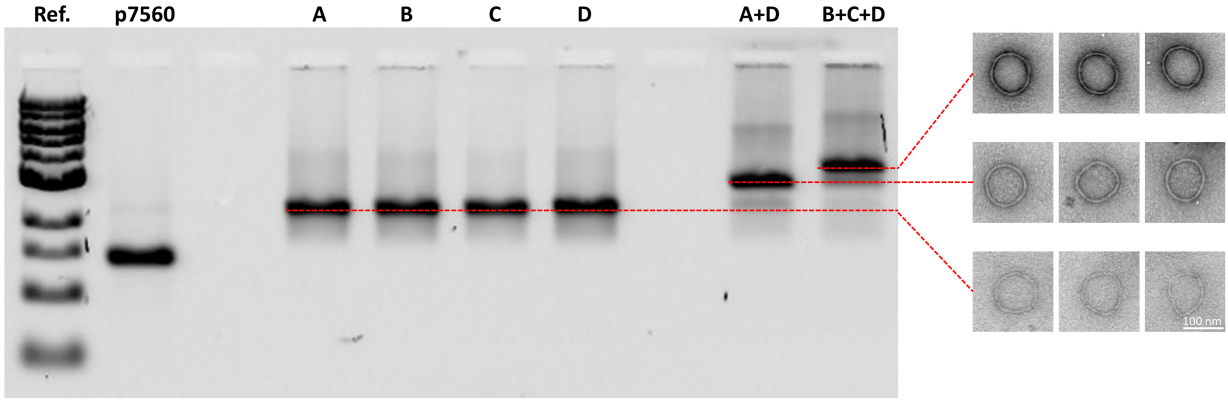
All the rings were assembled from a scaffold strand (50 nM) and a pool of staple strands (300 nM each) in 1× TE buffer (5mM Tris• HCl, 1 mM EDTA, pH 8.0) with 10 or 12 mM of $MgCl_2$, using a 36-hour annealing program (80℃–65℃, -1℃/5 min; 64℃–24℃, -1℃/50 min; 15℃ hold). Typically, 400–800μl of assembly product were concentrated to 200 μl using Amicon Ultra-0.5 mL Centrifugal Filters (EMD Millipore) with 30-kD nominal molecular weight limit (NMWL), loaded on top of a 15%–45% (v/v) quasi-linear glycerol gradient in a polycarbonate centrifuge tube (13×51 mm, Beckman Coulter Inc.), and spun at 50krpm for 40–180 minutes (Table S1) on a Beckman SW-55-Ti rotor. The contents of the tube were fractionated from top to bottom (200 μL per fraction, labeled in Box 2 as F1–F15). 4 μL of each fraction was loaded onto a 1.5% agarose gel containing 0.05% ethidium bromide and run in 0.5× TBE, 10 mM $MgCl_2$ for 1.5 hours at 5 V/cm. The fractions containing well-formed DNA rings were combined and concentrated using Amicon Ultra-0.5 mL Centrifugal Filters with 30-kD NMWL. Ring concentration was determined using a NanoVue Plus Spectrometer (GE Healthcare) and adjusted to 50 nM. The concentrated rings were stored in 1× TE-Mg^{2+} buffer at -20℃.

To assemble the 94-nm dimer or trimer ring (see design details in Figure S1), purified monomers were mixed at equal molar ratio in 1× TE buffer with 20 mM $MgCl_2$ and annealed from 42℃–25℃ over 17 hours. Well-formed dimers and trimers were purified by rate-zonal centrifugation (Box 3). The concentrated rings were stored in 1× TE buffer with 20 mM $MgCl_2$ at -20℃.

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Box 2: A gel image showing 60-nm DNA ring assembly products separated by rate-zonal centrifugation. Well folded DNA rings are enriched in Fractions 6–8 (smaller numbers are assigned to lighter fractions).



Box 3: A gel image showing purified 94-nm monomer, dimer and trimer rings. Lane A–D: monomers, Lane "A+D": dimer, Lane "B+C+D": trimer. Representative electron micrographs are shown for the major bands.

Preparation of DNA-ring templated liposomes

Lipidated anti-inner-handle and Cy5 modified anti-outer-handle were added to purified DNA-origami rings displaying inner and outer handles at 3:1 and 1.5:1 anti-handle:handle ratio, respectively. The worksheet for preparing a 40 µL hybridization mixture containing 10 nM DNA ring is given as Table S2. The mixture was incubated at 37℃ for 1 hour. Optionally, excessive anti-handles were removed *via* rate-zonal centrifugation as described above.

Table S2: Worksheet for anti-handle hybridization.

	Ring with ***a***× inner and ***b***× outer handles	Lipidated anti-handle	Cy5 modified anti-handle	OG	HEPES	KCl	$MgCl_2$
Final concentration	10 nM	3 × ***a*** × 10 nM	1.5 × ***b*** × 10 nM	1%	25 mM	0.4 M	10 mM

To form DNA-ring templated liposomes, 15 µL of 1× hydration buffer (25 mM HEPES, 400 mM KCl, 10 mM $MgCl_2$, pH 7.5) and 5 µL of 15 mM rehydrated lipid (lipid compositions shown in Table 1) were added to 40 µL of 10 nM lipid- & Cy5- labeled DNA ring to reach 60 µL total volume. The solution was

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gently shaken for 30 min at room temperature, diluted with 60 µL of 1× hydration buffer containing 0.67% OG, and dialyzed against 2 L of 1× hydration buffer for 16 hours. 100 µL of recovered solution was mixed with 200 µL of 45% iodixanol in 1× hydration buffer and placed at the bottom of a centrifuge tube (5×41 mm, Beckman Coulter Inc.). Six additional layers of iodixanol solution in 1× hydration buffer were added to the centrifuge tube (Table S3). The tube was spun in a SW-55-Ti rotor at 50 krpm and 4℃ for 5 hours, after which the contents were fractionated (52 µL per fraction). For a larger scale preparation, a similar centrifugation protocol was used but with 13×51 mm tubes and 200 µL fractions. Optionally, desired fractions were subject to another centrifuge cycle to improve the sample purity.

Table S3: Iodixanol (IDX) gradient recipe for purifying liposomes templated by different types of rings.

Layer	Volume (µL)	29-nm ring	46-nm & 60-nm ring	94-nm ring (dimer)
1	60	0% IDX	6% IDX	2% IDX
2	60	8% IDX	10% IDX	6% IDX
3	60	10% IDX	14% IDX	10% IDX
4	60	12% IDX	18% IDX	14% IDX
5	60	14% IDX	22% IDX	18% IDX
6	60	18% IDX	26% IDX	22% IDX
7	300	30% IDX + liposomes	30% IDX + liposomes	30% IDX + liposomes

To determine the contents of the fractions, 4 µL of each fraction was loaded onto a 1.5% agarose gel that contained 0.5×TBE, 10 mM $MgCl_2$ and 0.05% SDS. The gel was run at 5V/cm for 1.5 hours in the same buffer and imaged on a Typhoon FLA 9500 multi-fluorescent gel scanner (GE Healthcare). Optionally, DNase I (Thermo Scientific) was used per manufacturer's recommendation to remove DNA rings. The fractions were stored at 4℃.

Electron Microscopy

For negative staining transmission electron microscopy (TEM), a drop of sample (~5 µL) was deposited on a glow discharged formvar/carbon coated copper grid (Electron Microscopy Sciences), incubated for 1–3 minute and blotted away. The grid was then washed briefly and stained for 1 minute with 2% (w/v) uranyl formate. Images were acquired on a JEOL JEM-1400 Plus microscope (acceleration voltage: 80 keV) with a bottom-mount 4k×3k CCD camera (Advanced Microscopy Technologies).

For cryoEM observation, templated vesicles were assembled and purified in a buffer containing 25 mM HEPES, 100 mM KCl, 10 mM $MgCl_2$, pH 7.5. Amicon Ultra-0.5 mL Centrifugal Filters (30-kD NMWL) were used to concentrate the purified vesicles to ~50 nM and remove iodixanol from the sample. Commercially available holey carbon grid, Quantifoil R2/2 200 Cu (Quantifoil MicroTools GmbH) with a thin layer of carbon over the holes was glow-discharged to spread out the sample solution on the grid. After waiting for 30 seconds to let 4 µL of sample to adsorb to thin layer of carbon, the grid surface was washed with a buffer with 10% reduced salt concentration to swell vesicles to maintain its spherical shape, then the residual solution was blotted for 5 sec at -1.5 mm offset in 100% humidity at 8°C and flash frozen into liquid ethane using an FEI Vitrobot (FEI). Grids were examined using an FEI Tecnai-F20 electron microscope equipped with Field Emission Gun and operated at 200 keV acceleration voltage, using a nominal magnification of 80,000. We recorded images on a 4k×4k CCD camera (Gatan) at a pixel size of 2.34 Å with hardware binning using low-dose mode with an exposure dose of 20–30 $e^{-}Å^{-2}$. The images were taken with two defocuses, -2.5 and -10 µm and merged into one by image processing (*29*).

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Time-course study of liposome formation

The remaining OG concentration at various time points ($C_{OG,\tau}$) during dialysis was determined as follows. 240 μL of lipid-labeled 60-nm ring containing 1% OG (non-radioactive) was mixed with 60 μL of extruded liposome (lipid concentration ≈ 15 mM), 60 μL of 1× hydration buffer, and 1.8 μL of ^{14}C-labeled OG (~0.01%, American Radiolabeled Chemicals, Inc.) for a total volume of 361.8 μL. After being agitated for 1 hour, a 10 μL aliquot of this mixture was diluted 5-fold with 1× hydration buffer, and mixed with 18 mL of scintillation cocktail (Opti-Fluor, PerkinElmer). The radioactivity of the resulting mixture was measured on a Beckmann-Coulter LS6500 multi-purpose scintillation counter *via* beta-counting (R_0). The remainder of the mixture was then diluted with 411.4 μL of 1× hydration buffer with 0.67% OG and split into six cassettes (volume = V_0 each) for dialysis against 1× hydration buffer. After 0.5, 1, 3, 3.5, 5 or 16 hours, 50 μL of the dialyzed solution (total volume V_τ) was mixed with 18 mL of the scintillation cocktail and the radioactivity was measured (R_τ). The remaining OG concentration was calculated using Equation S1.

$$C_{OG,\tau} = \frac{R_\tau \times \frac{361.8\ \mu L - 10\ \mu L + 411.4\ \mu L}{50\ \mu L} \times \frac{V_\tau}{V_0}}{R_0 \times \frac{361.8\ \mu L - 10\ \mu L}{10\ \mu L}} \times 0.67\% \quad \cdots (Equation\ S1)$$

Such an experiment was repeated four times and the data were fitted into a single-exponential curve as shown in Figure 4A. The OG concentration at any time point during dialysis can then be calculated based on this fitting curve (examples are shown in Table S4).

Table S4: Calculated OG concentrations (w/v) during dialysis.

Dialysis time	0 hour	0.5 hour	1 hour	1.5 hours	16 hours
OG concentration	0.67%	0.25%	0.093%	0.037%	0.00%

In order to capture intermediates during detergent removal, 10 μL of 1× hydration buffer and 10 μL of extruded liposomes (lipid concentration ≈ 15 mM; lipid composition 0, Table 1) were added into 40 μL of lipid- & Cy5- labeled DNA rings (C_{OG} = 0.67%). The solution was gently shaken for 30 min at room temperature, diluted with 60 μL of 1× hydration buffer containing 0.67% OG, and dialyzed against 2 L of 1× hydration buffer for 0, 0.5, 1, 1.5 or 16 hours. Such dialyzed solutions were fractionated after isopycnic centrifugation and characterized by SDS-agarose gel electrophoresis and TEM. It is important to note that specific amounts of OG (Table S5) were included in the density-gradient media and negative-stain solutions (2% uranyl formate) to reach the same detergent concentration as the samples recovered from dialysis cassettes. Gel and TEM images were analyzed manually using ImageJ software.

Two different methods were used to calculate the percentage of different membrane structures in a dialyzed sample. The first method was used for samples after 0-, 0.5- and 16-hour dialysis, where each fraction was imaged by TEM separately (Figure S12-1 – S12-4). Membrane structures found in the electron micrographs were sorted into seven different categories. The percentage of a specific type of membrane structure in a fraction was noted as $P_{type\text{-}Fx}$. The amount of DNA rings in each fraction was estimated by the corresponding band intensity in the gel image (I_{Fx}). An example is given in Table S5 and S6 (60-nm ring with 4 lipid seeds, dialysis time = 16 hours). The percentage of each membrane structure in the dialyzed sample (TP_{type}) was calculated using Equation S2 and plotted in Figure 4b.

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Table S5: Distribution of DNA rings in a density gradient after centrifugation (60-nm ring with 4 lipid seeds, dialysis time = 16 hours)

Fraction	F2	F3	F4	F5	F6	F7	Total
I_{Fx}	17.7%	20.2%	17.9%	13.9%	17.4%	12.9%	100.0%

Table S6: Population of different types of membrane structures found in the TEM images ($P_{type\text{-}Fx}$) for 60-nm ring with 4 lipid seeds after 16-hour dialysis

	Empty	With worm-like micelles	Wrapped by lipid bilayers	Encompass a donut vesicle	With multi-vesicles	Filled by one liposome	Other
F2	20.9%	2.3%	0.0%	0.0%	31.1%	21.5%	24.3%
F3	10.7%	5.4%	0.0%	0.0%	48.2%	26.2%	9.5%
F4	2.6%	1.6%	0.0%	0.3%	54.8%	27.9%	12.8%
F5	1.1%	0.4%	0.0%	0.0%	93.2%	4.9%	0.4%
F6	19.6%	1.8%	0.0%	0.0%	73.6%	4.3%	0.6%
F7	18.7%	0.8%	0.0%	0.0%	80.5%	0.0%	0.0%

$$TP_{type} = \sum_{x=2}^{7} \left(I_{Fx} \times P_{type-Fx} \right) \cdots (Equation\ S2)$$

The second method was used for samples after 1- and 1.5-hour dialysis. After isopycnic centrifugation, 5 μL of each fraction (F2–F7) were mixed thoroughly and imaged by TEM. Different types of ring-associating membrane structures were counted directly from the resulting micrographs (Figure S12-5). The normalized population of each type was calculated and plotted in Figure 4b.

It is important to note that in both methods, category "Other" includes rings with overfilled vesicles, large DNA-membrane aggregates and all ambiguous structures. Together they represented a relatively small population (~15%) and were not included in the bar graphs shown in Figure 4.

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Technical notes

Size controllability *versus* reported methods

In table S7, we compare the size distribution of liposomes generated by DNA templated membrane self-assembly with those resulted from a selection of established methods. In table S7, we compare the size distribution of liposomes generated by DNA templated membrane self-assembly with those resulted from a selection of established methods. Note that although the sonication[1] and the lipid composition tuning[7] methods have provided relative standard deviation (RSD) similar to this work, their products are limited to liposomes of either small sizes or a particular lipid composition.

Table S7: Liposome mono-dispersity comparison

Year	Ref #	Preparation Method	Purification Method	Mean size (nm)	RSD (std/mean)	Measurement
1969	1	Sonication	Gel filtration	25	8%	Electron Microscopy (EM)
1973	2	Solvent injection	Gel filtration	89	51%	EM
1979	3	Sequentially extrusion	None	260	39%	EM
1985	4	Extrusion	None	70–94	21%–46%	EM
2007	5	Microfluidic directed formation	None	50–120	20%–42%	Light scattering
2009	6	Detergent removal	Size exclusion chromatography	47–103	13%–36.3%	EM and Dynamic Light Scattering
2009	7	pH jump and tuning lipid composition	Differential centrifugation	22–57	4%–8%	Photon correlation spectroscopy
2015	This work	DNA-origami templated assembly	Isopycnic ultracentrifugation	29–94	7.8%–14.3%	EM

Material cost

To make a typical batch of DNA-ring templated liposome (40 µl × 10 nM = 0.4 pmol), the material cost is the following (in US$). Calculations are based on the listed price provide by the vendors: Integrated DNA Technologies, Inc. and Avanti polar lipids, Inc.

Label-free oligonucleotides:	$0.1 per base, 4 nmol final product per oligo
Cy5- anti-outer handle:	$150 per strand, 250 nmol final product per oligo
SH-anti-inner handle:	$113 per strand, 250 nmol final product per oligo
M13-based DNA scaffold:	Negligible (homemade)
Maleimid-PE (18:1 MPB PE):	$440 for 25 mg (~25 µmol).
DOPC:	$48 for 200 mg (254 µmol)
DPPC:	$53 for 200 mg (272 µmol)
DOPS:	$84 for 25 mg (31 µmol)
18:1 PEG-2k-PE:	$215 for 200 mg (71 µmol)
18:1 Liss Rhod PE:	$150 for 1 mg (0.77 µmol)
Cholesterol:	$30 for 100 mg (257 µmol)

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Label-free oligonucleotides (staples):

(Number of bases)× (synthesis cost per base) × (DNA rings in pmol) × (times in access for assembly) / (assembly recovery yield) / (synthesized amount) =
(8064+21×16+21×8)×0.1×0.4×6/20%/4000≈ $2.6

Cy5-anti-outer handle:

(Label cost per oligo) × (DNA rings in pmol) × (outer-handle number) × (times in access for assembly) / (synthesized amount) = 150×0.4×8×1.5/250000 ≈ $0.0028 (negligible)

To synthesis lipid/anti-inner handle conjugate:

Part I: Maleimide lipid
(Cost of lipid) × (times in access for cross-link reaction) × (DNA rings in pmol) × (inner-handle number) / (cross-link reaction yield)/ (purchased amount) =
440× 20× 0.4× 16/0.05/25000000≈ $0.045

Part II: SH-DNA oligo
(Label cost per oligo) × (DNA rings in pmol) × (inner-handle number) / (cross-link reaction yield) / (purchased amount) =
113×0.4×16/0.05/250000 ≈ $0.058
Cost of lipid-anti-inner handle = Part I + Part II ≈ $0.1

Other lipid:

$\sum_{i=1}^{4}$(Volume of lipid × lipid concentration × molar percentage(i) × lipid price (i)) =
(5 μL×0.015 M)×((79.2%×$48/254 μmol)+(15%×$84/31 μmol)+(5%×$215/71 μmol)+
(0.8%×$150/0.77 μmol))
≈ $0.17

The total material cost is ~$2.9 for the preparation of liposomes containing ~90 μM lipid (see **Scalability**).

Scalability

In this work, a typical analytical scale preparation of DNA templated liposome yields ~0.23 pmol liposomes (40 μl×10 nM×58% ≈ 0.23 pmol). In a volume of 50 μl, the concentration of templated liposome is thus 4.6 nM and the total lipid concentration is ~90 μM, assuming a homogenous population of 50-nm vesicles (~19000 lipid molecules per vesicle). In a large scale (20×) batch of preparation for cryo-EM study, 1 mL of products in similar concentration was produced and further concentrated to 100 μl. The final liposome and lipid concentration of this sample are ~47 nM and ~0.9 mM, respectively. The cost of this large batch is estimated to be $58 based on the cost calculation above. Note that this production scale can meet the needs for many structural biology and biochemistry studies, where the samples are ultimately examined by cryo-EM, fluorescence microscope or fluorescence spectrometer.

In order to produce vesicles for applications (e.g. drug delivery) that demands milliliters of ~10 mM lipid, a simple scale-up of our current protocol becomes expensive both in material and labor. However the cost could be driven down by (1) purchasing a larger synthesis-scale DNA and lipid at substantially lower per micromole price (e.g. synthesizing DNA at 1 μmole scale costs >80% less per mole per base than 25 nmole scale) and (2) employing automated liquid handling techniques such as a gradient making and fractionating station.

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Figures

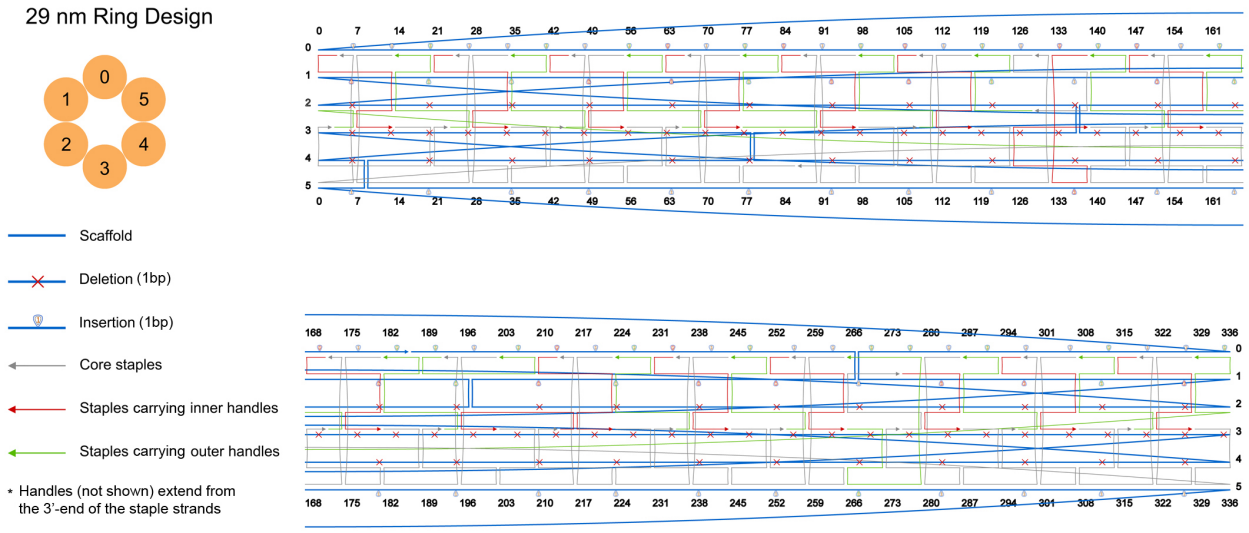


Figure S1-1: Strand diagram illustrating the design of the 29-nm ring.

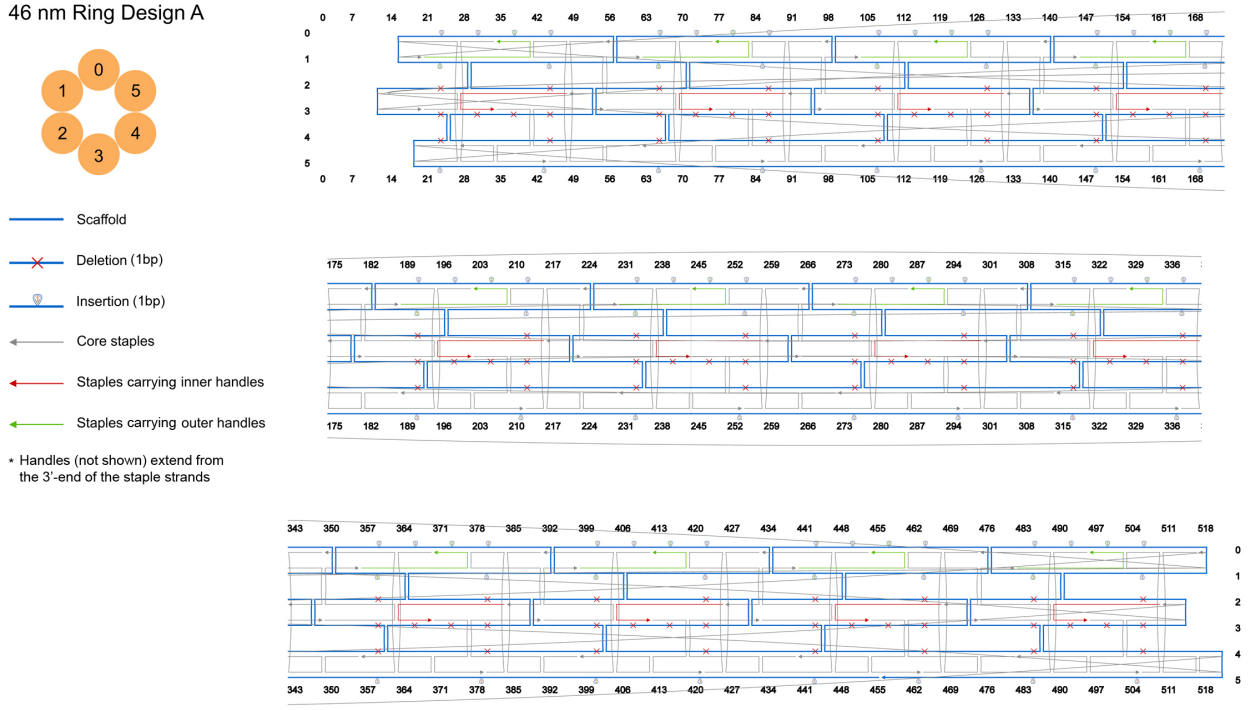


Figure S1-2: Strand diagram illustrating the design of the 46-nm ring.

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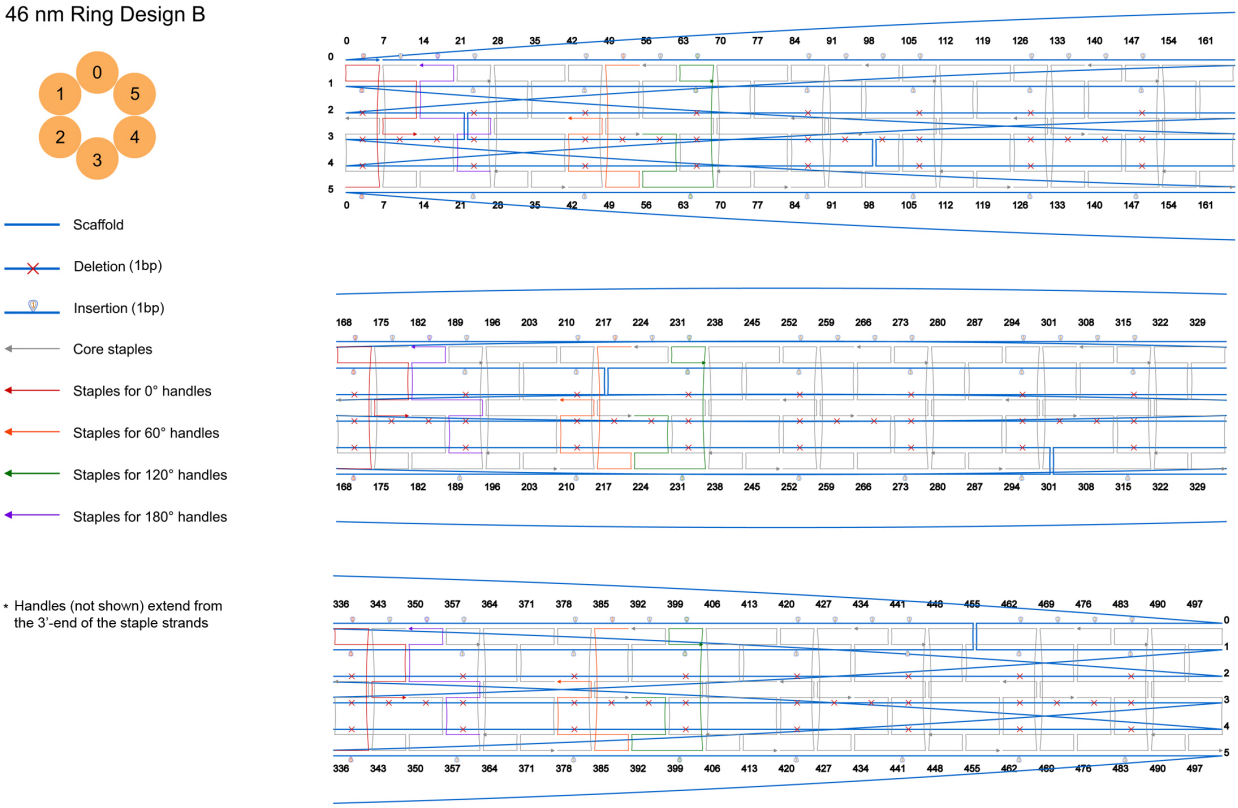


Figure S1-3: An alternative design of the 46-nm ring to position lipid seeds toward different directions.

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Figure S1-4: Strand diagram illustrating the design of the 60-nm ring.

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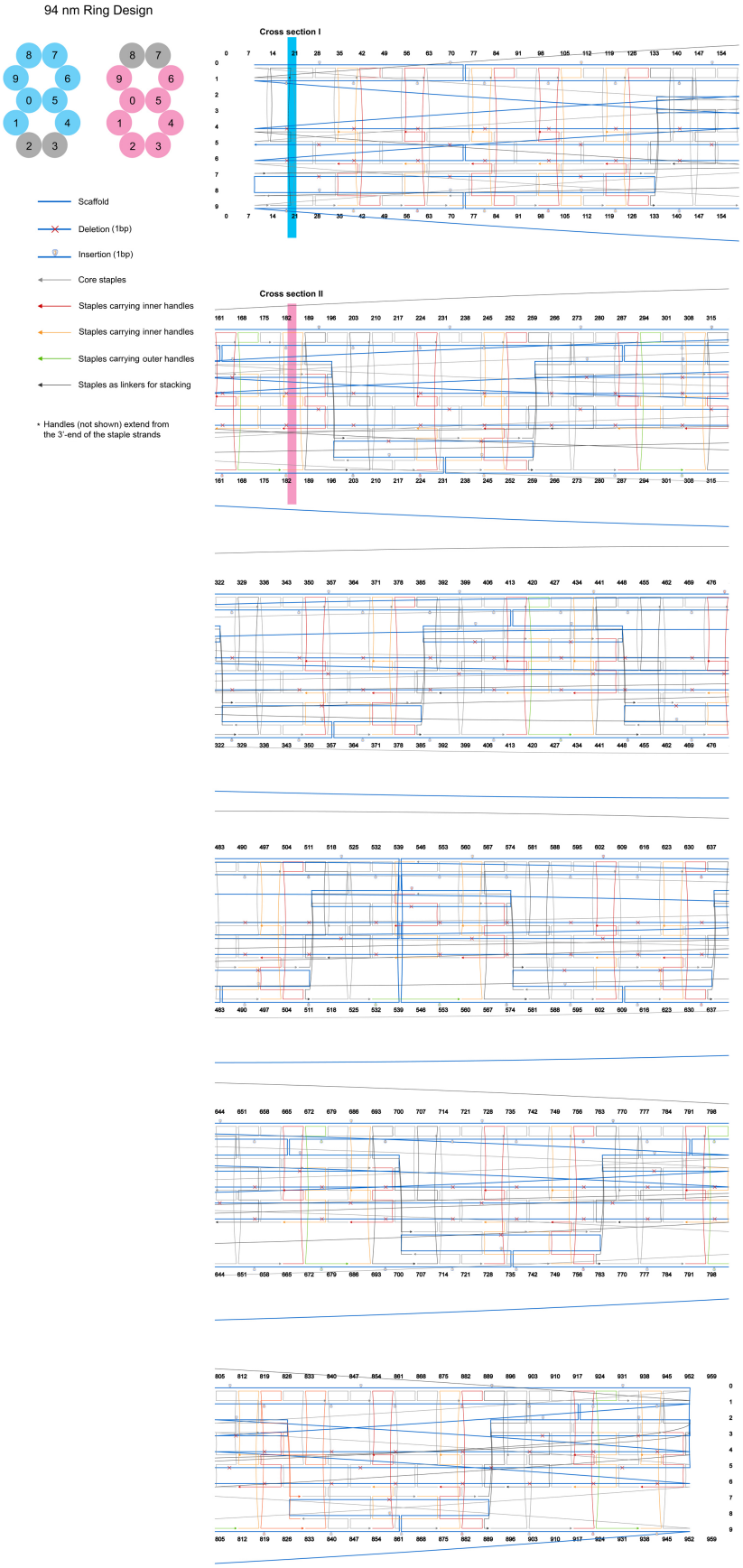


Figure S1-5: Strand diagram illustrating the design of the 94-nm ring.

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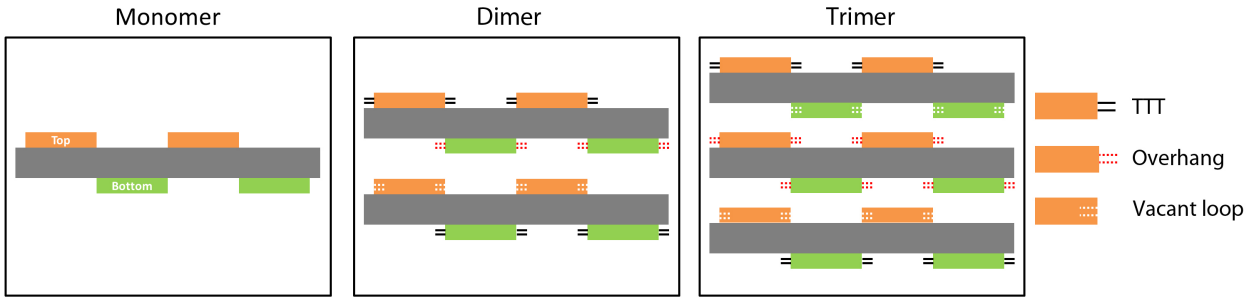


Figure S1-6: Cartoon models illustrating the multimerization of 94-nm ring by stacking. Grey rectangle: main body of the 6-helix-bundle DNA ring; Orange and green rectangles: "teeth" made of two DNA helices; Dotted lines: sticky ends (red: staple strand overhangs, white: unpaired scaffold loops); Solid line: dT_3 staple extensions.

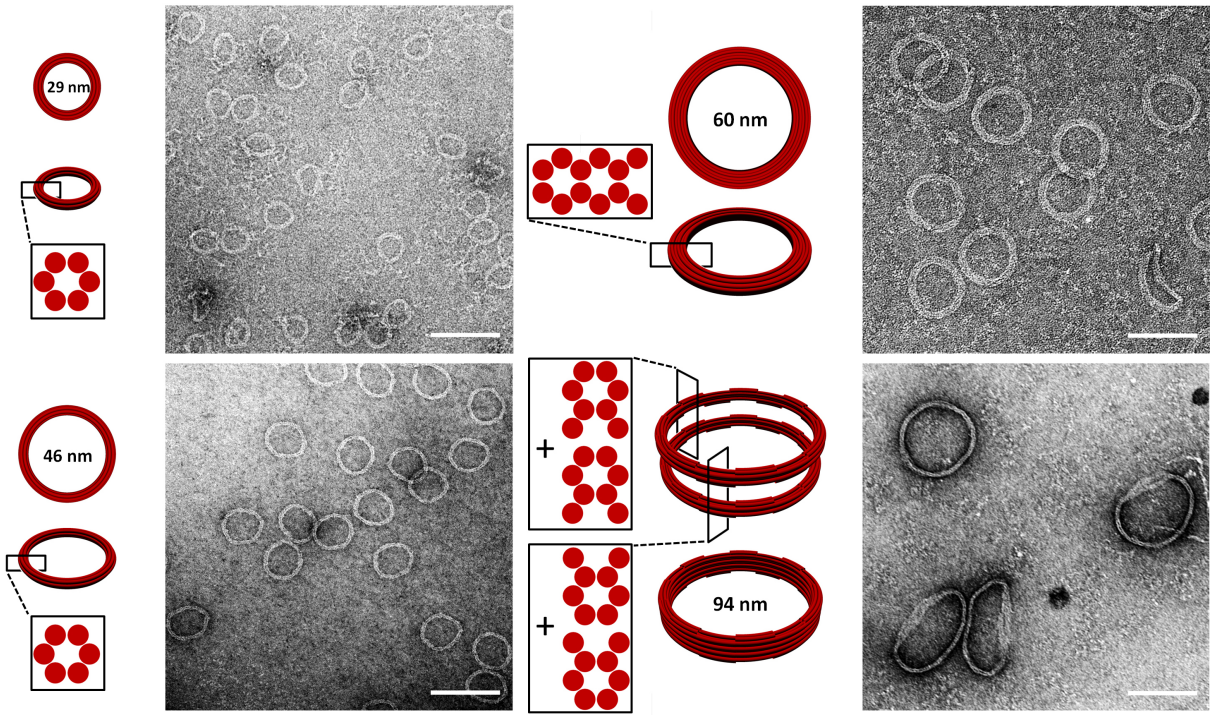


Figure S2: Cartoon models and representative TEM images of DNA-origami rings. Each red cylinder circle represents a DNA double-helix. Scale bars: 100 nm.

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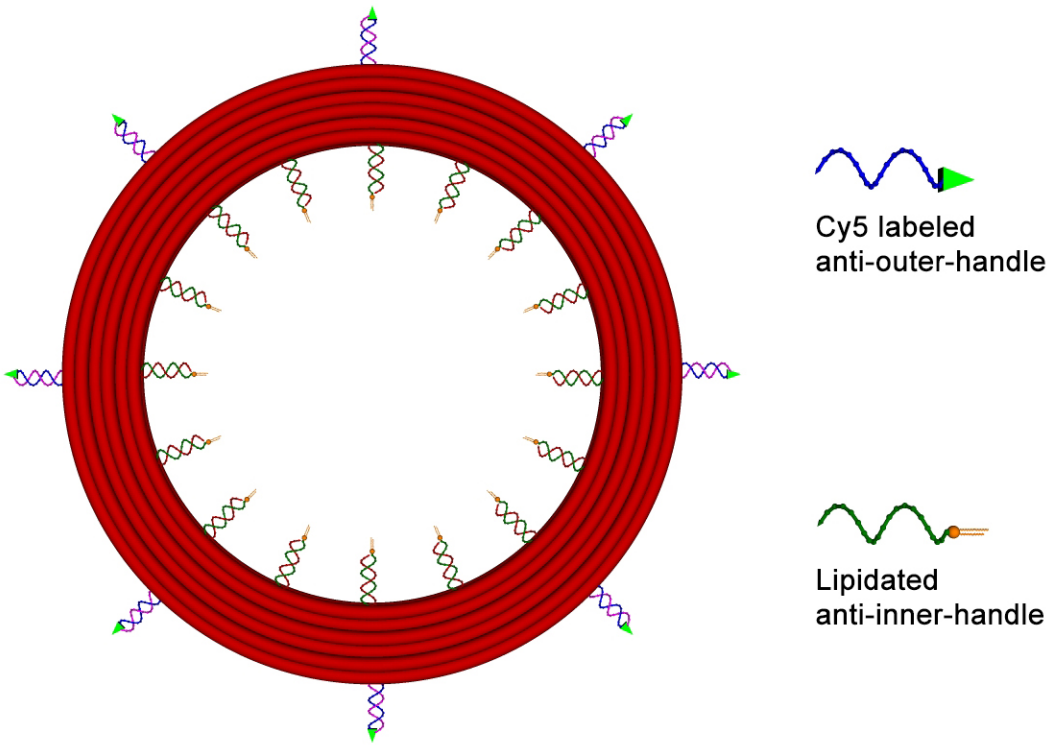


Figure S3: A schematic diagram illustrating one design of a 60-nm ring labeled by eight Cy5 fluorophores and sixteen lipid molecules. Cy5-labeled anti-outer-handles and lipidated anti-inner-handles hybridize with outer- and inner- handles of the ring, respectively.

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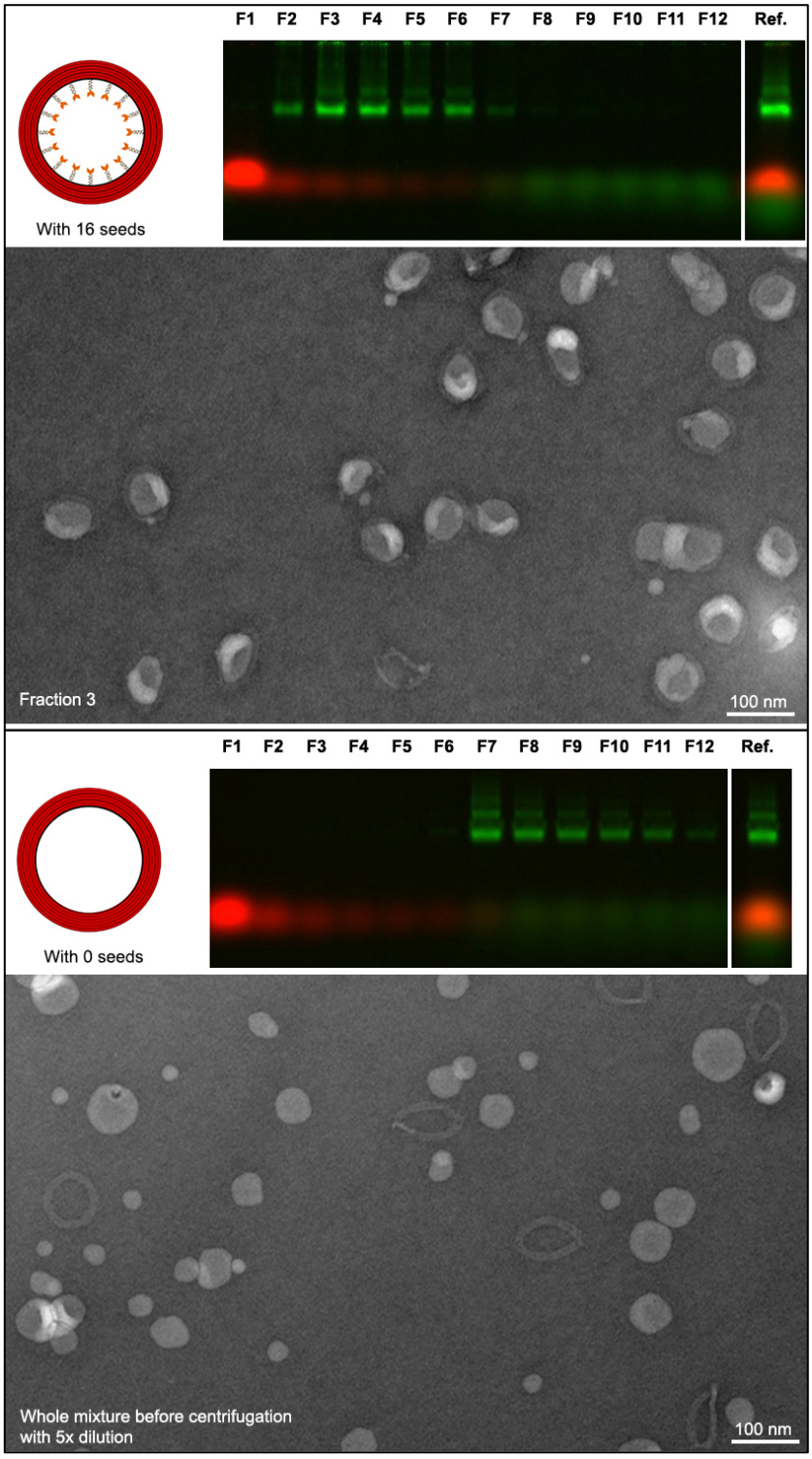


Figure S4: Comparison between liposome assembly results: (Top) DNA rings with 16 lipid seeds; (Bottom) DNA rings without any lipid seed. Gel images show the content of each fraction collected after isopycnic centrifugation (pseudo-fluorescence color: green–DNA, red–lipid). Smaller numbers are assigned to lighter fractions. A representative TEM image is shown for each assembly result.

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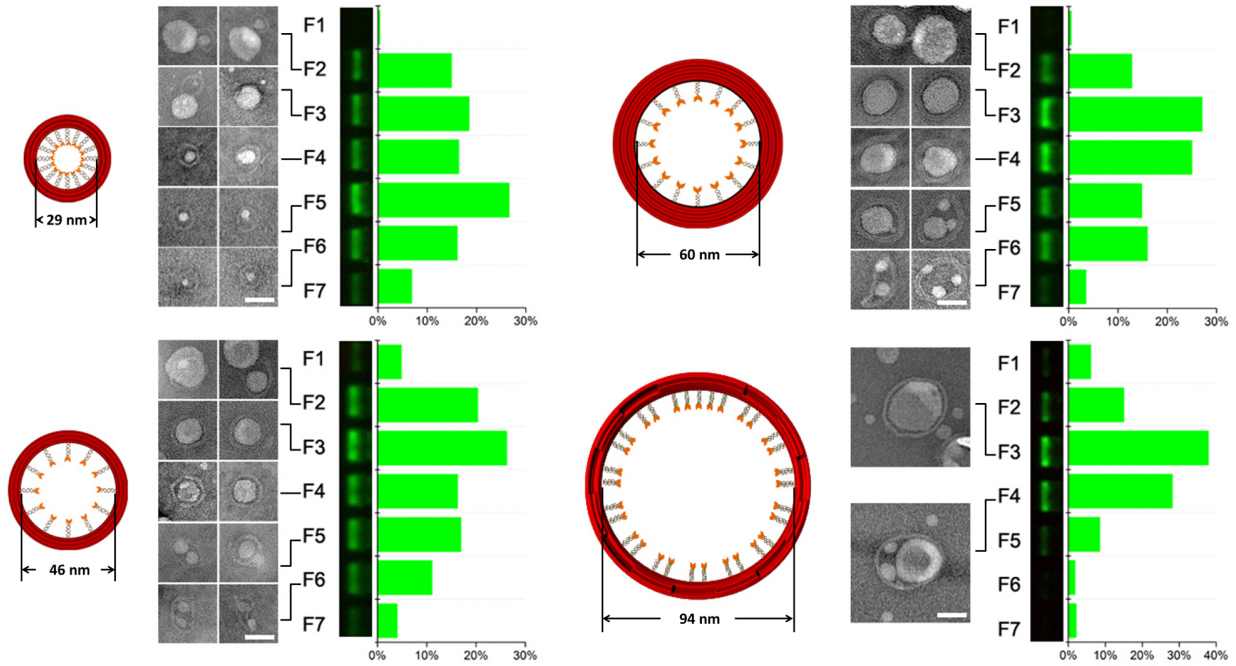


Figure S5: DNA-ring-associating liposomes separated by isopycnic centrifugation. For each ring type, a cartoon model of the lipid-labeled ring, representative TEM images of major fractions, a gel image and a bar-graph showing the normalized DNA-ring population of each fraction are shown from left to right. Smaller numbers are assigned to lighter fractions. Scale bars: 50 nm.

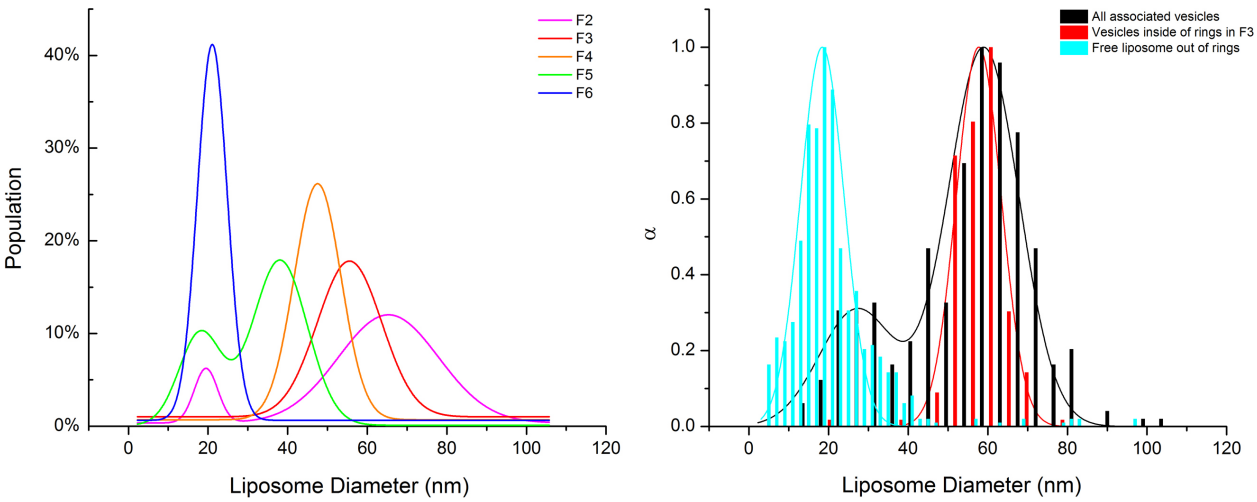


Figure S6-1: Vesicle size distribution in the 60-nm ring system (ref. Figure S5). Left: Liposome size distribution (fitted by Gaussian function) in fractions 2–6. Right: histograms and their fitted curves of: the overall size distribution of vesicles associated with the DNA ring (black), the size distribution of vesicles inside DNA rings found in fraction 3 (red) and the size distribution of free liposomes (without DNA ring association, light blue).

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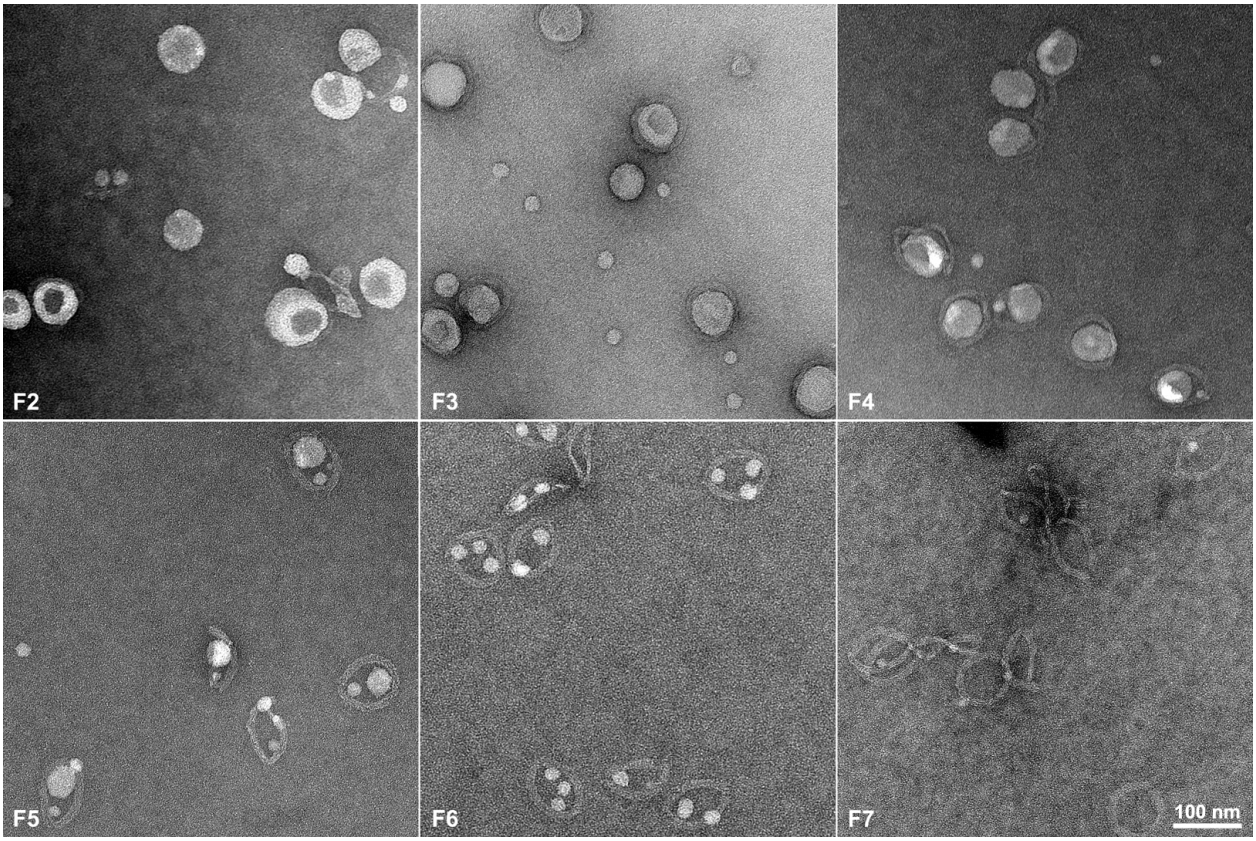


Figure S6-2: Representative TEM images of fractions 2–7 in the 60-nm ring system (ref. Figure S5).

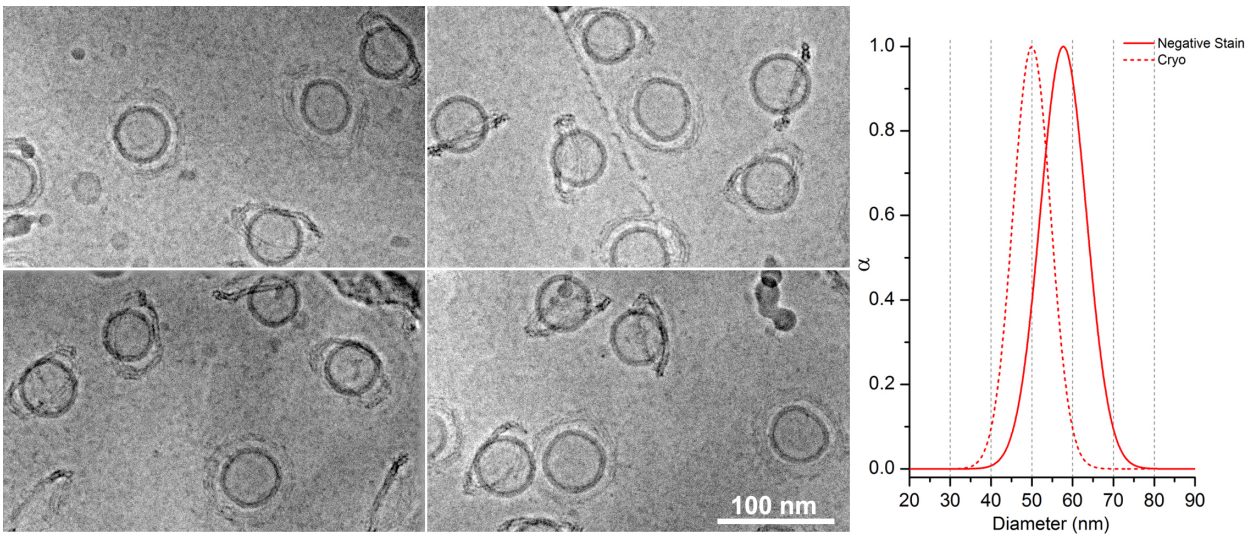


Figure S7: Left: Cryo-EM images of liposomes assembled on the 60-nm ring. Right: Liposome size distribution measured from negative-stained TEM images (solid line) and cryo-EM images (dashed line).

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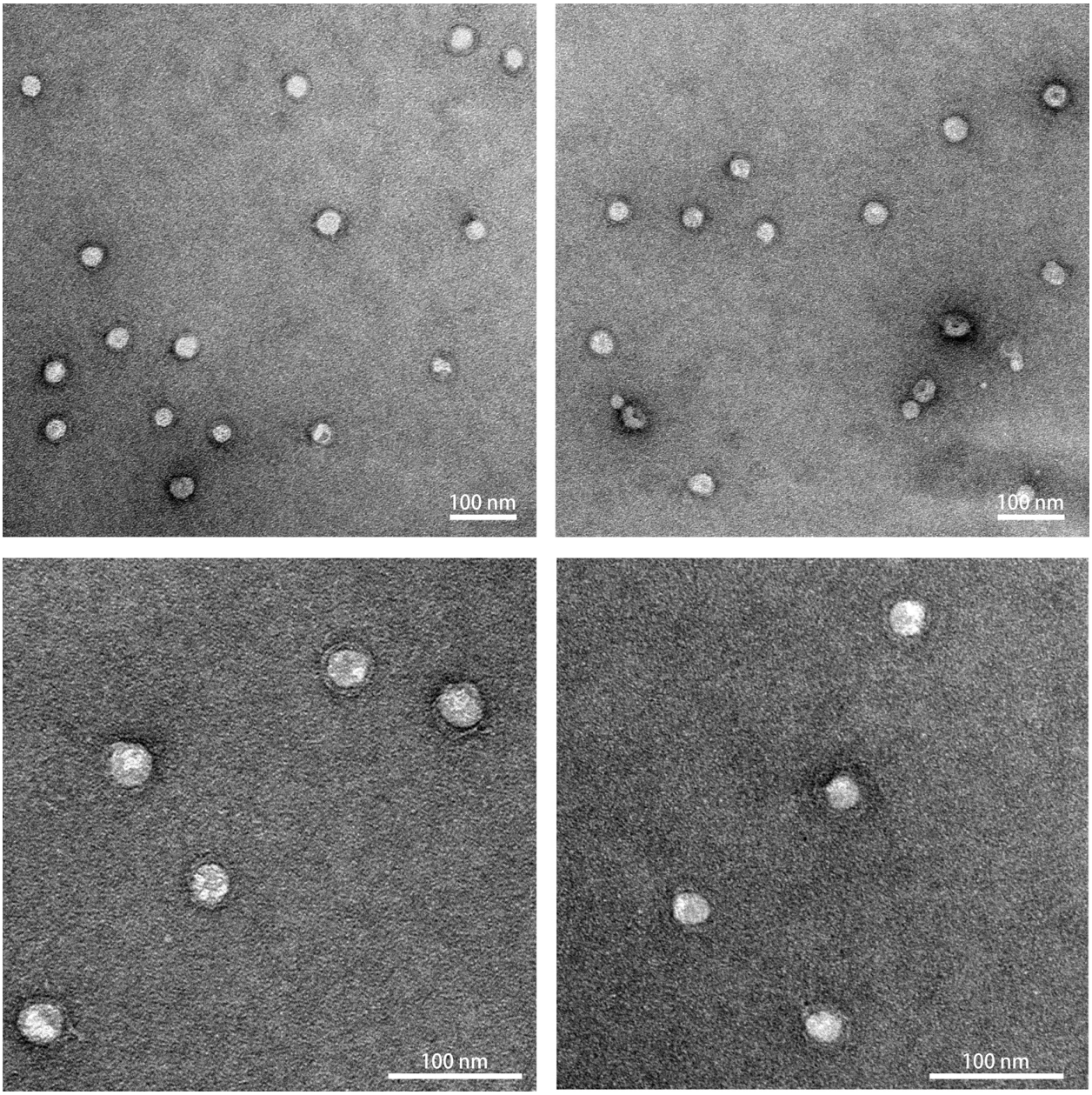


Figure S8-1: TEM images of the liposomes templated by the 29-nm ring.

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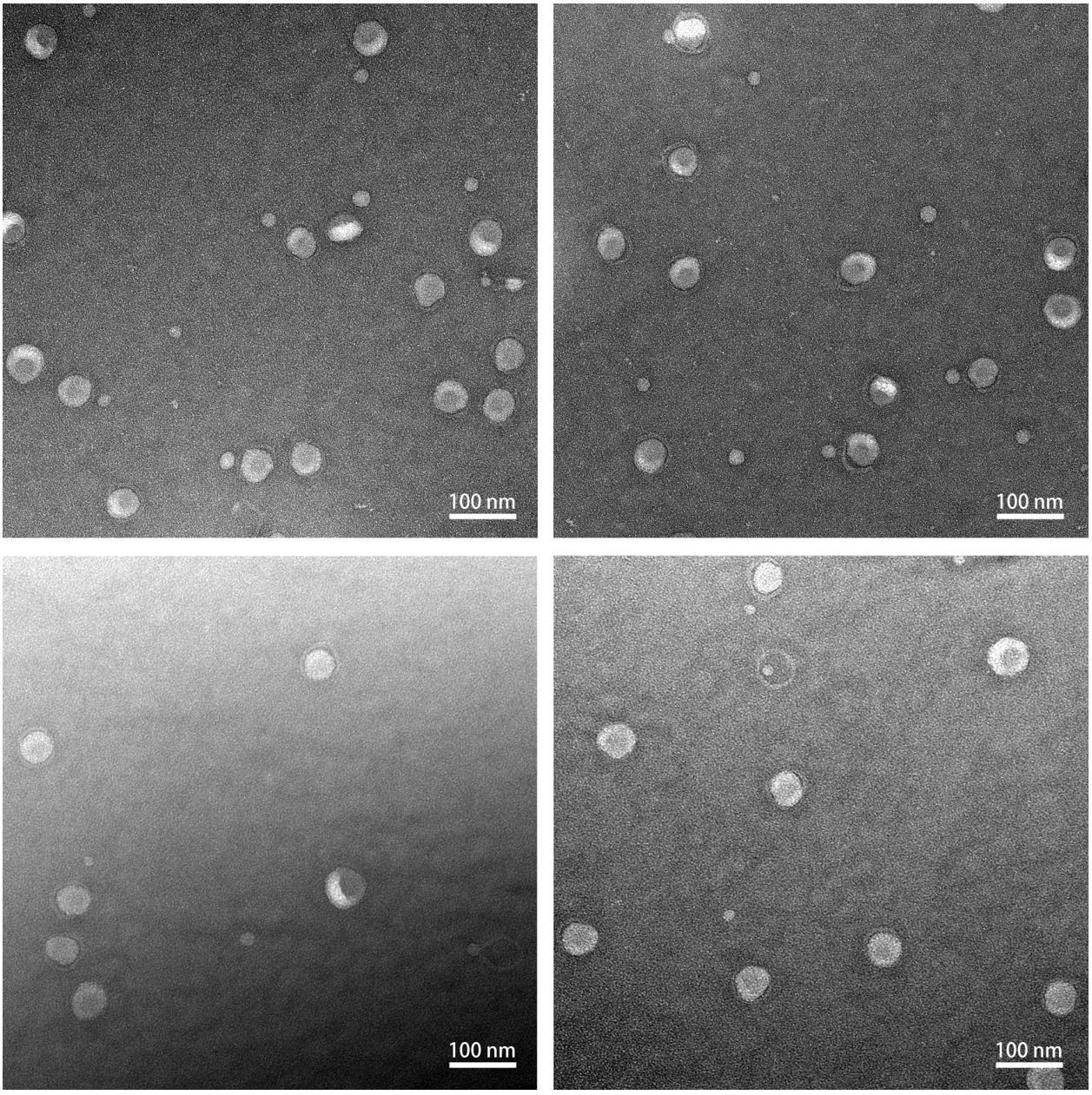


Figure S8-2: TEM images of the liposomes templated by the 46-nm ring.

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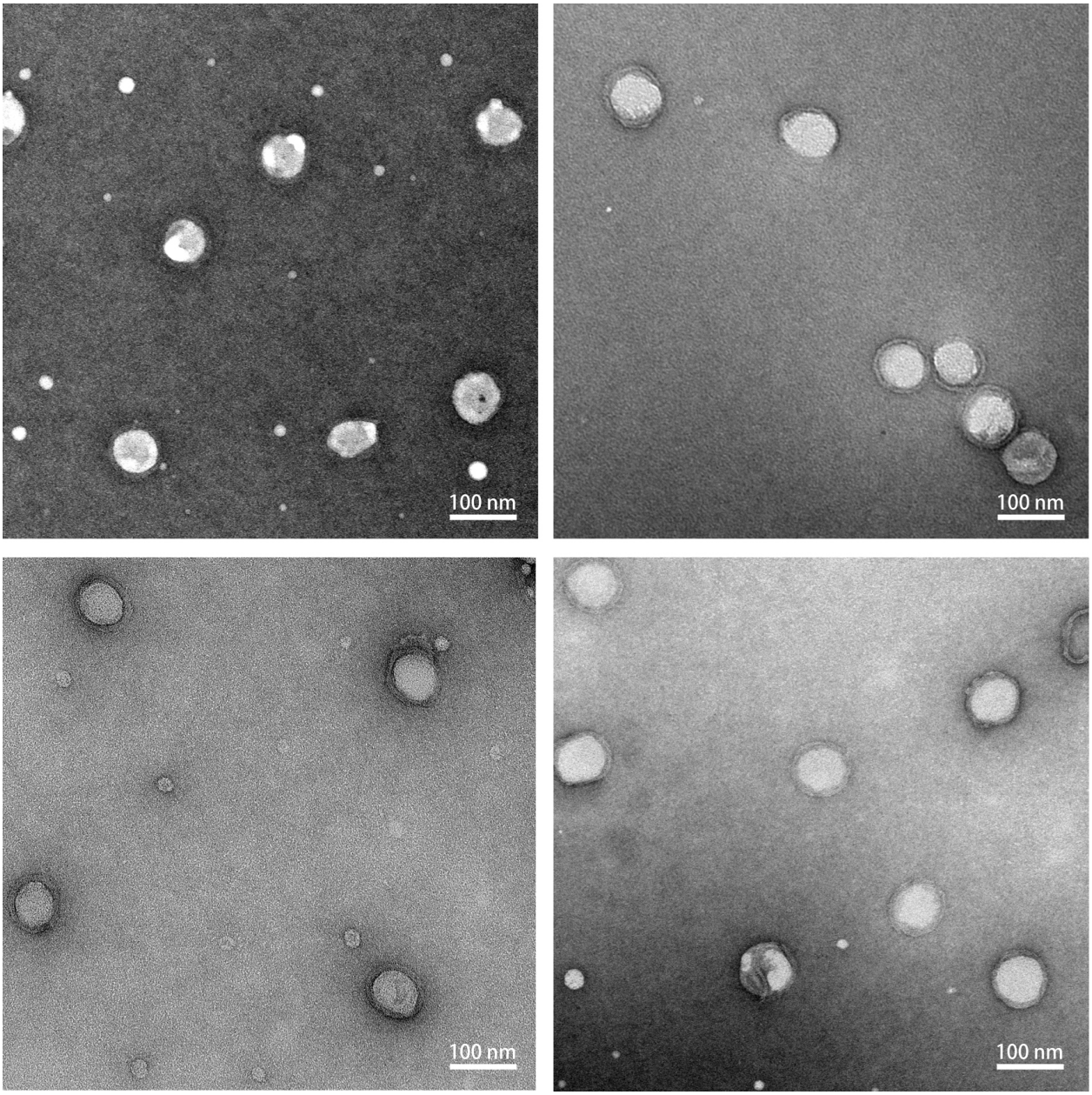


Figure S8-3: TEM images of the liposomes templated by the 60-nm ring.

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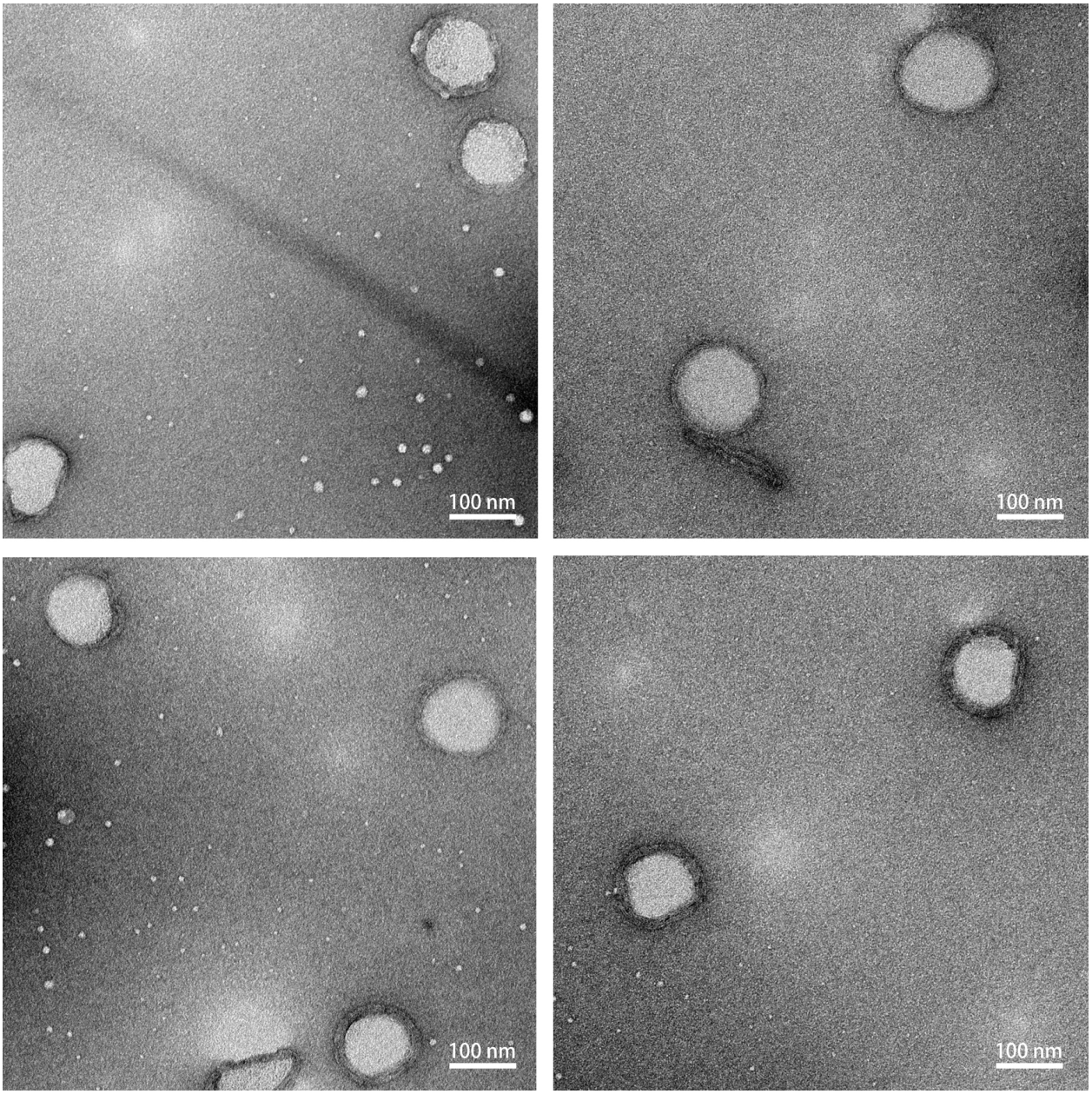


Figure S8-4: TEM images of the liposomes templated by the 94-nm ring.

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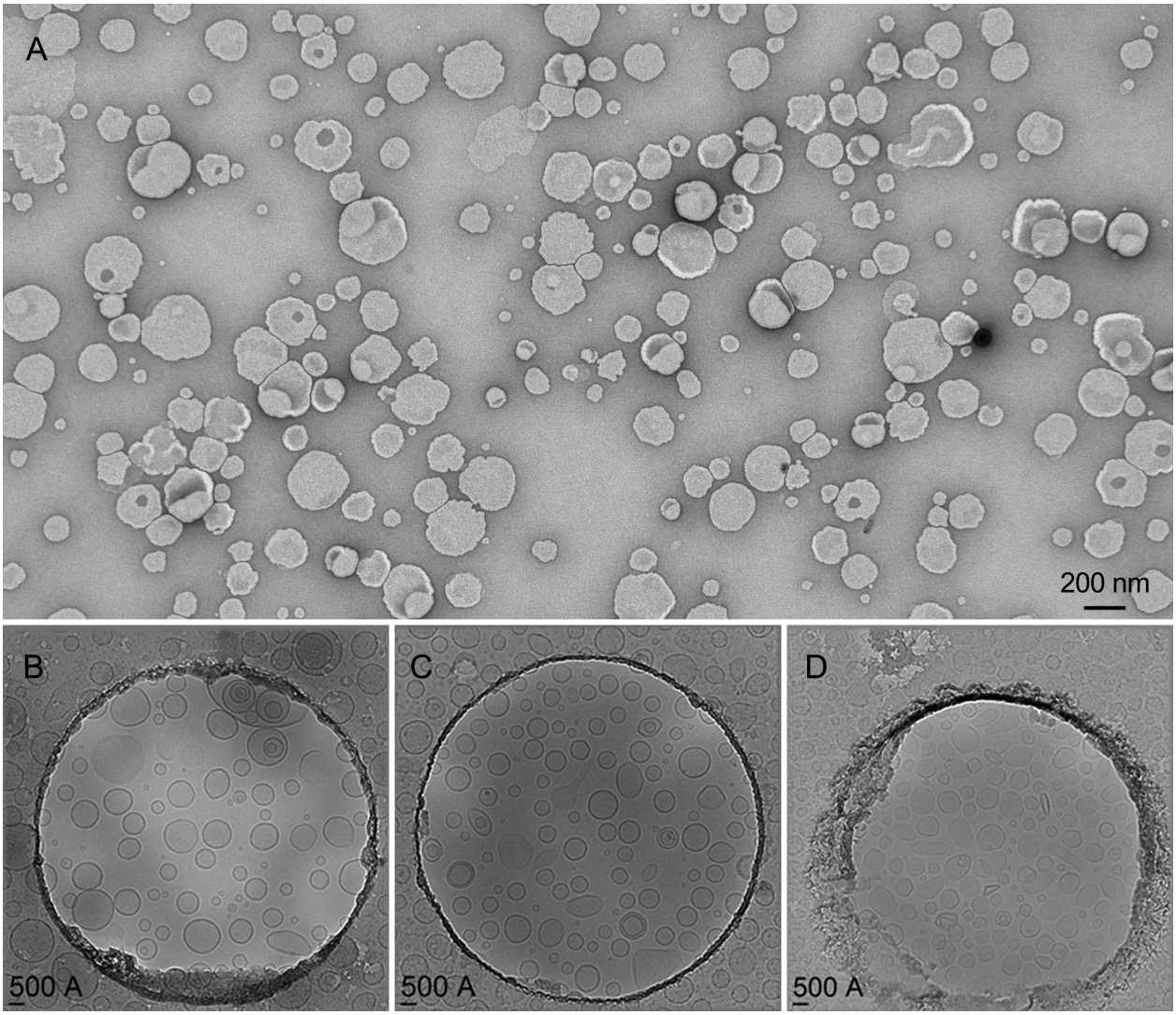


Figure S9: Electron micrographs of extruded liposomes. (A): A negative-stain TEM image of liposome extruded through membrane with 200 nm pore size. (B) – (D): Cryo-EM images of sequentially extruded liposomes through (B) 100 nm, (C) 50 nm and (D) 30 nm pore size membrane.

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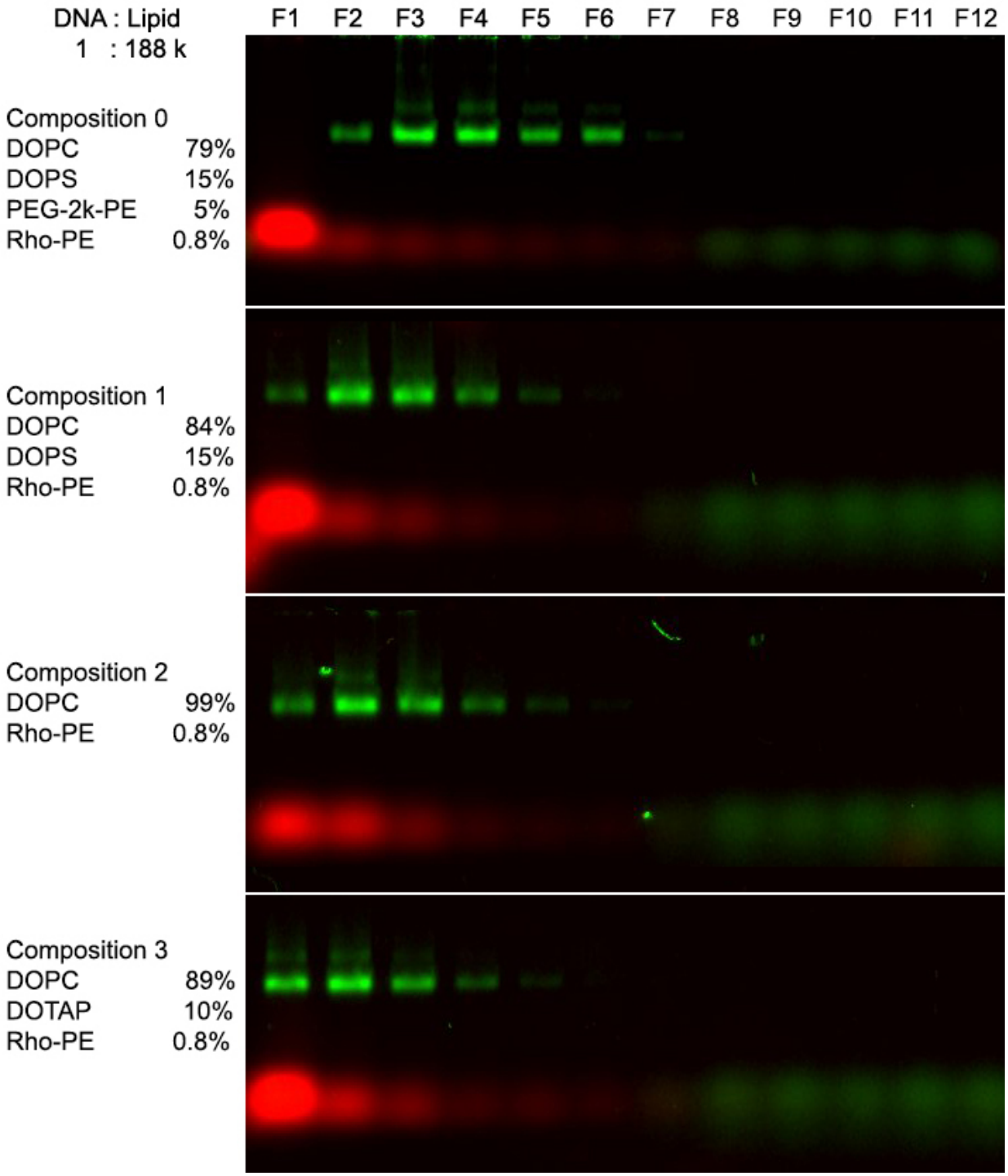


Figure S10-1: Liposome assemblies resulting from different lipid compositions (60-nm-ring:lipid ratio fixed to 1:188,000) characterized by isopycnic centrifugation. Lipid composition is labeled to the left of the corresponding gel images (pseudo-fluorescence color: green–DNA; red–lipid; smaller numbers are assigned to lighter fractions). When PEG-2k-PE is removed from the lipid composition, more products shift to the lighter fractions. Changing negatively charged lipid (DOPS) to positively charged lipid (DOTAP) makes such shift more obvious.

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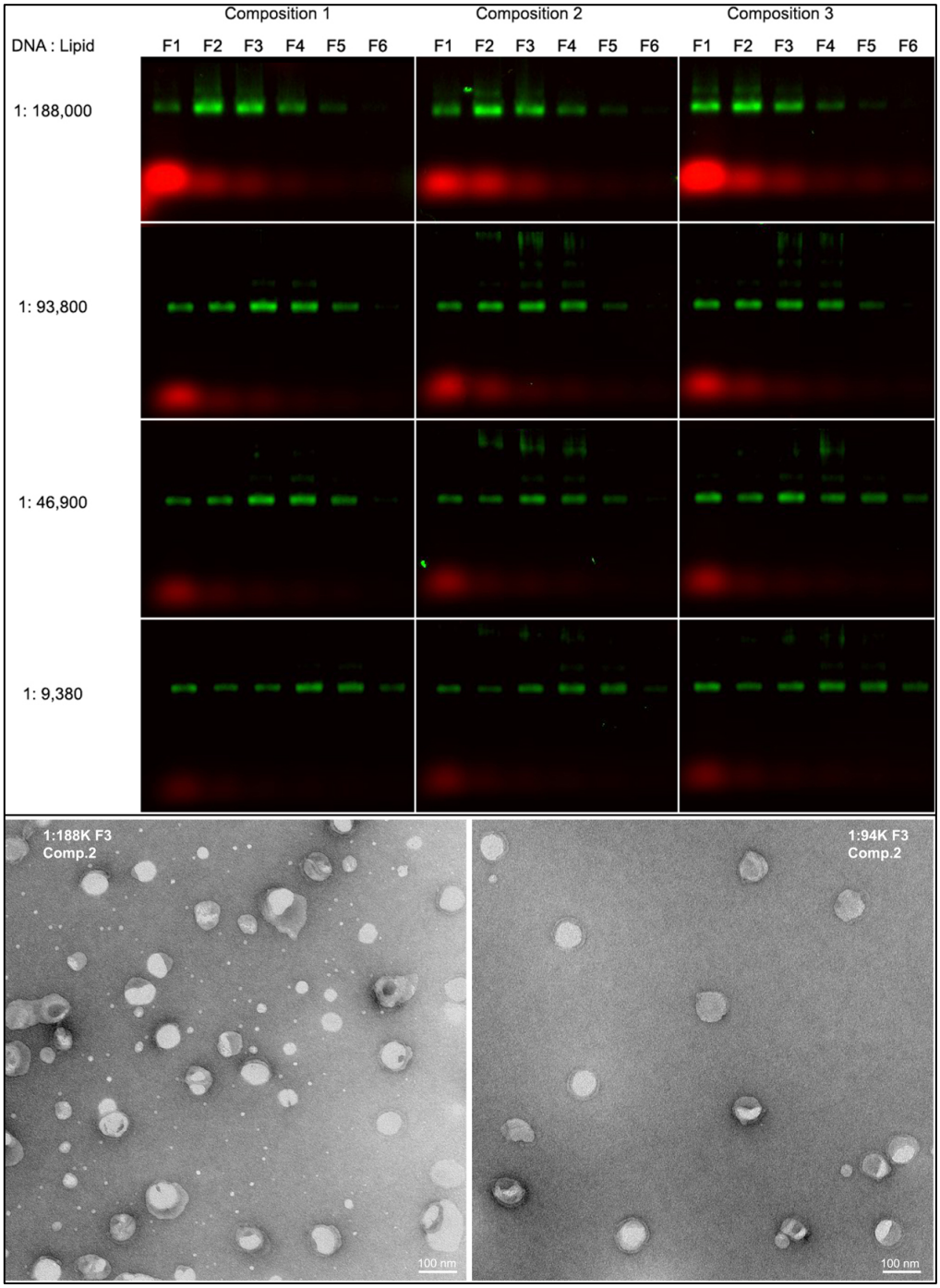


Figure S10-2: Rescuing the yield of templated liposomes by varying the DNA-ring:lipid ratio. Top: gel images showing the content of centrifugation fractions (green–DNA; red–lipid; smaller numbers represent lighter fractions). Bottom: representative TEM images of fraction 3 formed from lipid composition 2. The left electron micrograph shows a result of the 1:188,000 ring:lipid ratio and the right micrograph shows the result of halving the lipid supply.

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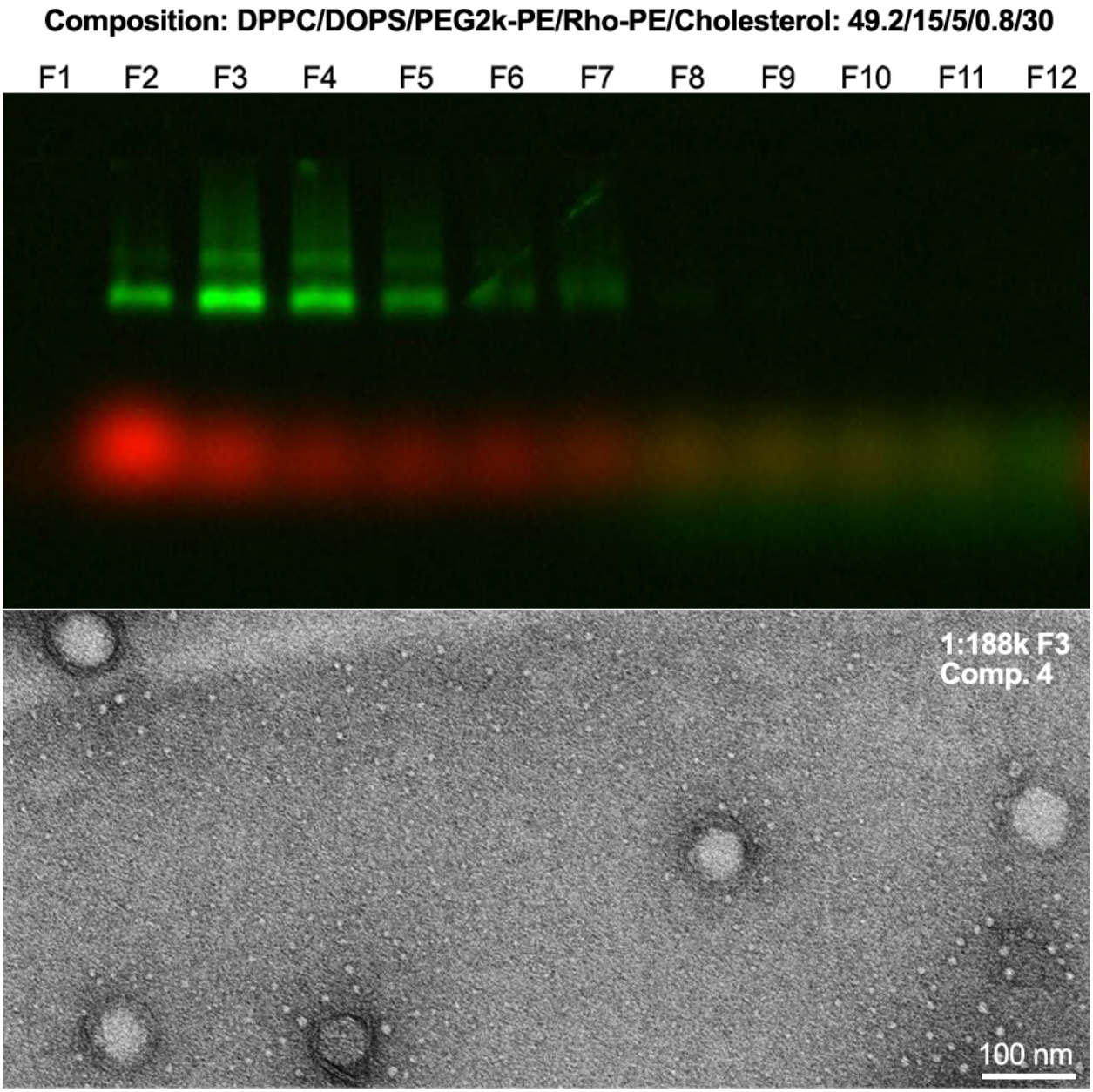


Figure S10-3: Liposome assemblies resulting from lipid composition 4, which contains 49.2% DPPC and 30% cholesterol (60-nm-ring:lipid = 1:188,000). Top: gel images showing the content of centrifugation fractions (green–DNA; red–lipid; smaller numbers represent lighter fractions). Note the similar buoyant density between liposomes with lipid composition 0 and 4. Bottom: A representative TEM image of fraction 3.

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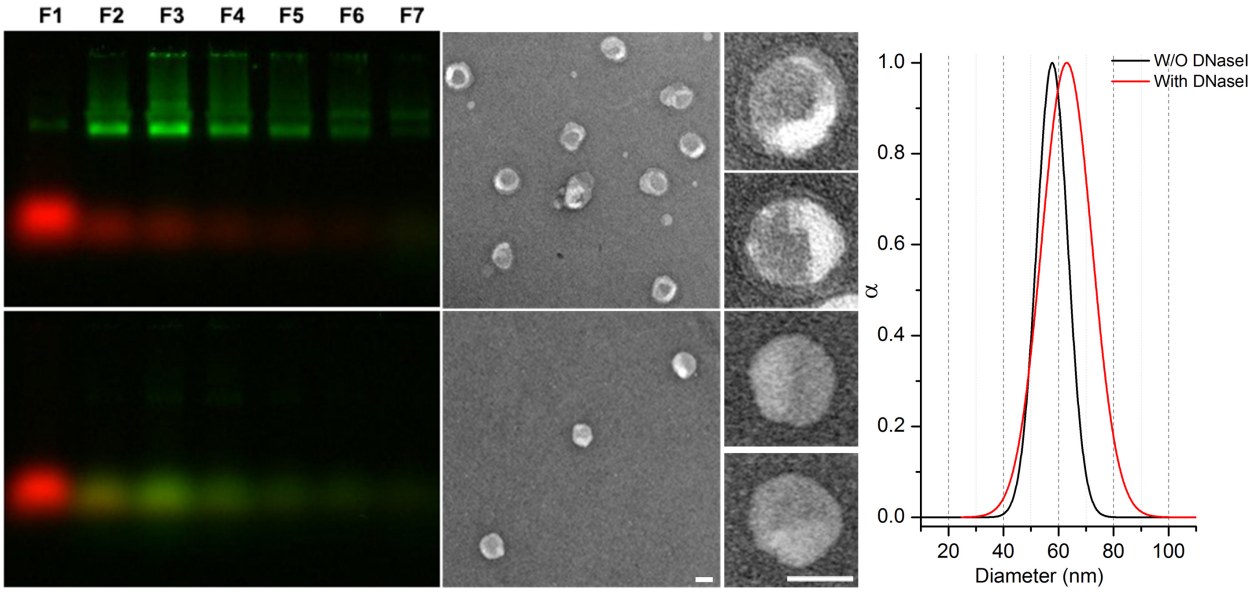


Figure S11-1: Enzymatic removal of DNA rings after templated vesicle formation. Left: Gel images of the centrifugation fractions before (top) and after (bottom) DNaseI digestion (green–DNA; red–lipid; smaller numbers represent lighter fractions); Center: TEM images of fraction 3 (F3) before/after DNaseI digestion; Right: Liposome size distribution in F3 before/after DNaseI digestion. Scale bars: 50 nm.

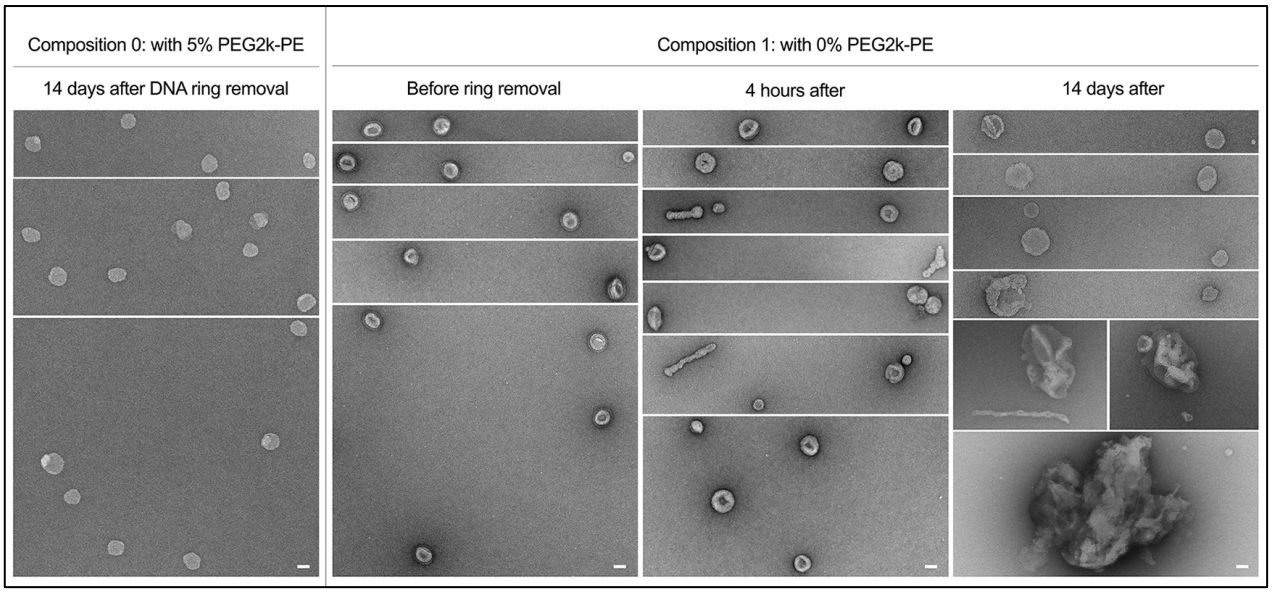


Figure S11-2: TEM analysis of liposome stability after enzymatic removal of DNA rings. Column 1: templated liposomes containing 5% PEG-2k-PE (composition 0) imaged 2 weeks after DNAseI treatment. Note that the vesicle uniformity remains comparable to those before and shortly after digestion (Figure S11-1). Column 2−4, from left to right: templated liposomes without PEG-2k-PE (composition 1) before, 4 hours after and 14 days after DNAseI treatment. Note the obvious morphology change shortly after digestion and the emergence of large aggregates after 2 weeks. Samples were stored at 4℃. Scale bars: 50 nm.

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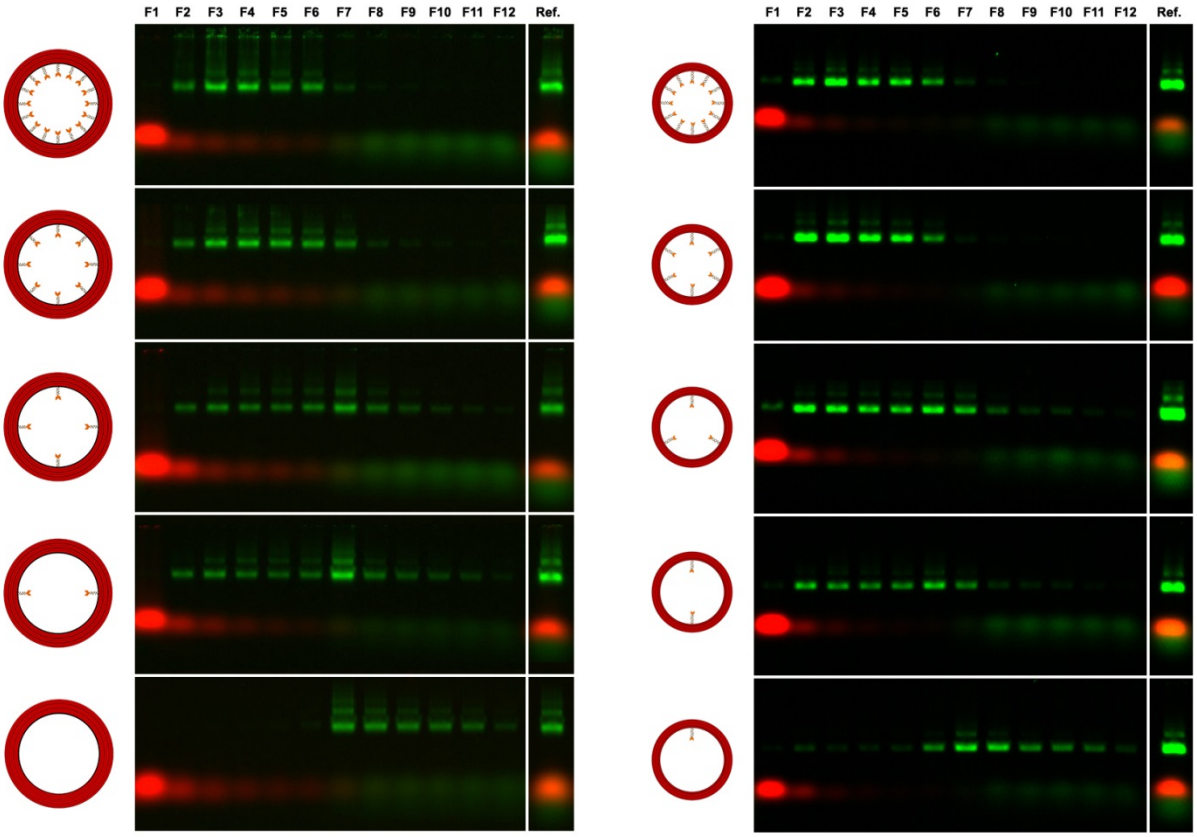


Figure S12: Liposome formation resulting from 60-nm (left) and 46-nm (right) DNA rings with various numbers of lipid seeds. Cartoon models are place to the left of the corresponding gel images that show the content of each fraction collected after isopycnic centrifugation (pseudo-fluorescence color: green–DNA; red–lipid; smaller numbers are assigned to lighter fractions).

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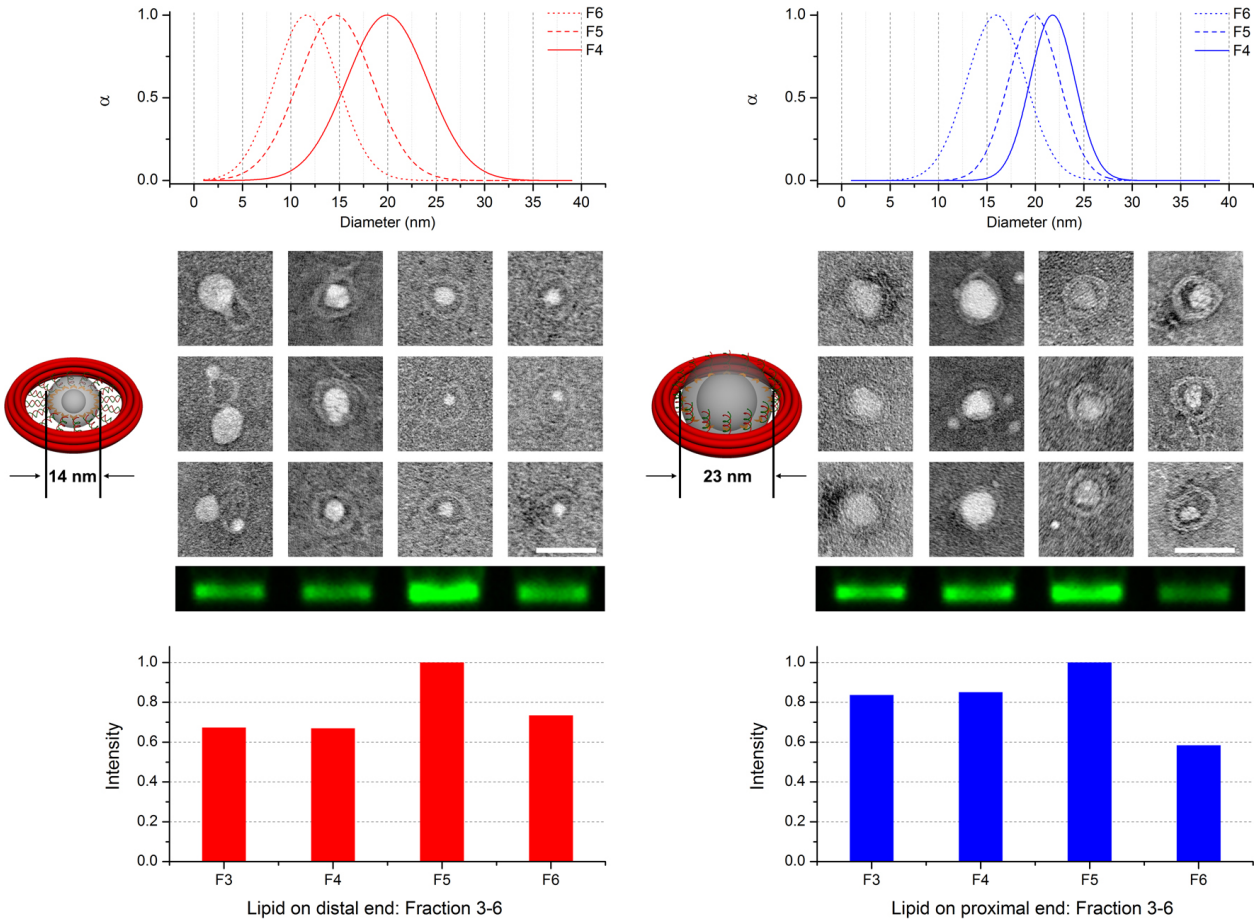


Figure S13: Comparison of the 29-nm ring with the lipid seed decorating the distal (left) *vs.* proximal (right) end of the handle/anti-handle duplex. For each lipid seed configuration, the top panel shows the liposome size distribution in fractions 4 to 6, the middle panel shows typical TEM images of fractions 3 to 6, and the bottom panel shows the relative population of DNA rings in fractions 3 to 6. A smaller number denotes a lighter fraction. Scale bar: 50 nm.

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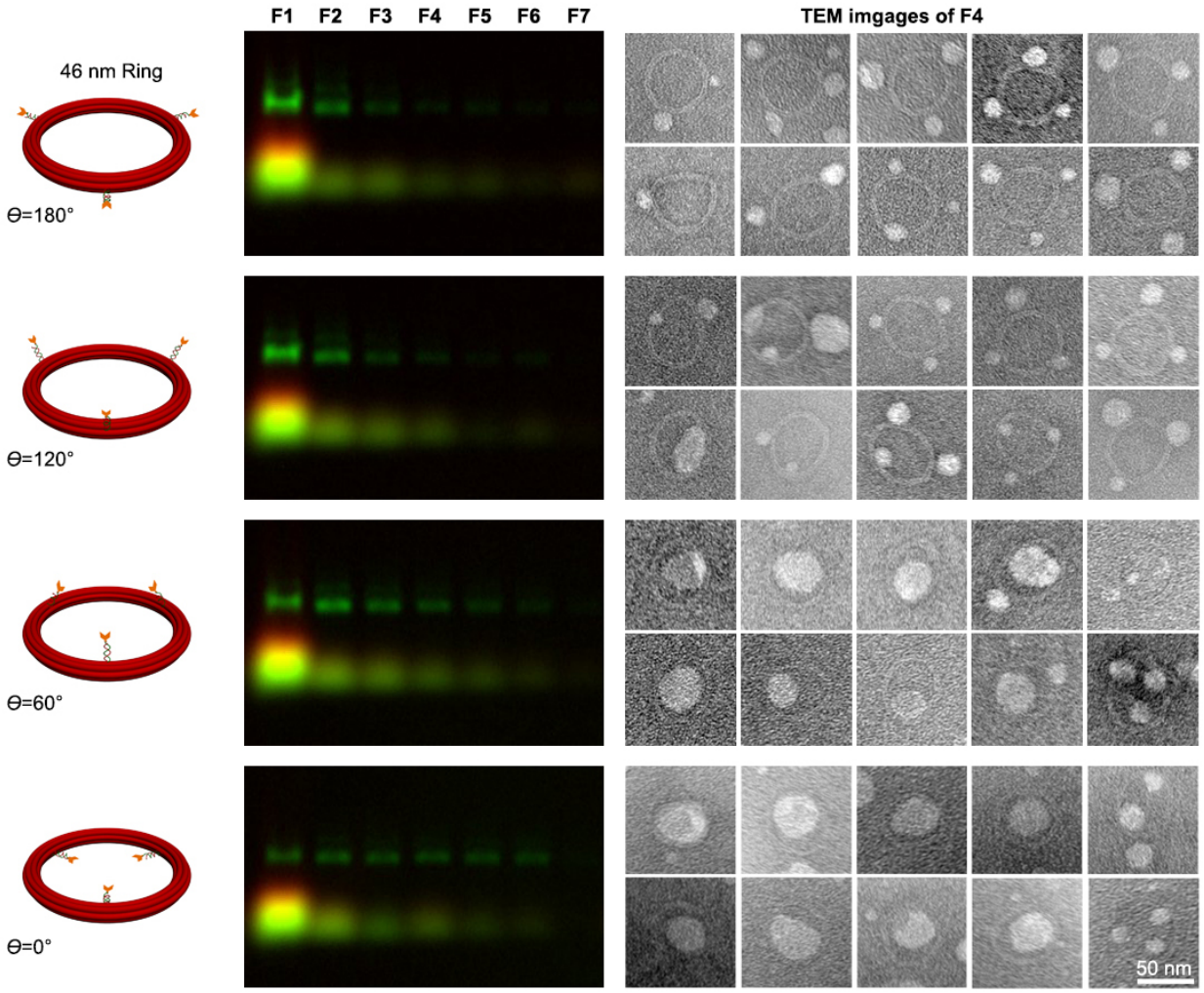


Figure S14: The vesicle assembly products resulted from 46-nm rings with lipid seeds inside or outside of the ring. Left column: cartoon models of 46 nm DNA rings with 3 lipid seeds pointing outward (180°/120°) or inward (60°/0°). Center column: gel images showing the assembly products in fractions 1–7 collected after isopycnic centrifugation (pseudo-fluorescence color: green–DNA; red–lipid; smaller numbers are assigned to lighter fractions). Note that the anti-inner-handle DNA strand is duo-labeled with lipid on its 5'-end and Cy5 on its 3'-end. Right column: selected TEM images of fraction 4 for each lipid-seed configuration.

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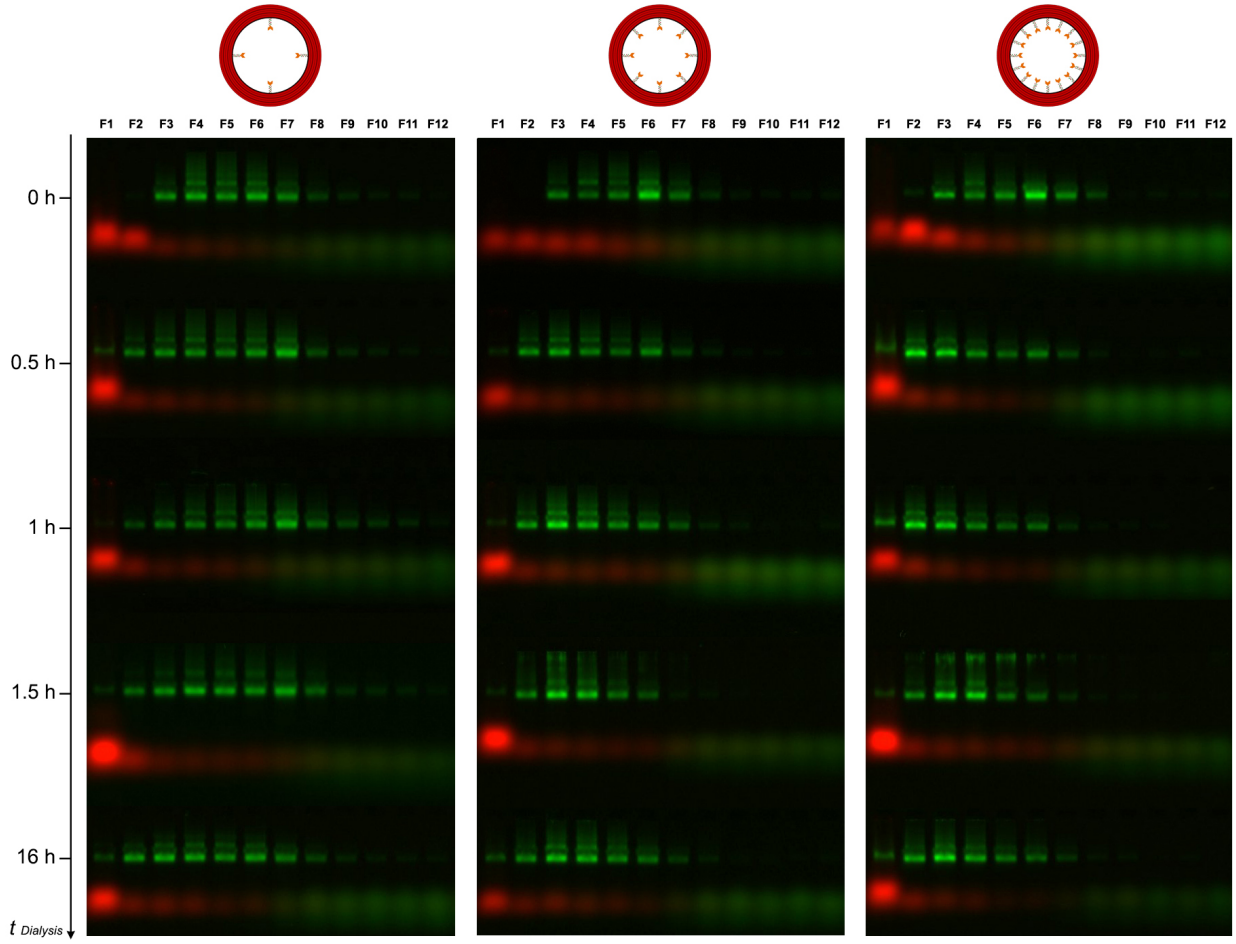


Figure S15-1: Dialysis time-course study performed on 60-nm rings with 4, 8 and 16 lipid seeds. Gel images show the content of each fraction collected after isopycnic centrifugation (pseudo-fluorescence color: green–DNA; red–lipid; smaller numbers are assigned to lighter fractions). A cartoon model of lipid-seed labeled DNA ring is shown on top of each column.

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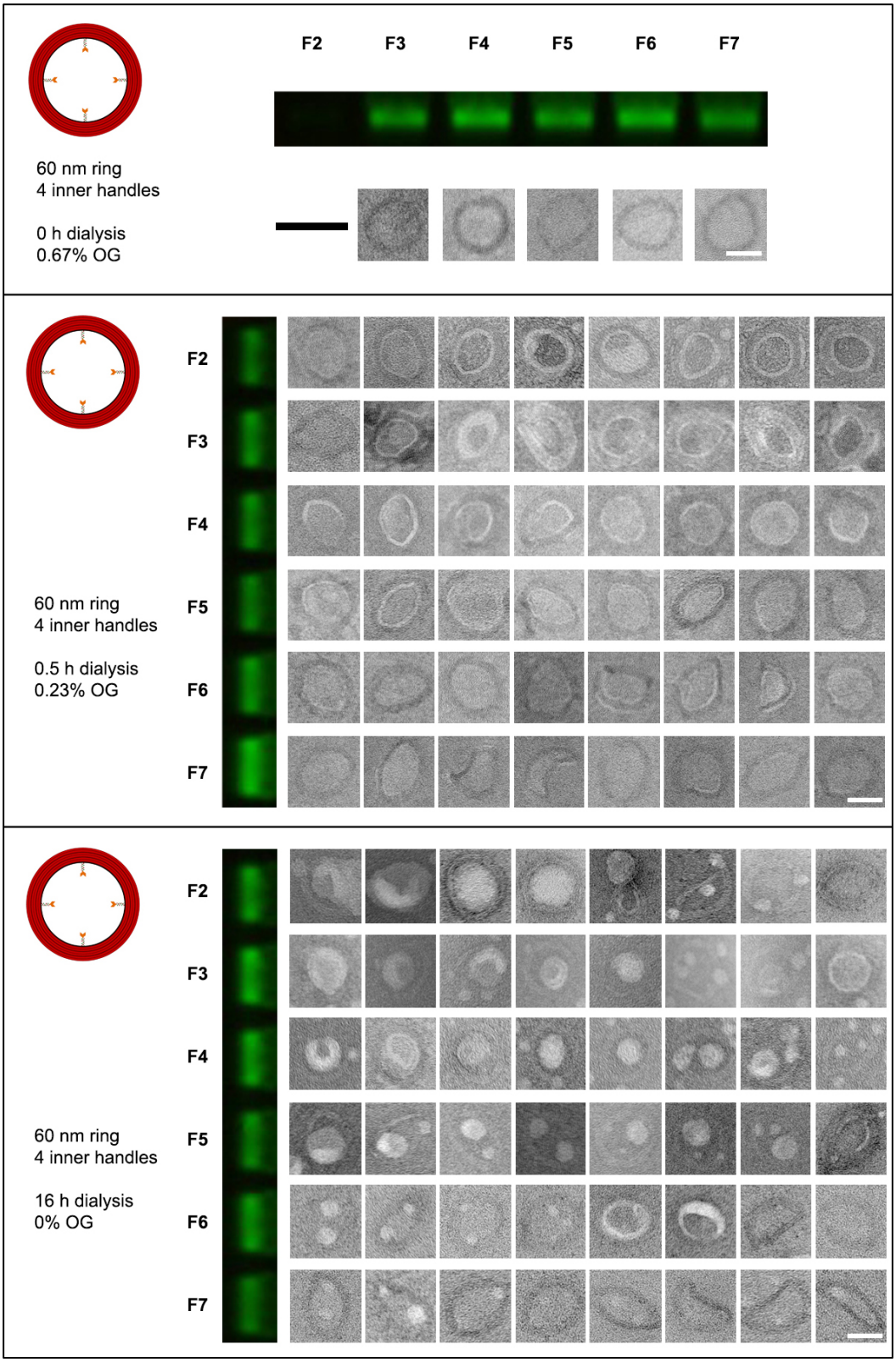


Figure S15-2: Selected negative-stain TEM images of the DNA rings (4 lipid seeds) in each fraction after 0-, 0.5- and 16-hour dialysis. Scale bars: 50 nm.

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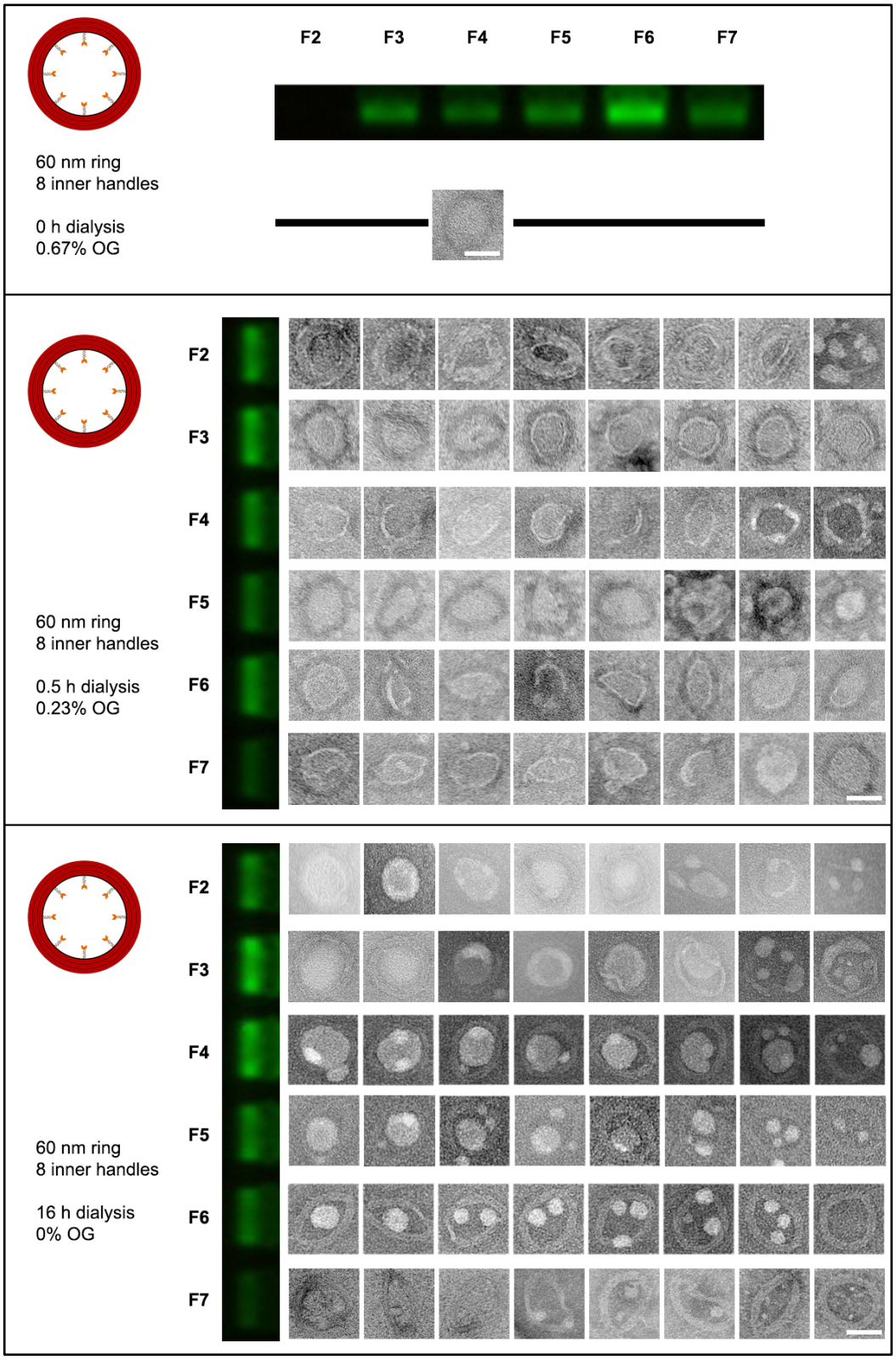


Figure S15-3: Selected negative-stain TEM images of the DNA rings (8 lipid seeds) in each fraction after 0-, 0.5- and 16-hour dialysis. Scale bars: 50 nm.

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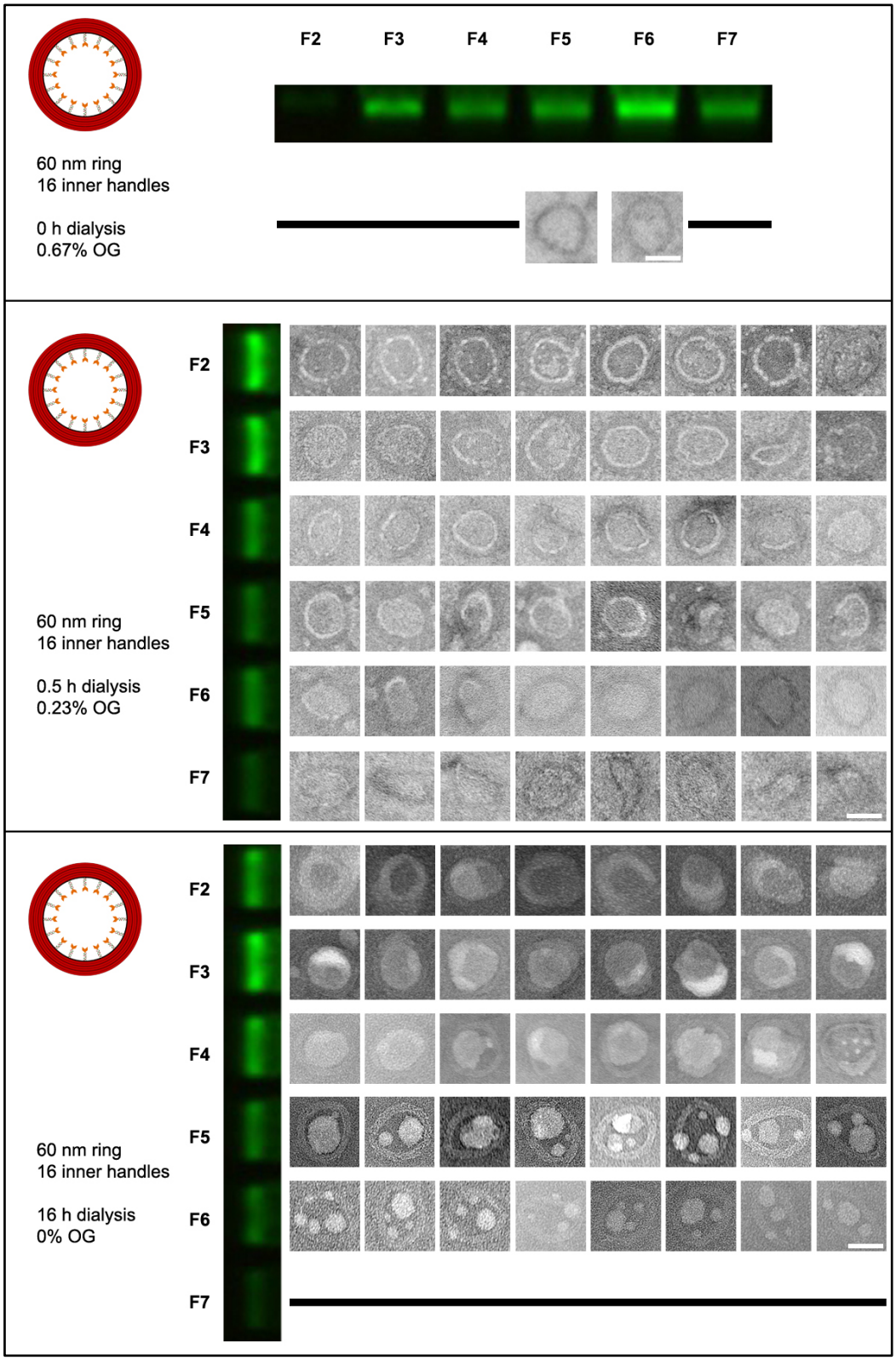


Figure S15-4: Selected negative-stain TEM images of the DNA rings (16 lipid seeds) in each fraction after 0-, 0.5- and 16-hour dialysis. Scale bars: 50 nm.

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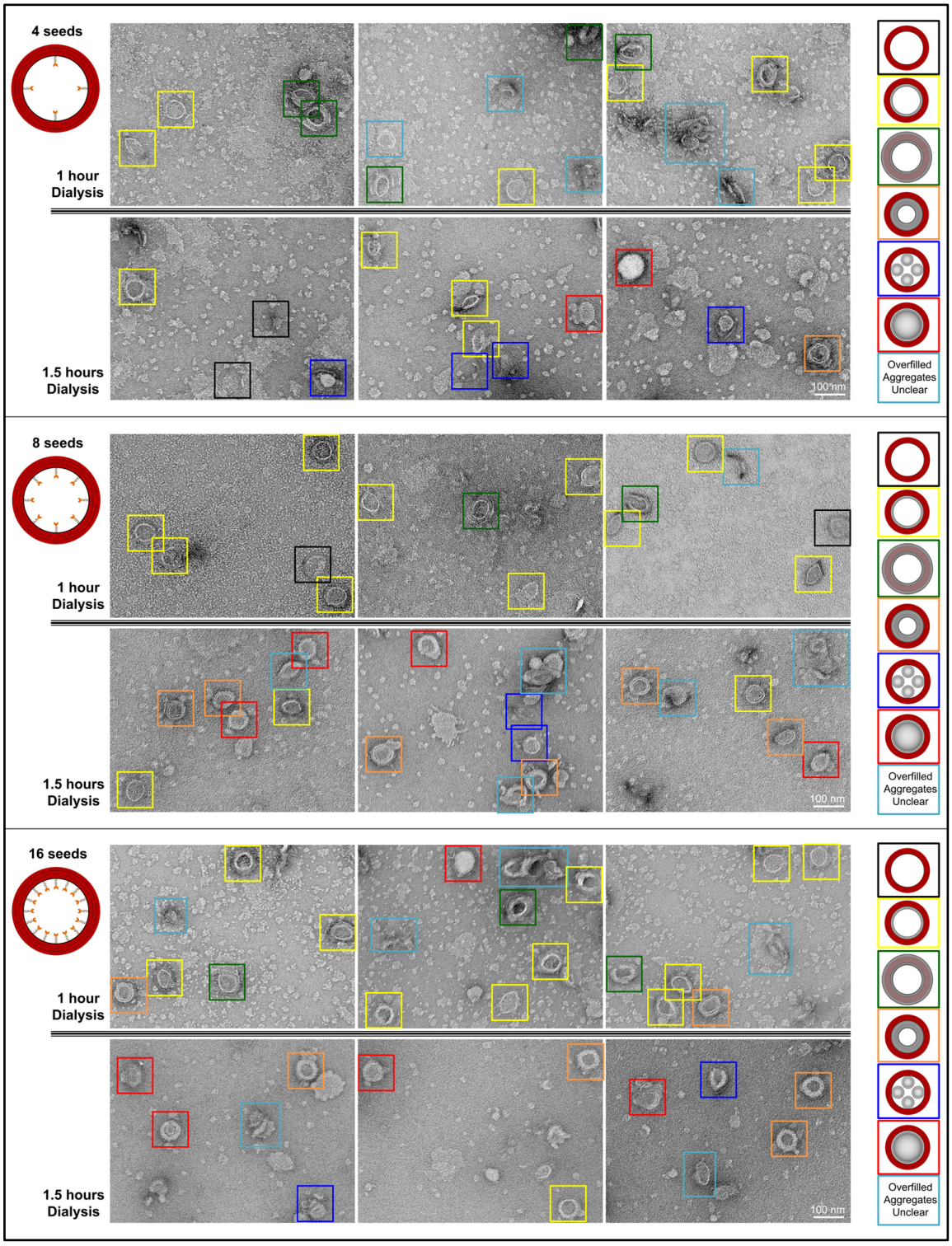


Figure S15-5: Representative TEM images of mixtures of fractions 2–7 for DNA rings with 4-, 8- or 16-seeds after 1 or 1.5 hours dialysis. Different types of ring-associating membrane structures were labeled with colored boxes. Cartoon models of lipid-seed labeled DNA rings are shown on the left and the color scheme used to denote different membrane structures is shown on the right. Scale bars: 100 nm.

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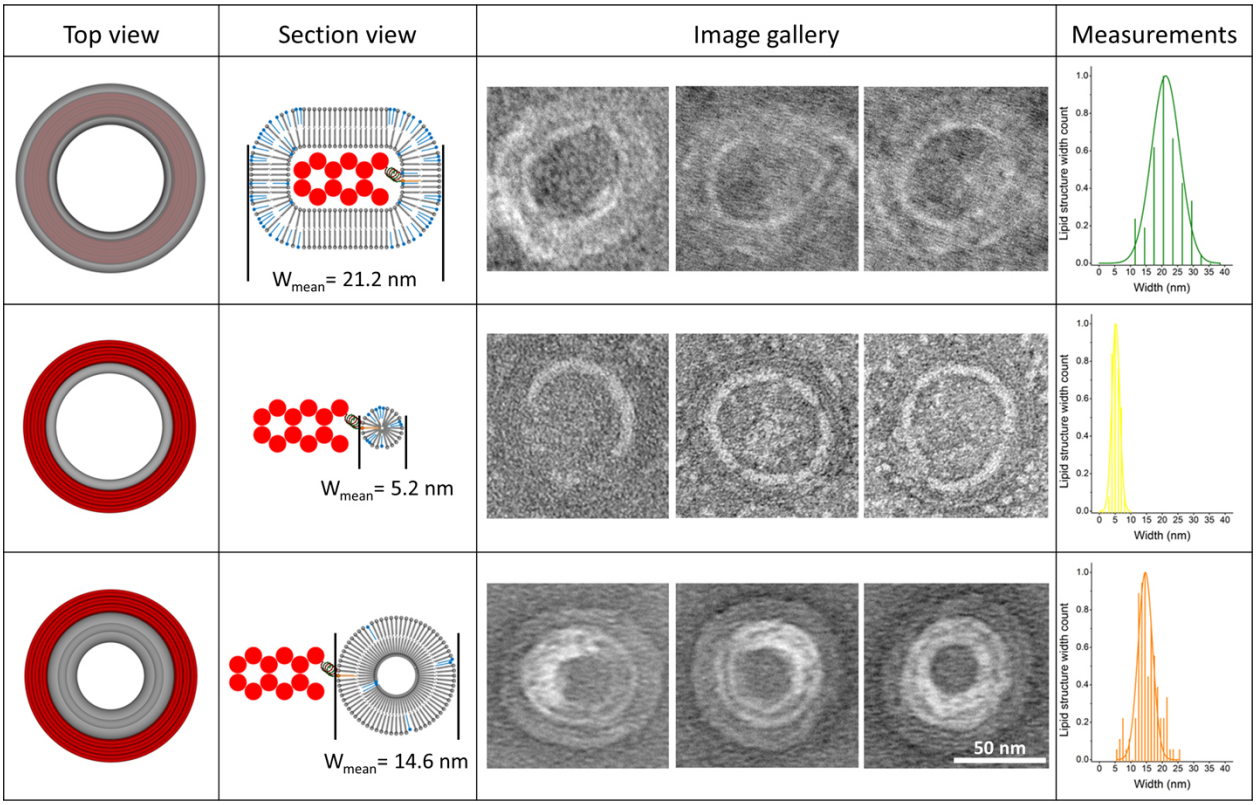


Figure S15-6: Measured dimensions of three intermediate membrane structures.

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Handle and scaffold strand sequences

Inner handle (5'-end connected to a staple strand):
5'-AAATTATCTACCACAACTCAC-3'
Outer handle (5'-end connected to a staple strand):
5'-CTTCACACCACACTCCATCTA-3'
p7308 (the first 2016 bases were used for 29-nm ring)
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