Supporting Information

DNA-Corralled nanodiscs for the structural and functional characterization of membrane proteins and viral entry

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Supporting Experimental Procedures

Expression and Purification of triple cysteine NW11 (3C-NW11)

3C-NW11 construct in pET-28a containing a tobacco etch virus (TEV) protease-cleavable N-terminal His6 tag and a C-terminal sortase-cleavable His6 tag was transformed into BL21-Gold (DE3) competent *Escherichia coli* cells (Agilent). 3L cell cultures were grown at 37 °C with agitation at 200 r.p.m. in Luria broth (LB) medium supplemented with 50 g/ml kanamycin. Expression was induced at an OD600 of 0.6 with 1 mM IPTG, and cells were grown for another 3h at 37°C. Cells were harvested by centrifugation (7,000 x *g*, 15 min, 4 °C), and cell pellets were stored at −80 °C.

3C NW11 was purified as follows; Pellets of cells were resuspended in Buffer A (50 mM Tris–HCl, pH 8.0, 500 mM NaCl, 8 mM BME) plus 1% triton X100 and lysed by sonication on ice. Lysate was centrifuged (35,000 × *g*, 50 min, 4 °C), and the supernatant was loaded onto a Ni^{2+}-NTA column. Resin was washed with 10 CV of the following buffers: buffer A + 1% Triton X-100, buffer A + 50 mM sodium cholate, buffer A, and buffer A + 30 mM imidazole. Proteins were eluted with buffer A + 500 mM imidazole.

Reconstitution of 3C-NW11 nanodiscs

We used ratio of 1:75 3C-NW11:lipid to assemble nanodiscs. Lipids (POPC:POPG, 3:2; solubilized in sodium cholate) and 3C-NW11 were incubated on ice for 1 h. After incubation, sodium cholate was removed by incubation with Bio-beads SM-2 (Bio-Rad) for 1 h on ice followed by incubation overnight at 4 °C. The nanodisc preparations were filtered through 0.22 m nitrocellulose-filter tubes to remove the Bio-beads. The nanodisc preparations were further purified by size-exclusion chromatography while monitoring the absorbance at 280 nm on a Superdex 200 10 × 300 column equilibrated with 20 mM Tris–HCl, pH 7.5, 100 mM NaCl, 8 mM BME, 0.5 mM EDTA. Fractions corresponding to the size of each nanodisc were collected and concentrated. The purity of nanodisc preparations was assessed using SDS–PAGE.

Nanodisc-DNA conjugation and purification

The bifunctional cross linker Sulfo-SMCC (Thermo Scientific) was dissolved in anhydrous dimethylsulfoxide (DMSO) to give a final concentration of 100 mM. 10 nmoles of DNA oligo (with primary amine modification, /5AmMC6/TAGATGGAGTGTGGTGTGAAG) was incubated with a 100 times molar excess of the crosslinker in buffer B (100 mM NaPi, pH 8.0, 150 mM NaCl and 7.5% DMSO) for 1 h at 23°C. The reaction mix was applied to Amicon filter (Millipore, 3kD) and centrifuged at 7000 rpm for 50 min (repeat 3 times), and then went through a disposable Bio-rad P-6 spin column to remove excessive cross linker.

Next, 50 uL of 5 uM nanodisc was incubated with purified DNA oligo-SMCC from the first step at 23°C in buffer C (containing 100 mM NaPi, pH 7.4, 150 mM NaCl) for 2 h (DNA:nanodisc ratio 12:1). We

removed the BME from the nanodisc sample right before the incubation with DNA oligo-SMCC by applying it to Bio-rad P-6 spin column. The oligo-conjugated nanodisc was then purified by size exclusion chromatography (preferred, **Figure S1**) or by using Centricon concentrators (30 kDa MW cutoff, Millipore) and centrifuging at 4000 g for 10 min (repeat 5 times).

Design and assembly of DNA origami structures

The DNA origami/crystal nanostructures were designed using the software caDNAno.[1] DNA Origami was folded by mixing p7308 scaffold at 10 nM with 10-fold excess of staples in folding buffer (containing 5 mM Tris-HCl, 1 mM EDTA, 12 mM $MgCl_2$, pH 8) and subjected to a thermal annealing ramp (from 65°C to 25°C over 20 h). Well-folded DNA origami was purified by a rate-zonal centrifugation procedure using a 15-45% (v/v) glycerol gradient.

Assembly of oligo-conjugated nanodisc with DNA Origami

Excess of oligonucleotide-conjugated nanodisc was incubated with the DNA corrals containing handle strands (5'-CTTCACACCACACTCCATCTA-3'). Nanodisc assembly was performed in buffer containing 5mM Tris-HCl, 1mM EDTA, 10 mM $MgCl_2$, using an annealing protocol, in which the temperature was gradually decreased from 37 °C to 4 °C over 2 h.

Large lipid nanodisc reconstitution

DNA corrals containing small nanodiscs were mixed with 9X amount liposomes (POPC:POPG:cholesterol:DGS-NTA(Ni) ratio of 51:34:10:5, for the poliovirus experiment) then diluted with tris buffer containing octyl glucoside (5 mM Tris, 1 mM EDTA, 12 mM $MgCl_2$, pH 8, 0.7% OG). Next, this solution was incubated on a Thermomixer at 300 rpm at room temperature for 1 h. The entire solution was then transferred into a 7K MWCO Slide-a-Lyzer dialysis cassette (Thermo Scientific). The cassette was dialyzed against 2 L of tris buffer for 48 h. After dialysis, the sample was recovered from the dialysis cassette and concentrated using an Amicon column. Reconstituted nanostructures were separated from excess lipids by equilibrium centrifugation using sucrose gradient (30, 25, 20, 15, 10 from bottom to top). The gradient solutions were layered into ultracentrifuge tubes and centrifuged at 48,000 rpm for 5 h at 4°C. The gradient was fractionated, and aliquot of each fraction was checked for the presence of assembled DCND.

VDAC-1 production

Human VDAC1 was expressed, purified and refolded as detailed previously.[2-3] Briefly, the plasmid containing pET21d:hVDAC1 (VDAC1(1–283)-Leu-Glu-His_6) construct was transformed to BL21 (DE3) competent cells. Expression of hVDAC1 was carried out in LB medium and induced by 1mM IPTG at 37 °C for 3~5 hours. Cells were lysed and the inclusion bodies containing hVDAC1 were collected and solubilized in denaturing buffer (8 M urea, 50 mM Tris-HCl, pH 8.0, 250 mM NaCl, 20 mM imidazole). hVDAC1 was subsequently purified with Ni-NTA resin and precipitated through dialysis against dialysis buffer (50 mM Tris-HCl, pH 8.0, 50 mM NaCl, 1 mM EDTA, 5 mM DTT). The precipitate was collected and dissolved in 6M guanidine hydrochloride buffer. Refolding of hVDAC1 was carried out at 4 °C by very slow, dropwise dilution into 10x volume of refolding buffer (50 mL; 50 mM NaPi, pH 6.8, 100 mM NaCl, 1 mM EDTA, 5 mM DTT, 1% (43 mM) lauryldimethylamine oxide (LDAO)). The refolded hVDAC1 was further purified through cation exchange chromatography, from which the fractions containing properly folded hVDAC1 were pooled and concentrated for nanodisc reconstitution.

Transmission electron microscopy

For imaging, particles were adsorbed onto glow discharged carbon-coated TEM grids (Ted Pella) and then stained using a 0.7% (for the poliovirus samples) or 2% aqueous uranyl formate solution. The samples were visualized with a JEOL JEM-1400 TEM, operated at 80 kV in the bright-field mode.

Cryo electron microscopy

Gatan CP3 system was used to plunge-freeze a glow-discharged Quantifoil grids (EMS, Hatfield, PA) after the application of 3 µl of the poliovirus-DCND solution (blot times set to 3, 4 or 5s). Grids were transferred into an FEI F20 electron microscope operating at an acceleration voltage of 200 kV. Micrographs were acquired on a K2 Summit camera (Gatan, Pleasanton, California) in super-resolution mode.

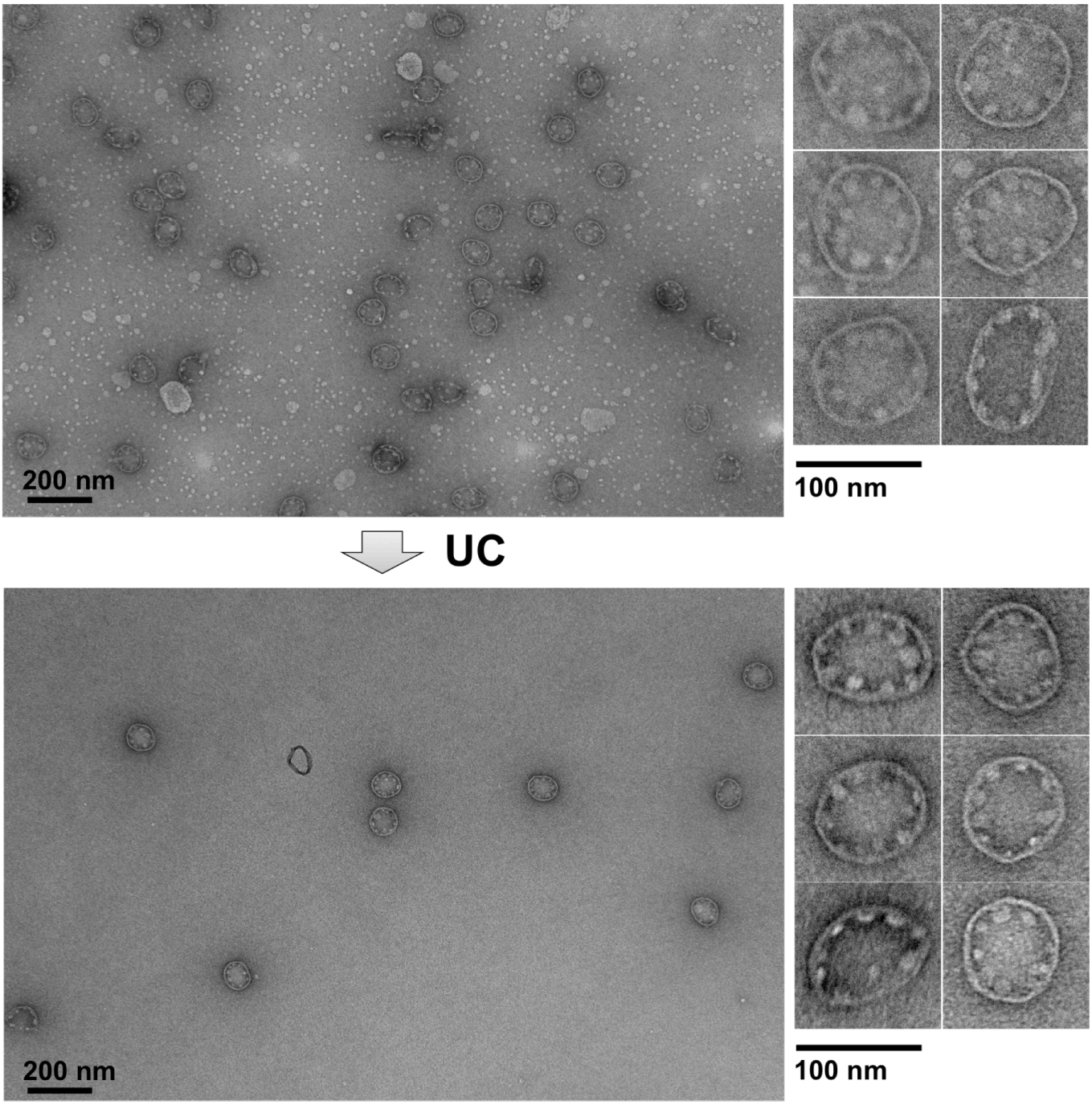

Figure S1. TEM analysis of 90 nm DNA-origami barrel after assembly with small nanodiscs. (top) TEM image of DNA-origami barrel after assembly with small nanodiscs. (**bottom**) TEM image after the ultracentrifugation (UC) step to remove uncoupled, free nanodiscs.

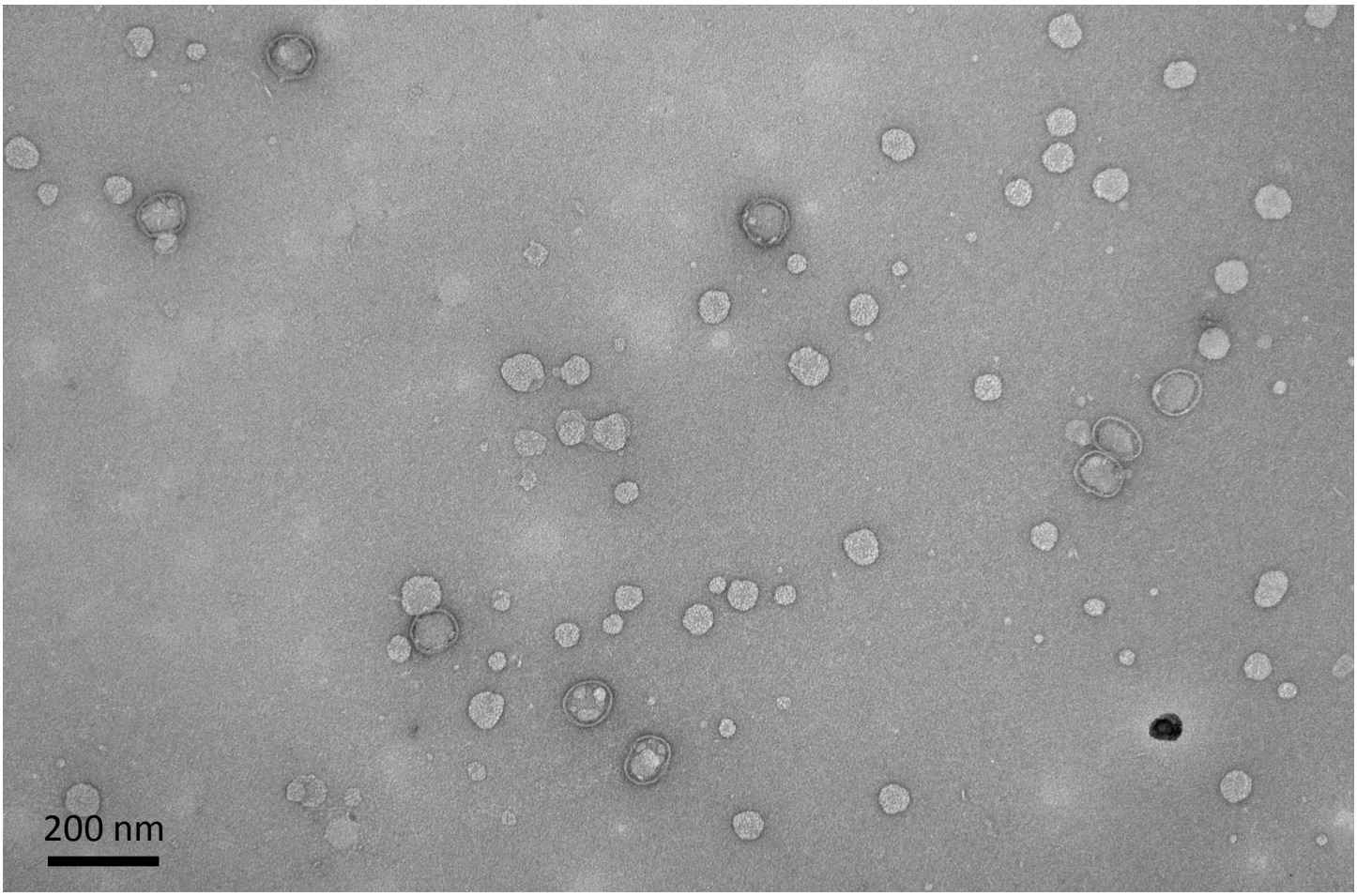

Figure S2. **TEM of DCND reconstituted inside 90-nm barrel**. Negative-stain images show the formation of integrated large sized nanodiscs inside the DNA barrel. The image also shows the formation of free lipid vesicles outside the DNA barrels. Reconstituted DNA nanostructures were separated from excess lipid vesicles by sucrose gradient at a later step. The POPC/POPG lipid mixture was used in the reconstitution of DCND.

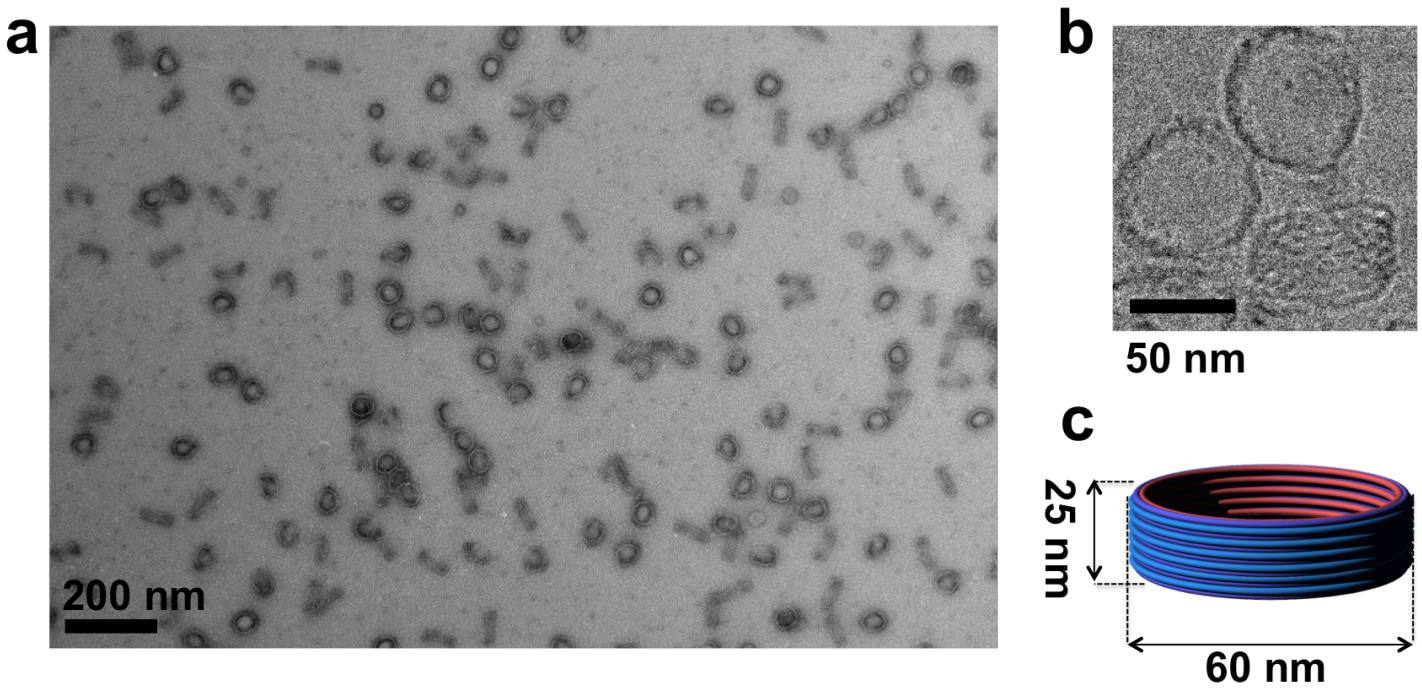

Figure S3. **TEM characterization of 60-nm DNA-origami-barrel without a bilayer.** (**a**) Negative-stain EM for the 60 nm barrel. **(b)** Cryo-EM of empty 60 nm DCND particles (lacking membranes). The Image shows the side and top views side by side. **(c)** The dimensions of DNA origami barrel**.**

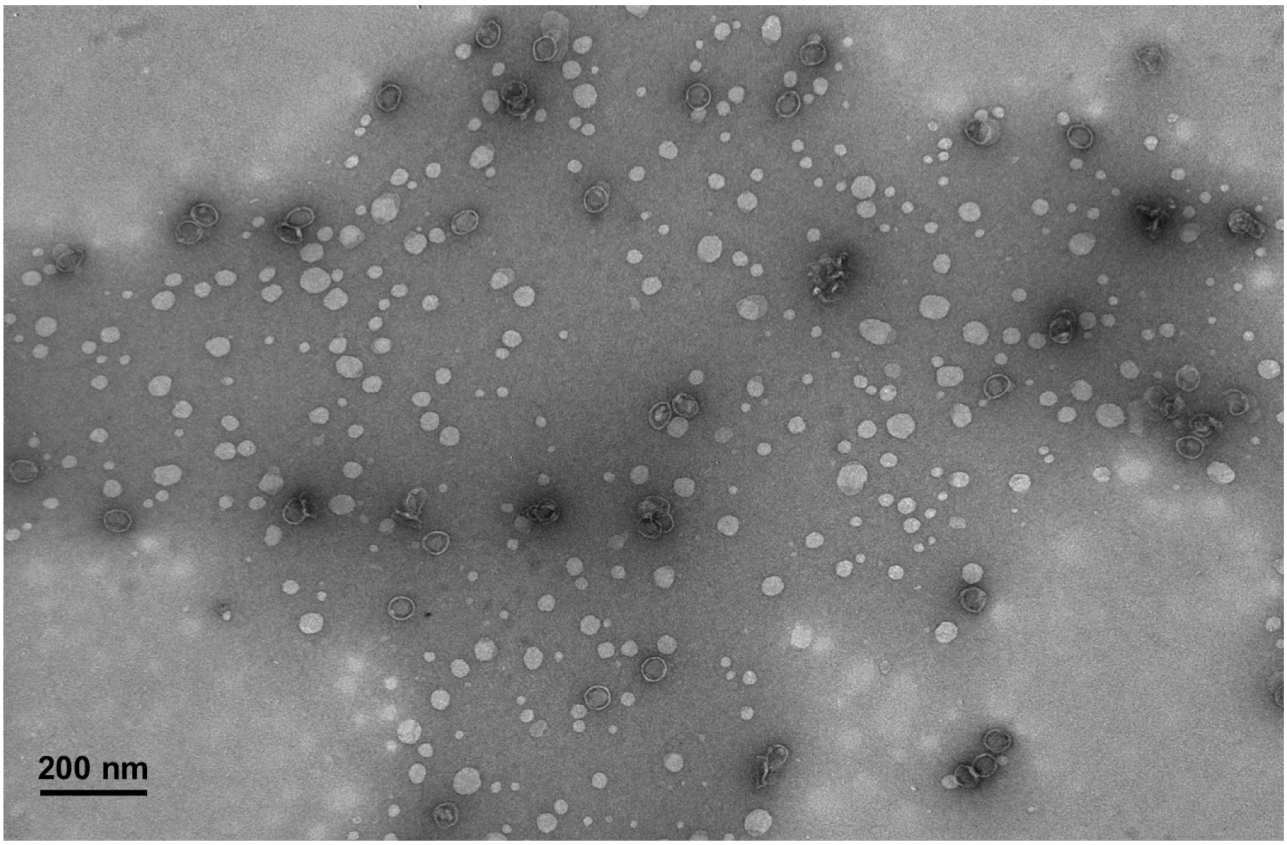

Figure S4. **TEM analysis of DCND reconstituted inside 60-nm barrel**. The image also shows the formation of free lipid vesicles outside the DNA barrels. The free lipid vesicles can be removed at a later step by sucrose gradient.

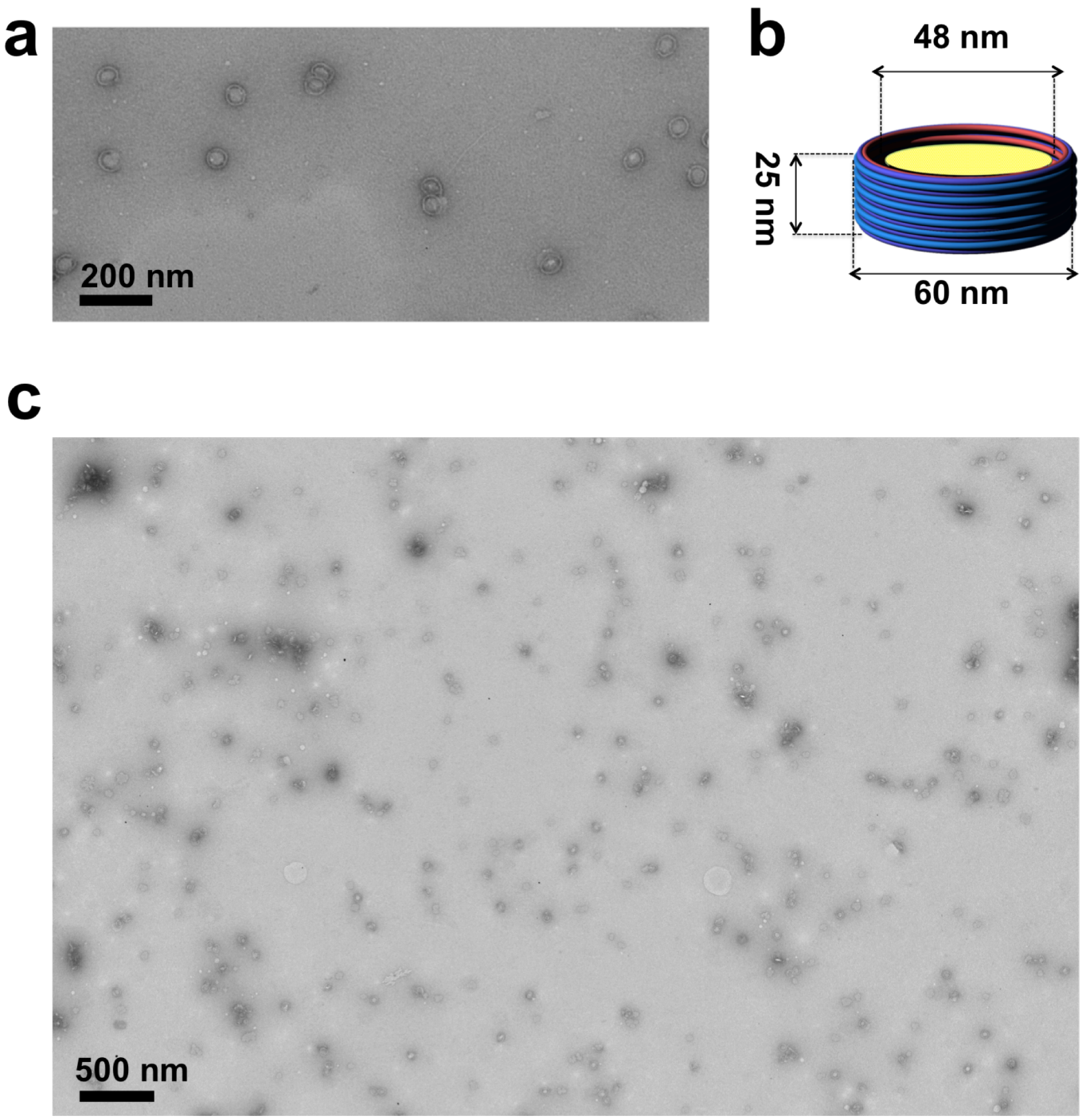

Figure S5. TEM analysis of 60 nm DCND after the sucrose gradient step. (a) Negative-stain image for the 60 nm DCND **(b)** The dimensions of the 60 nm DCND. **(c)** Zoom-out view of 60-nm DCND.

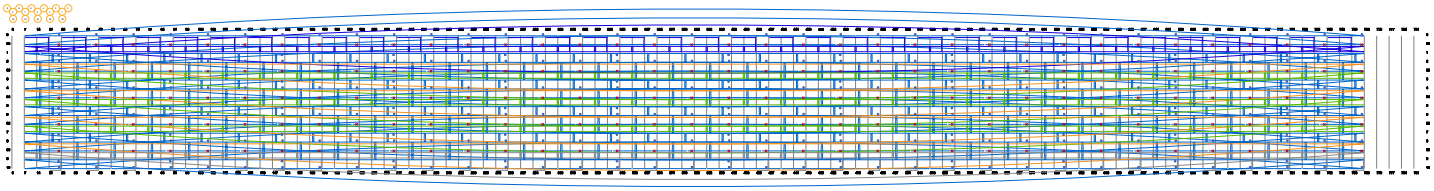

Figure S6. caDNAno design of 90 nm DNA barrels.

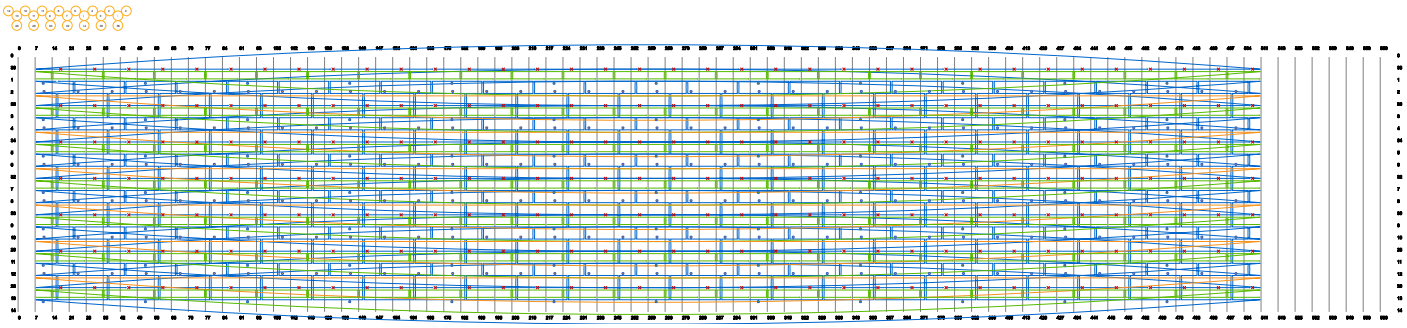

Figure S7. caDNAno design of 60 nm DNA barrels.

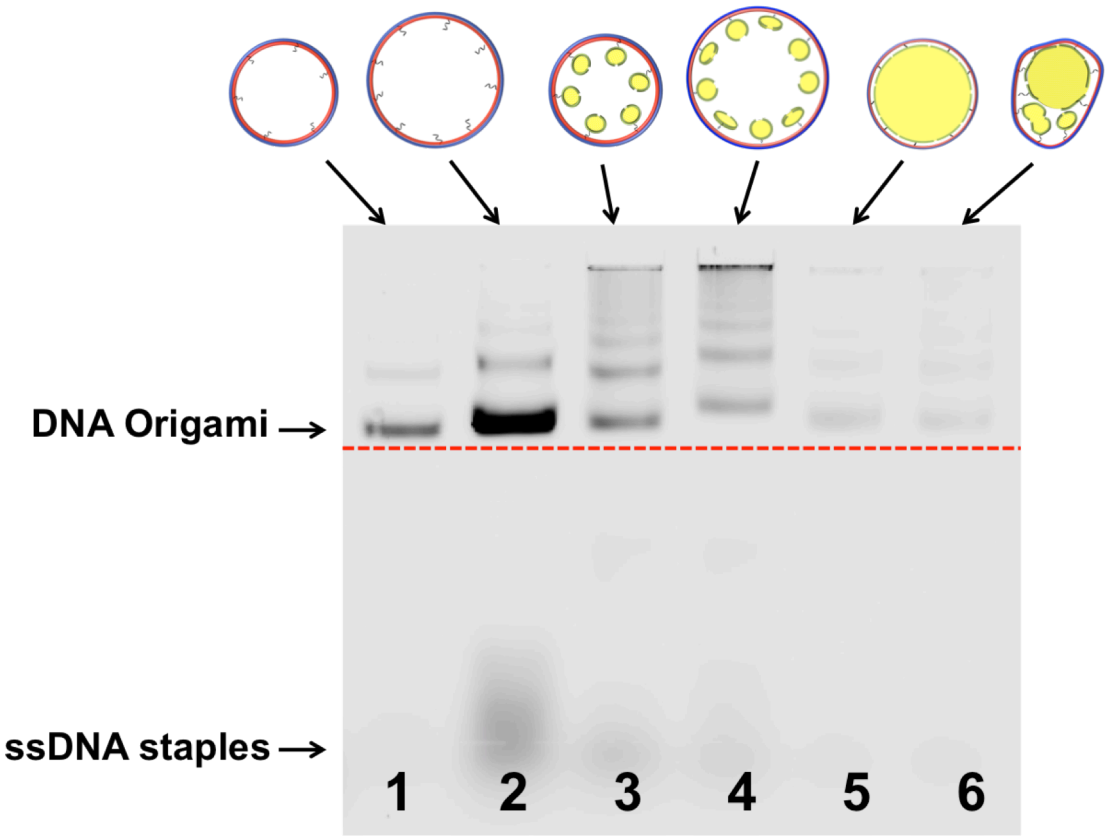

Figure S8. Native Agarose gel electrophoresis for DCND. Lane 1: folded 60-nm DNA barrel only, lane 2: folded 90-nm DNA barrel only, lane 3: folded 60-nm DNA barrel containing small nanodiscs, lane 4: folded 90-nm DNA barrel containing small nanodiscs, lane 5, 6: 60 nm DCND reconstituted with different amount of lipids.

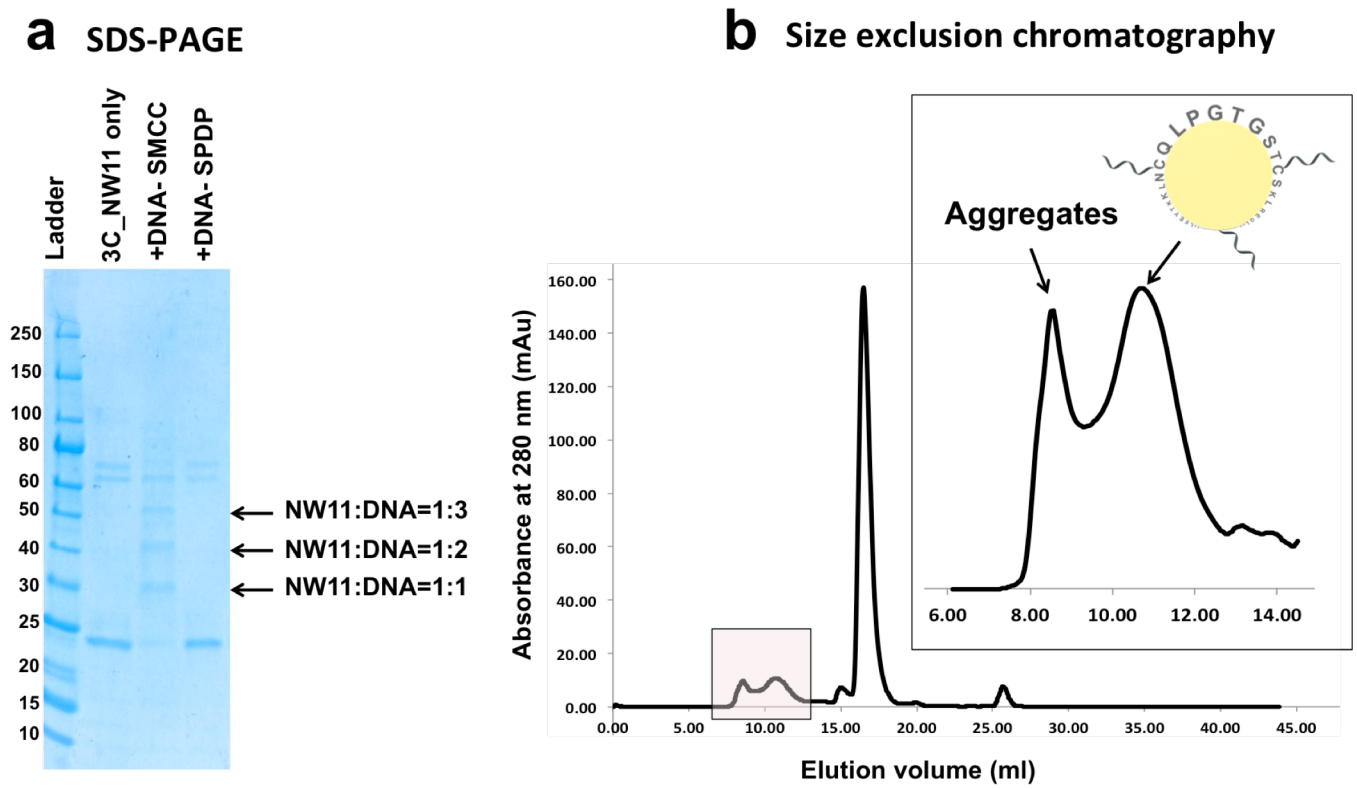

Figure S9. Coupling of DNA oligos to nanodisc. (**a**) SDS-PAGE of SMCC and SPDP coupling. SMCC coupling resulted in better yield. **(b)** Size exclusion chromatography was performed to purify oligo-nanodiscs from free oligos and aggregates.

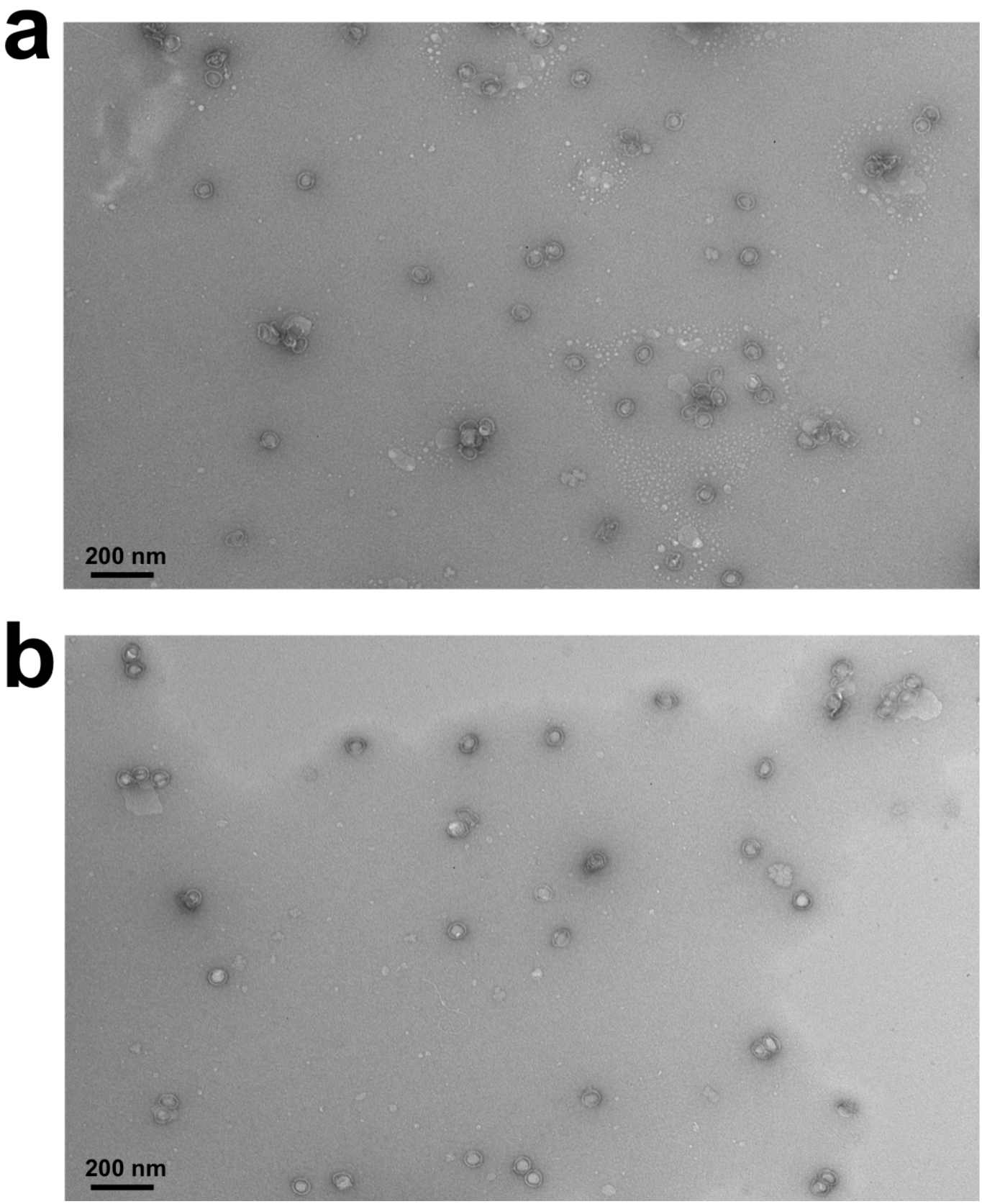

Figure S10. TEM analysis of 60 nm DCND before and after storage for 7 days at 4°C. (a) TEM image for the 60 nm DCND taken on day 1 after assembly. **(b)** TEM image for the 60 nm DCND after storage for 7 days at 4°C.

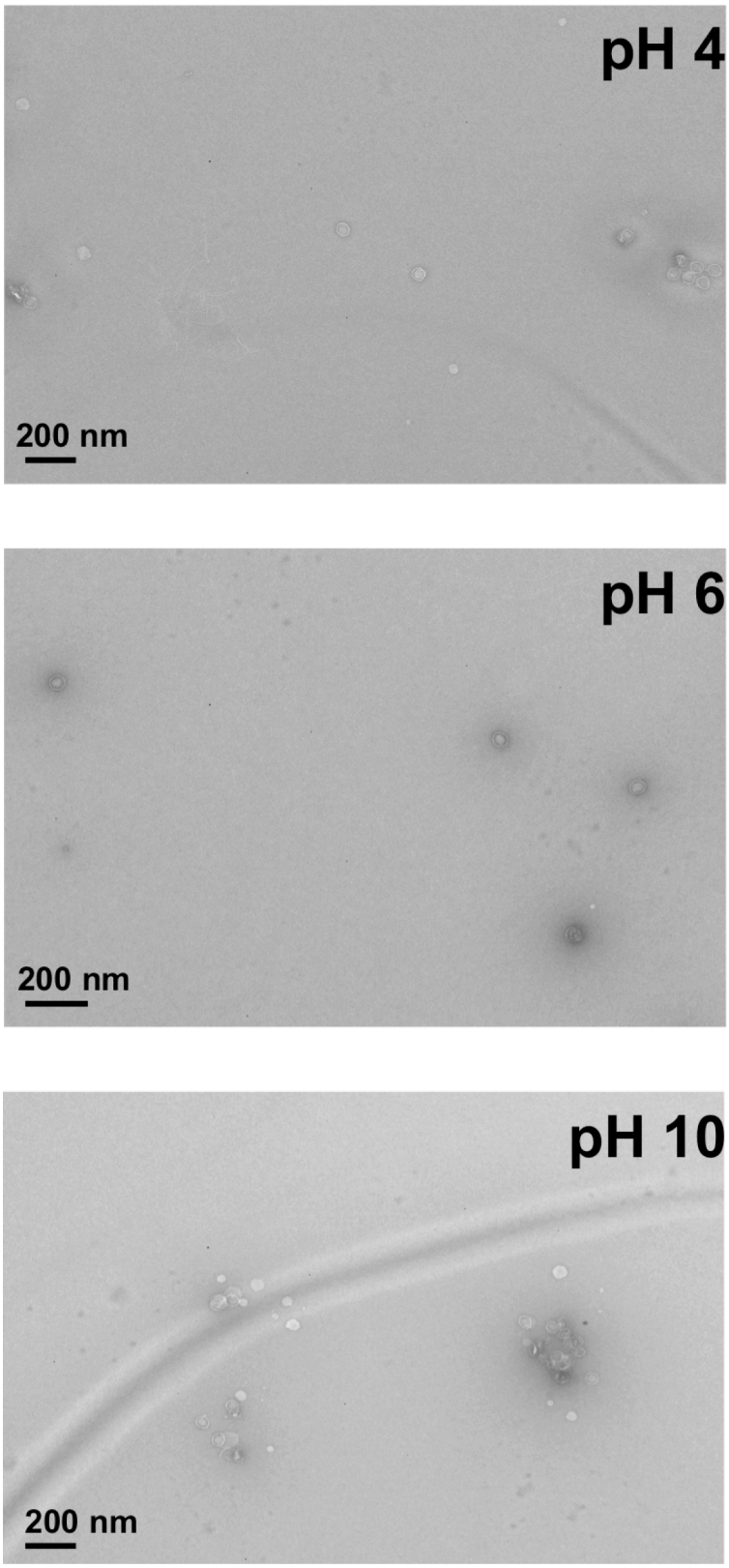

Figure S11. TEM characterization of 60-nm DCND samples in 1X TE-Mg^{2+} buffer at different pH.

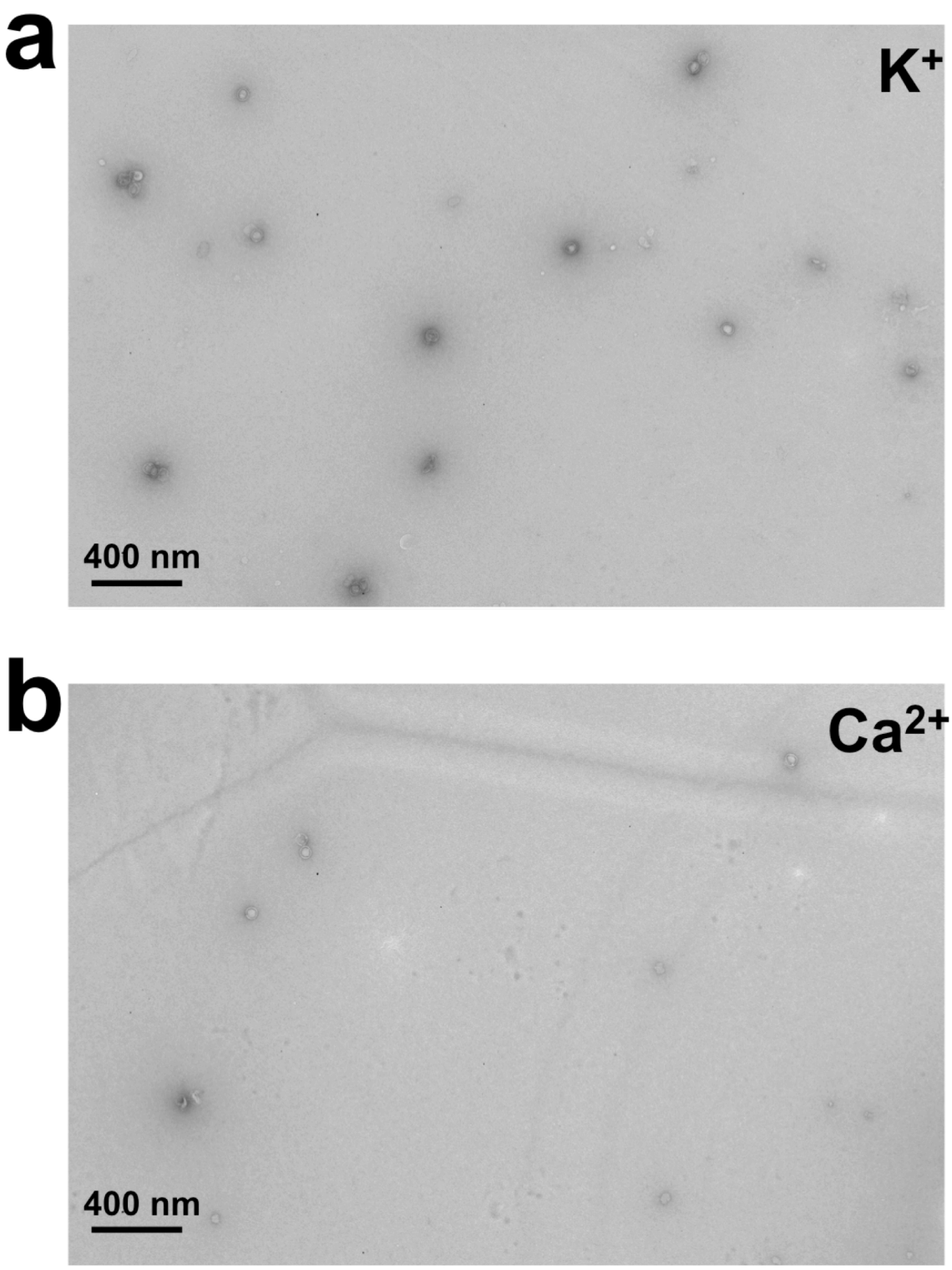

Figure S12. TEM characterization of 60-nm DCND in presence of 50 mM K^+ (a) and 10 mM Ca^{2+} (b) buffers.

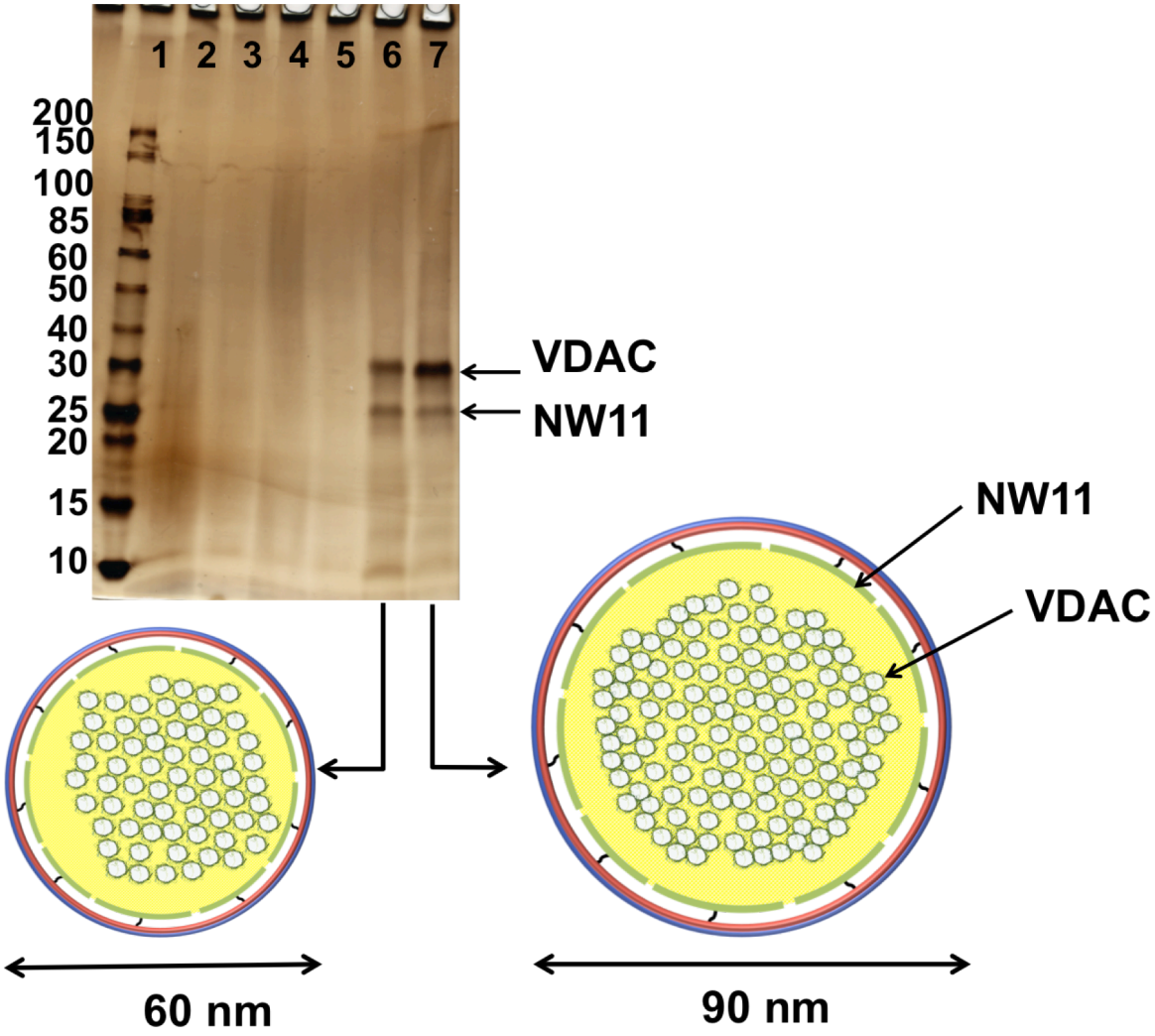

Figure S13. SDS-PAGE analysis for purified 60 and 90 nm DCND. Samples were visualized by silver stain. Lane 6: 60 nm DCND containing VDAC. Lane 7: 90 nm DCND containing VDAC. The analysis confirms the incorporation of VDAC into both 60 and 90 nm DCND.

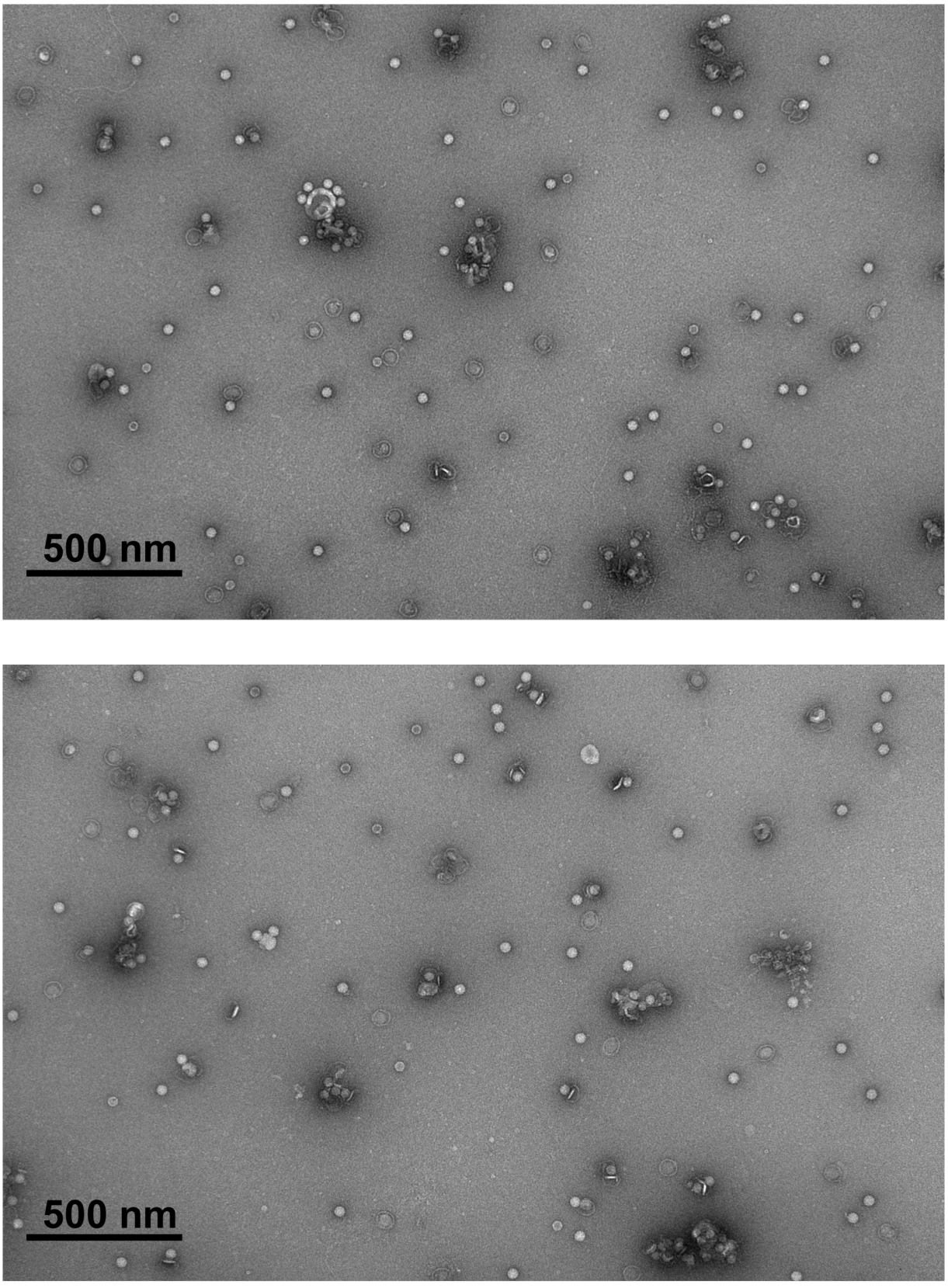

Figure S14. TEM images showing individual viral particles tethered to 60 nm DCND. The 60 nm DCND were prepared and stored for 6 weeks at 4°C before the incubation with the poliovirus.

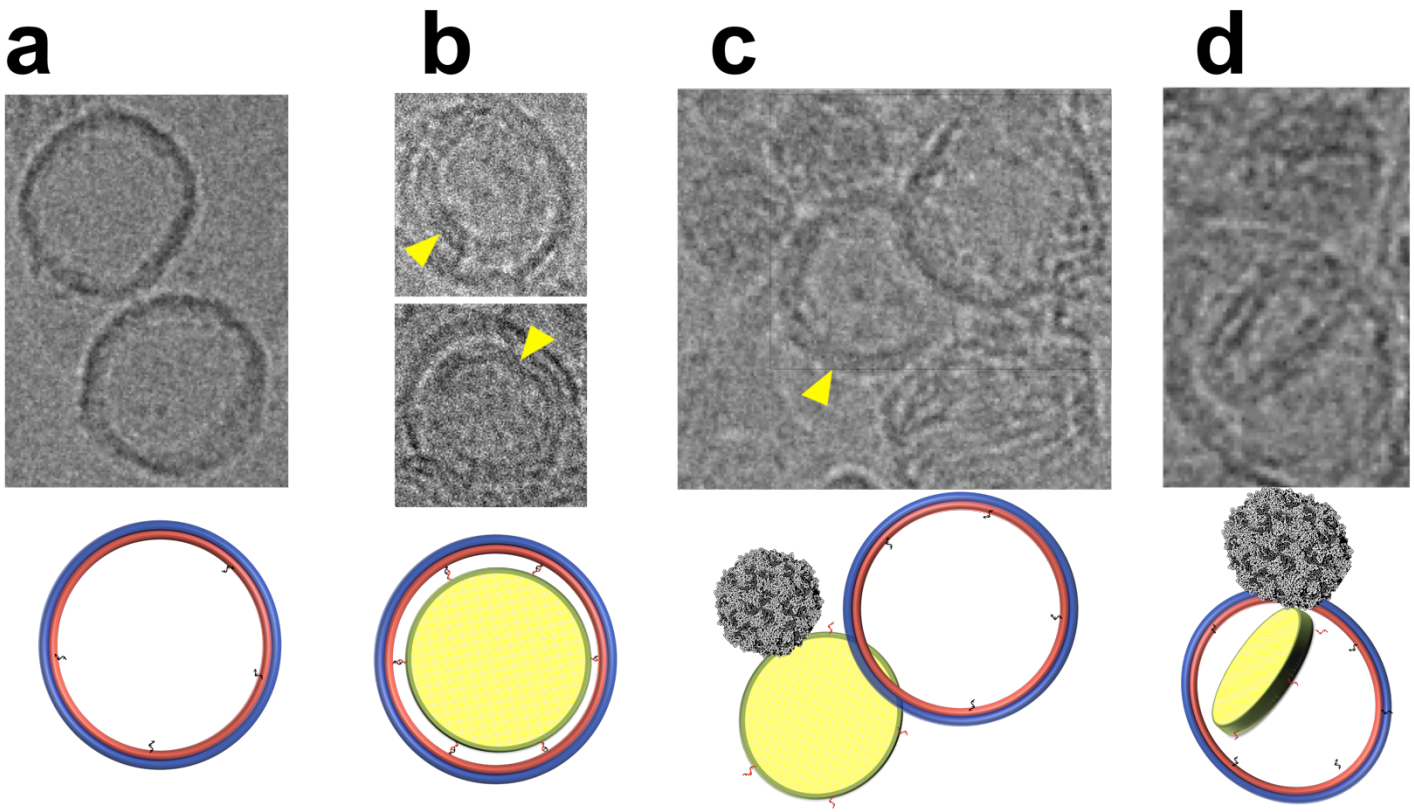

Figure S15. Cryo-EM analysis of the 60 nm DCND with and without poliovirus. (a) Membrane-free DNA barrel**. (b)** DCND particles. The yellow arrows point to the lipid bilayer boundaries. **(c)** Poliovirus plus DCND. The bilayer is partially separated from the DNA. **(d)** The bilayer is tilted within the DNA barrel.

3C-NW11 sequence

MGSSHHHHHHENLYFQGSTFSKLREQLGPVTQEFW**C**NLEKETEGLRQEMSKDLEEVKAKVQPYLD
DFQKKWQEEMELYRQKVEPLRAELQEGARQKLHELQEKLSPLGE**C**MRDRARAHVDALRTHLAPYS
DELRQRLAARLEALKENGGARLAEYHAKATEHLSTLSEKAKPAL**C**DLRQGLLPVLESFKVSFLSALEE
YTKKLNTQLPGTGAAALEHHHHHH

DNA Origami sequences

90 nm DNA-origami-barrel

Core staples

Oligo0	CGTGGACCGACCAGCAGTCCTCCGTCTGATTGCCCTAAGAA
Oligo1	CATCACTGGAGGCGAACTACGATGCGATCCGTATAATAATAA
Oligo2	GCCAACAACCCGCTCGGTTGCCCACCATGCCTGAGTTTCTG
Oligo3	CCACGCTGAAGCGCCCTTGAGCCATCAGAGATAGAACAGTG
Oligo4	GAAGTATTACGGTTAACCCCTTGACTTGAGAGCCAGGATTTA
Oligo5	ACTTCTGCCGCCTCGTCACTGGGCACATAGACTTTAATTAT
Oligo6	CGAATTAGTGGTTGAGCCGCAGTACGGAATAATGGAAGAGG
Oligo7	CATAGCGTGCCCAGATTCCGTGCGGTATTCATTTCATGAAAA
Oligo8	CTTCTGACCGAGTCTGTCGTAGCACCGATAGCTTAGTTCAT
Oligo9	ACATGTAGCGCACATGGAGCACAGAAGCCTAAATTTCGCCA
Oligo10	CAATCAACAACCCGCCGACGCGTGTCAATTTAGGCAAGAAAC
Oligo11	CTTGCGGTGCCGGTATTTGCTGCCAATTAATCGGCTCCCGA
Oligo12	TAGCAGCGCGCTCGCCTCATGTGCCAGGAGGTTTTGGAAAA
Oligo13	GAAAAGTCTGGATGGGCTCAGTTCGCACTTTACAGATTTTAA
Oligo14	TCACAATAGTGCCGGAGTCCTAGCTCTAAGCAGATATTTTG
Oligo15	CGGAAACCAGCAGACACAACGCCGCGCCAATAGAAAAAGGC
Oligo16	CACCCTCTAGGTAGTCCTCAAAAGAGAGTCACCAATAGCCGC
Oligo17	ATACATGTCGCGGCGGGACAGGTCACCAGAACCGCCGCGTC
Oligo18	CCAGGCGTGCAGCGACACAAGCCTTCGGCTTTTGATCAGTA
Oligo19	GTACAAAGCGATTGGAAGCTCGTACTGGATAAGTGCTCACCA
Oligo20	CGTTGAACGTGCCGGGCGGTTGTCCCGCTACAACGCTTTCA
Oligo21	GATCGTCTCCTGAAAAATGGGCGCTCAAATCTCCAATGCGG
Oligo22	ATACCAATCAGGATCGAAGGTGTAGGGACCCTCAGCGCGATT
Oligo23	TCATCAAGCCCGAGGAGGTGAGTTCGCGCGCGAAACGACCT
Oligo24	CCAGTCATGAACGGCAGCGTCGACCATGAGTAATCTTTATA
Oligo25	ACCAAAAGTCTTCCGGCGCGTACCTCGGGACGTTGGATAAAA
Oligo26	TCAGAAGCATCGGGCCACAATGTCCAGTAGCGAGAGTATAG
Oligo27	TATAATGATCGCGTCAATCCTCTCCAGCAAAGCGGACTGAA
Oligo28	TACTAATTACGGAGGACGGCGTATTGCCTGTAGCTCCAATTC
Oligo29	AAATGCAGCTCCAACCCGCCAGCCCGGAGTAGTAGCATTTT
Oligo30	ATGAACGAGGTTGCTCGATAGACGTCAATGCCTGAGAATCG
Oligo31	GGCCTTCACACTAGGGTTTGGATGCGTGTAATCGTAGCGTCT
Oligo32	CCAGCTTCAAACGTCGTTGGGCCGGCCCTGTAGCCATCCAG
Oligo33	ACGGCCATGGAGGAGGCACGAGGCGCATCCGGCACCAAACG
Oligo34	GGAAGCACTCAGAAGACGCACTTTGTCGTGCCAAGCCGAGCC
Oligo35	AACAGCTAGTACCGGTAGCCGCGCGCCTAAAGTGTACGGGC
Oligo36	GTTTGGACTCGGGCCTGCAACCGATAATCCTGGCCCTTCCA
Oligo37	TTGTAGCGGTGGTAGGCCACTCGCTGAACTCCTCGTTAACCG
Oligo38	ACACGACGGCGAGATGGGTCGACGGGAAACTCAAACCAGTC
Oligo39	GGTCAGTCCTTGTTCTCTTGAGCTACGCAGACCTGAGAGGC
Oligo40	CCGTCAAGCTCTCGGCAGTCGGCGTTTATTGAAAAATTAGAG
Oligo41	ATCAATAGTGGGCACGGGTTGGGCCAGTATTCGACAAATTC
Oligo42	GAATACCGGTTCATCAGCGCTGGGTGGTAGAACCTAGCTTT
Oligo43	TAATTAAGCTGAACTGGGTCTGCATAAAAGAGCAAACGCTAT
Oligo44	TTTTTCAGTGGAACTGCGCCCGCCGTATTCGCTGAGAAAAC
Oligo45	ATTGAGAACTCGCCCAGCAGTGTGACTAATGAAATAGCTTA
Oligo46	TCCCATCTTCACAACGCCGCGACTCCCATTTTCGAGATAATA
Oligo47	AACGCGATGTCTCCTGCATAGGTCGTGCTCTTATCACTAAG
Oligo48	GATTTTTGGGTTTGTGGGTATGGGTCCGGAAATCAAGAAAC
Oligo49	CAATAGCTCACGAAATTCTCGGCCGACTGACATAAAAAATAG

Oligo50 CAAAGACGTGAGACCGGGCCTTGCGCGTAAAAGTTAAAACG
Oligo51 ACCAGTACGTGGCCGACGCTACGGAGAACTGGTTTAAAATC
Oligo52 CACCACCCTGATCCAGCGGGAGTCTGCGCTCGATAGCAGAGC
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Oligo122 AGTATGTGCAGCCATGCCGAACGACCGACGCAATAATACGC
Oligo123 AAGGTGACTCCATGGCTCCAGGATAGCTACGACATTCATTA
Oligo124 TCATAGCCGGCGGGCTTGGCGGATTAGATCAGAATCTTTCGG
Oligo125 TTGGCCTGTGTCCCACCACTTCGTATGCCAACCACCGACGA
Oligo126 CGGAACCACAAAGTAGCCACCGCGTCCTGGTCAGTGTATTT
Oligo127 GAACCGCCAAGTCCCGGTCTGAAGGCGTAGGTGTATCCCTCA
Oligo128 GGGATTTTGGCGAGGAATTCCTACGACCATAACGATTGTAT
Oligo129 CGCCGACTCCCGCCACCCCGGCAGGGATGGTTTATCAGTTG
Oligo130 AAAATACGCGCGTGTCGACAAGACCTAAACTACAGAACGGGT
Oligo131 CGGTCAAGAGGCCAGCAGTGTGATGAGGTATTGTGTGCAGA
Oligo132 GAGTAGTACCGAGCGATACCAGAAAGCTCTAACAAAAGAAC
Oligo133 AACTAATGATGGCCGCGCTCAGGACCGAAAACAACAACATTC
Oligo134 ATTGAATCACTTAACAATGGCGCTTCTGCAGTAAAATATTC
Oligo135 CCAACAGGCTGGTCACCGTCGAGCGGACCTTCAAATAAACT
Oligo136 TTTGACCTAACGGCCGGCCTGGTTAGCGTTGGAAGTATTTAG
Oligo137 AAAAACAATACCGGGTCCCAAATCCCAATAATTAGCGTACC
Oligo138 TTAATGCTCCTGTCCGACGTCTGTTGCTTGCCGGAGATAAA
Oligo139 GTTAATAACGAATCCGCCGGGTGGGACCGAATCAGAGTAAAC
Oligo140 GTTGGTGTACCTGGCATGACGCAGGCCTTCCGTCGGGTCAC
Oligo141 TTACGCCACGGTGGCGTTCCCTGGACATAGCCATTCCGCTA
Oligo142 CGGGTACCTAGGTACGGTCGGCCTTGAAGGCGAGGCGATCCC
Oligo143 TGCATTAGCACAGAGACCCGCTATGGACGTTAATTGCCAGC
Oligo144 AAAATCCCGCAGTTGGGGCTCCTTCCGTCCCCCAGCAGGCG
Oligo145 ACAGGAACTTGTTACCTCGGCACGTCCTGAAAGGGATTTTAG
Oligo146 GCAACAGCGCCCGGCCCGACGGATTGCCGCCGCCAGCCATT
Oligo147 TCTTTAAGAGCGCTTTACGAGTTGGCTGAAATGGCTATTAG
Oligo148 ATCTGGTGCATTGGGCAGCCAAGTATATGACCCTCAATCAAT
Oligo149 AGTAACAGCGGTTGCCTGGAGGCCTGGCATTTAAAAGTTTG
Oligo150 AGATTTTGACACCACATTAAGAGCTTTTTAAGAAATTGCGT
Oligo151 CAATTTCCGCCTGACCCGCGAGATCCGCATAATTACATTTAA
Oligo152 TTTTTAATGAGCTCGGGGCTACATGGAATTGAGAGACTACC
Oligo153 CATAATTGGATCGGGCGGTGCACTGTCCCAAACACCGGAAT
Oligo154 TCCAGACCCGGTGCGCACACCCAATAGACTAAAGTAATTCTG
Oligo155 CAAGCCGATGGACCAGGCCGAGCTAGTGAATCGAGAACAAG
Oligo156 CCAACGCCATGGCCACTGCTAAGTGAGTTTCCTGAATCTTA
Oligo157 AGTCAGATAGCCATCCGGCGTACGTTTTAACACCCTGAACAA
Oligo158 ACTGGCACTATGTCGGATCCCGCGAGGGGATACCCAAAAGA
Oligo159 AGGTAAATGCTGGGTTAGCTCAGCGGGTGTTGAGGGAGGGA
Oligo160 ACTGTAGTCAGACGCCTCACCTGGACGTACCTTTAGCGTCAG
Oligo161 TTGACAGGATTGTCAGGGCACCGCTAGCGCCGCCGCCAGCA
Oligo162 TATAAACACGTGGGACCCCTTCGGACCGATAACAGTGCCCG
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Oligo165 TTTCTTAATCCAACACAGGCGCGGAGGTTTTTCGAGGTGAA
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Oligo180 GAGTCCACTATTATCACCGCTTTTCACCAGTGAGAAAGCCTGAT
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Oligo189 GCGGGGTTTTGCTGATACAGTTACCGTTCCAGTAAACCCTCAAA
Oligo190 CGAATAATAATTTCTGTAGCTAACACTGAGTTTCGCGTCGAGTA
Oligo191 TCTTTGACCCCCAAGCGAAAGTTAAAGGCCGCTTTAAAAAAGTG
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Oligo196 AGGAAGATCGCACGCTTTCACATCAAAAATAATTCAAACTAGTG
Oligo197 TCCACACAACATATTGCATGAGTCACGACGTTGTAGCTTCTGTC
Oligo198 CCATCACGCAAATTAGAATCTAGGGTTGAGTGTTGTGAGAGATT
Oligo199 ATAAAACAGAGGTAAGCGTACATTGGCAGATTCACTATCGGCGT
Oligo200 ATCAGATGATGGCACTCGTACTAACAACTAATAGATCTAAAGAG
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Oligo204 GCAAGAAACAATGAACAGGGTCCCAATCCAAATAAGATTAGTGG
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Oligo208 ATATTCGGTCGCTAAGGAGCTGAGAATAGAAAGGAAGACAGCAT
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Oligo228 TGGGCTTGAGATGATCAACGATAAGGGAACCGAACCTGATAAAA
Oligo229 CTCAAATGCTTTAGGGTAATAGATACATAACGCCACTAACGGAT
Oligo230 GATACATTTCGCACGGTGTCCAGGATTAGAGAGTAGAAAGACCC
Oligo231 GAGGGTAGCTATTGAGAAAGATGACCCTGTAATACGGCAAAGTA
Oligo232 TGGGCGCATCGTATAACAACTTGTTAAAATTCGCAGGTTGATGA
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Oligo241 GACGGAAATTATTCAACCGAATTAAGACTCCTTATTAACGGAGT
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Oligo243 CTCAGAACCGCCACACCGTATTAATGCCCCCTGCCCCTTGAGGG
Oligo244 GCTTGATACCGATAGCTTGCAGTAAATGAATTTTCCTAAAGTCC
Oligo245 GCCGGAACGAGGCCGAAATCGGAAGTTTCCATTAAGGCTTTGCA
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Oligo247 CCAGACCGGAAGCATCGCGTCGGAATCGTCATAAATGTTTAGAG
Oligo248 AGCTAAATCGGTTAAAATTATCTGCGAACGAGTAGTTCATTCAA
Oligo249 AAATATTTAAATTAAAGCCCCAACCGTTCTAGCTGACAGTCAAA
Oligo250 GTGCGGGCCTCTTAGGCTGCACCGTAATGGGATAGATTCTCCGC
Oligo251 GAAACCTGTCGTGCGTTGCGTAGTACCCGTATAAGAAGTCCGCG

Short inner scaffolds

Oligo252 TTGTGGGTCTGTTCGCCTCAACTCTGCTGCGTCGGTGCGA
Oligo253 TGTGTAGGCTACAGACGCTTACTGTCCGGCCGGCAGCTCC
Oligo254 TGAGGAGTCCCTCGAAAGCCGAGCCAACGTCCTCGCACTC
Oligo255 AGGAAATATCCGGCACCGGGTGGCCCAATCCAGCGCAACA
Oligo256 CTCGATGTCGGGATGGTGACGCGCGGACACGCTTGAGAAG
Oligo257 CGCGACCAGGGTTGGGACGGGATACTTGCGGCTAACGAAC
Oligo258 TGTCATGAAACGAACTCCGCGTCACGACTGGGACCGCCGA
Oligo259 CGGCGTTTCCGGTTGCATGTGTTCACATCGCTGGACGGAC
Oligo260 GTCGCAGCGATCGCACCTGTGAAACCATGCCAAGATGGCG
Oligo261 TCCATGACAGGCGCTAGAAATAACCCGGGTCGCGCGAACG
Oligo262 GGGTTCCACCGTGCTCGAGGGCGCTTGTCTCTAGCTGCAA
Oligo263 CACGTCAAGCGCAACTGGATGCAGACACCCGCAGCCCTTT
Oligo264 CCCATGCCACCCATCACCGTCCGCCCTCTAACGAACCTCC
Oligo265 CGCCACACCCAGGAGTATGCTCGCCTTAGCCTACTGGCCG
Oligo266 TGGCCGCCTCATGGGAAGCCTAAGACGTACGTGACCCACG
Oligo267 TGTACTATGGAGTGTCCTTGGGTGCATGGTGGGCCGCCCG
Oligo268 GCAGTAAGGCAGCTGAACAGTGATACCGGTGCGGGCCTGG
Oligo269 GGCGGAGAATACACAGGCTCGCTCAGGCTTCGCGTGGGAT
Oligo270 CTGCCCATGTCGGCGGTCGCAAGAGACTCAGGTTCCACGA
Oligo271 ACGTTGGCATGAACGCCGACTGACGAGCTGCTGATTTGCG
Oligo272 ACACTTCGCTTAGCCGCCGCTTCTCAGCAGTCTTGCCTCC
Oligo273 TTCTCCGCATAAGCCGCCCACCTCTCCGGATCGCACCAGA
Oligo274 CATTGTCACCGCGCGTCAAGCAGCCACCAGAACTCGCACT
Oligo275 CAGCTCGAGGAGCGCGGATGCAGCCATTCGTACGTCCTGA
Oligo276 GGGAAAGTGTCGGTCGTTCGGCATGGCTGCGCTATCCTGG
Oligo277 AGCCATGGAGCTAATCCGCCAAGCCCGCCGCATACGAAGT
Oligo278 GGTGGGACACGGACGCGGTGGCTACTTTGTCGCCTTCAGA
Oligo279 CCGGGACTTGGTCGTAGGAATTCCTCGCCATCCCTGCCGG
Oligo280 GGTGGCGGGATAGGTCTTGTCGACACGCGCCTCATCACAC
Oligo281 TGCTGGCCTCGCTTTCTGGTATCGCTCGGTCGGTCCTGAG
Oligo282 CGCGGCCATCAGAAGCGCCATTGTTAAGTGTCCGCTCGAC
Oligo283 GGTGACCAGCGCTAACCAGGCCGGCCGTTATGGGATTTGG
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Oligo286 ACGCCACCGTTCAAGGCCGACCGTACCTAGTCCATAGCGG
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Oligo288 GGGCCGGGCGAGCCAACTCGTAAAGCGCTCTATACTTGGC
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Oligo292 TGCGCACCGGACTAGCTCGGCCTGGTCCATCTCACTTAGC
Oligo293 AGTGGCCATGAAACGTACGCCGGATGGCTACCTCGCGGGA
Oligo294 TCCGACATAGCCCGCTGAGCTAACCCAGCACGTCCAGGTG
Oligo295 AGGCGTCTGACTAGCGGTGCCCTGACAATCGGTCCGAAGG
Oligo296 GGTCCCACGTGACGTCGGTAGTGCTCGTGGCTGCACATGT
Oligo297 GGGTATCCAGCCTCCGCGCCTGTGTTGGATCTGCTGTTGC
Oligo298 ACGTTTGTCCATACACCGACAGGCGCACATCGTGGAGGCG
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Oligo300 ACGACACGACCCAGAACATACGGCGCACTCAGTCACGTGC
Oligo301 CCGGGCAACGCCTATGGCACCAAGACCTGACGGATGTTCG
Oligo302 TTTTGCCTGAACCGGGACGCCTCTCAGAGCGCGGCAAAGT
Oligo303 GTGTCCGAAAAACTGCTCGCCGGGCTAGCCACAGGAGACG
Oligo304 GTGGCAATAGCAATACGGGAGCCGCCGACACGGAAGGAGC
Oligo305 CCCAACTGCGGGACGTGCCGAGGTAACAAGGCAATCCGTC

Handles to hybridize with nanodisc-DNA conjugates

Oligo306 CCGAGCGGGTTGATGGCTCAAGGGCGCTTCAAGTCAAGGGTTTTTTCTTCACACCACACTCCATCTA
Oligo307 GTTAACCGTATGTGCCCAGTGACGAGGCGGCCGTACTGCGTTTTTTCTTCACACCACACTCCATCTA
Oligo308 GCTCAACCACTACCGCACGGAATCTGGGCACGGTGCTACGTTTTTTCTTCACACCACACTCCATCTA
Oligo309 ACAGACTCGGCTTCTGTGCTCCATGTGCGCTGACACGCGTTTTTTTCTTCACACCACACTCCATCTA
Oligo310 CGGCGGGTTGATTGGCAGCAAATACCGGCACTGGCACATGTTTTTTCTTCACACCACACTCCATCTA
Oligo311 AGGCGAGCGCTGCGAACTGAGCCCATCCAGAGAGCTAGGATTTTTTCTTCACACCACACTCCATCTA
Oligo312 CTCCGGCACTGCGCGGCGTTGTGTCTGCTGTCTCTTTTGATTTTTTCTTCACACCACACTCCATCTA
Oligo313 GGACTACCTAGGTGACCTGTCCCGCCGCGACGAAGGCTTGTTTTTTCTTCACACCACACTCCATCTA
Oligo314 TGTCGCTGCACAGTACGAGCTTCCAATCGCCGGGACAACCTTTTTTCTTCACACCACACTCCATCTA
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Oligo316 TCGATCCTGAGCGAACTCACCTCCTCGGGCATGGTCGACGTTTTTTTCTTCACACCACACTCCATCTA
Oligo317 CTGCCGTTCACGAGGTACGCGCCGGAAGACCTGGACATTGTTTTTTCTTCACACCACACTCCATCTA

Oligo318 TGGCCCGATGCTGGAGAGGATTGACGCGATGCAATACGCCTTTTTTCTTCACACCACACTCCATCTA
Oligo319 GTCCTCCGTACCGGGCTGGCGGGTTGGAGCTGACGTCTATTTTTTTCTTCACACCACACTCCATCTA
Oligo320 CGAGCAACCTACGCATCCAAACCCTAGTGTGGCCGGCCCATTTTTTCTTCACACCACACTCCATCTA
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Oligo322 GTCTTCTGAGGGCGCGCGGCTACCGGTACTAGACGGAGGATTTTTTCTTCACACCACACTCCATCTA
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Oligo324 GCCTACCACCTCCCGTCGACCCATCTCGCCCGTAGCTCAATTTTTTCTTCACACCACACTCCATCTA
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Oligo326 CCGTGCCCACCCACCCAGCGCTGATGAACCTTATGCAGACTTTTTTCTTCACACCACACTCCATCTA
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60 nm DNA-origami-barrel

Core staples

Oligo0 ACGTGGACGGGACTGCGCGAGGGTAGGACCAGCTGCAAAAGA
Oligo1 GTGGCACAGTGGGCACACTCGAGAACTCCCGTTGTAATAC
Oligo2 GAGCACTAACGGGCCCGAATTGAGGGCAAGACAATATTTTAG
Oligo3 CATATCACTGAGGATATGAGACGATGACAACTAATCCTAC
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Oligo65 GTCCAATCGCAAGGTTCTCCGACTCAACATTTAGGATAGC
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Oligo67 TTAAGCAGAGTGCTGTGTTTCGACGGGCTGATTCCCAAAA
Oligo68 CATTGCCTGAGAGTCGTGGTACGATGTGATAAGGATACAGGT
Oligo69 ACCCGTCAGATAGGCTGGCTCCGTGTGACCCAAAATAACA
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Oligo71 GTGAGCTGGACTGTGAGCGACCGGTCGGAGCGAGGAATGA
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Oligo81 TATTACGACATTCCCATTAGCCGGGATAAACAATGCTCCT
Oligo82 CGGAAACGCGACGATTTGCAGCGCTTCTCAAAGGGCGAAGGC
Oligo83 AGGTTGAACGAGCAGACCGGCACGGGTCATAATCAACAGG
Oligo84 GTTTTGCTGTGCGTCGAGGCGACTAACTGGGTAATAAGCGGG
Oligo85 TCTTTCCTAGTTGGGCCGGTCCCGTCCACCACCCTTGTCG
Oligo86 CAGGGAGTCGGGATGCCTTCTGCTTCTCAGAAGGCTCGCTTG
Oligo87 GAAATCCGGTGCGAACTGTGGCCGACTATAATGCCGTGTC
Oligo88 TGAATTACGGGAGTCTGCCGAGAATGGCGCGAGTAATCATTG
Oligo89 GTAATAGCGTGCTTTTCCATGTCGGTCTCATTCAAAGGGG
Oligo75 TCCTTTTGCTACCTGGGCCACTGCACGCTATTATAGTATTGC
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Oligo203 AACTGTTGGGAAGCGCACTCCAGGAACGCCATCAAAAATTGTAAT
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Oligo232 CAAAGTTACCAGACTTTTTAAAACAGCCATATTATTTCCAGAGCA
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Oligo238 GTTAAATCAGCTCATATTTTAAGGCCGGAGACAGTATGTGTAGCT
Oligo239 TAATCATGGTCATAAGGATCAGGCAAAGCGCCATTTTTCCGGCTT

Short inner scaffolds

Oligo240 ACTCCCTGATAGGCGTCTTTCAGGCGGGCGGGACTCAGG
Oligo241 GGGACGCCAGACTGGGCTCACGAGTGATAGGCTTTCCGC
Oligo242 TTCGGCTTAGGTCCTTCTCGTGTGGTGCCCGCTGCCCTT
Oligo243 GCGAGCCACGCGGCGTGTAACCAACAGCATTTTCTCGCT
Oligo244 TGCGGCAGCATCAAGGACCTACCATCTTGACCGGTCGCC
Oligo245 ACTCCAGTTGCTAGAACGGTCTCGCGCACACACGGTGGG
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Oligo247 GTGCCCGTTGTGAGTCGGAGAACCTTGCGCGGCCTATTG
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Oligo250 AACAGCCTTGGACCGGTCGCTCACAGTCCTTTCGTGCCC
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Oligo252 AGAAGGAAAGTCTCACCGGGCGCCCTAGGCCTTCCGAGG
Oligo253 GCCTCAATGCTCTTCACCTGGGTCCATGCCCGGACGGAG
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Oligo258 AGCAGCGGCATCTCCAGTTTTGGGCCAGGGTTGGGATCC
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Oligo264 GGCGCTATACTCGGCTCTCGGCTGGGTCCGTTCCTTCAC
Oligo265 TCACGCAGTTCGCCCGATCCTACACCTGTCCGTCACCAC
Oligo266 AGTTGATGCCGTGGCACAAGCAGAGGTCTGGACGCCCAG
Oligo267 TCGCTGGCGCGCGGTGAGTGTTGAGCAGAGAACTTCTGT
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Oligo270 ATGCCCGGATCGTAACTAAGACGCCGGCCGGTCCGTATG
Oligo271 CGCGGCCCGGAGCAACCACTGCCCAGTCAATATGCTACA
Oligo272 CGCGCGACGACGACAATCATCGAACGAACACCATGTGGC
Oligo273 CCGACAGGGTTTCTCGACGCCCACGTAAGCTCCGAGGTG
Oligo274 ACACAACAAGGCACACGTCCTCTTCGGGCCACCATCCGG
Oligo275 GCGGTGCACAAGCCTGGCGCTGTTGTCTTTATGCCCGAT
Oligo276 TGGTGTTCCCTTTCGCCGCCTTGCAAGTCCCATCATGCC
Oligo277 GCAAGAAGCCTACGCGAAGCTGTCGGGACCACGGCAATG
Oligo278 CTCCGTGCTTGTTACCTTCGAGTCGCGGCACACCACACG
Oligo279 TCTGCTCCCGTGAATCCCGCTCGTTCGTTCTCCCGGCTA
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Oligo285 GGTAATGCGTTCGGGCTACTGCGGTGTGAGATCGTGCGG
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Oligo287 CGAAGGGACGCGGGAGGCTAGGGAATGCTGCATCAGTGG
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Oligo289 AGAGGCCTCCTCAGCGGTCCTACGCGGTCTTGGACGATG
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Oligo292 CACCCGATGTGTTATGACGTGCGTCGCCCTCGAGCACCT
Oligo293 AGCATACAGAGGGCAGCTTGGCAGGGCCGCGAAGTTAGG
Oligo294 GACGCCCGATTGTCCTTGTTCGCCATGCCGCAAGGTGAG
Oligo295 GGTGGCGAGATGACGACAGTGCAGAAGGTGGCGGTAGGG
Oligo296 TCTGTGAAGCGGGTGGAGTACGGAGGTGTTCTTCGCGCC
Oligo297 TTTTGCGCAATCATGGCAGCGGACTCAACACCCTCCGGC
Oligo298 GACCTCGGAGCTAGACGGGAGTGTTGCGTGTGCAGGGTG
Oligo299 CGGTTCGAGTGCGCAGTGCTTAACGTGAAGCGAGGGAGG

Handles to hybridize with nanodisc-DNA conjugates

Oligo300 TCGGGCCCGTCATCGTCTCATATCCTCAGCAACTGCGGGTTTTTTCTTCACACCACACTCCATCTA
Oligo301 GGGCGACTGGCTACCGCTGCTACCCAGATATCACCGCACTTTTTTCTTCACACCACACTCCATCTA
Oligo302 TCGTGGGCAAGATATATGTGGTCCGGGATGGGCGGTGCGTTTTTTCTTCACACCACACTCCATCTA
Oligo303 CGGCCCACAGGGTCAATAGCCGTCGCCTCACCCGACTTATTTTTTCTTCACACCACACTCCATCTA

Oligo304 GAACCGCTAGCCTCGGCCGCGCATTTACACCTTGGTCCATTTTTTCTTCACACCACACTCCATCTA
Oligo305 CGGTCCAGCACCATGGTCCCGTACCCTCACTCGCCATTGTTTTTTCTTCACACCACACTCCATCTA
Oligo306 GCACAACTCCTGAACAAGGCGCCCAGTGCAGTCGACGTCTTTTTTCTTCACACCACACTCCATCTA
Oligo307 CGACTAGAGCTGCTGTGCGTCCCGTTGCGTGAGTTAGGCTTTTTTCTTCACACCACACTCCATCTA
Oligo308 CCTACCCTCGGTGCCGAGTCCCTAGACGCTGGGCCAGTATTTTTTCTTCACACCACACTCCATCTA
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Oligo322 CTCAGGAAGCGAGCAGTAAATCCTTGGCCGACCGCAGCGTTTTTTCTTCACACCACACTCCATCTA
Oligo323 GAGCAGAGCAGCTTATCCCTGGGTCTCCGGCATCTGCGCTTTTTTCTTCACACCACACTCCATCTA

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