Virus-Inspired Membrane Encapsulation of DNA Nanostructures to Achieve *In Vivo* Stability

Authors

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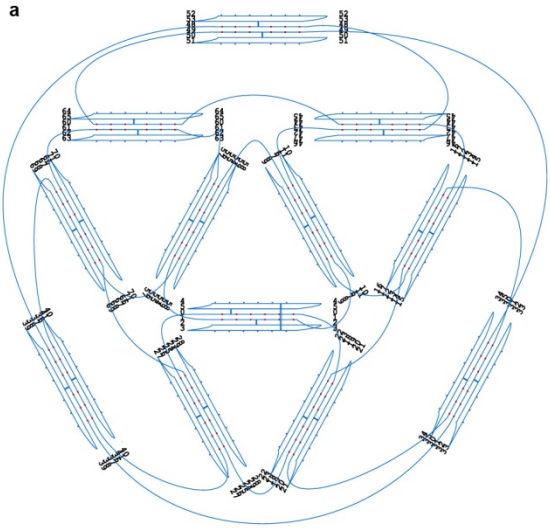
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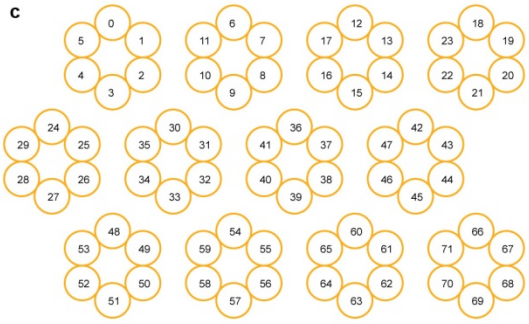
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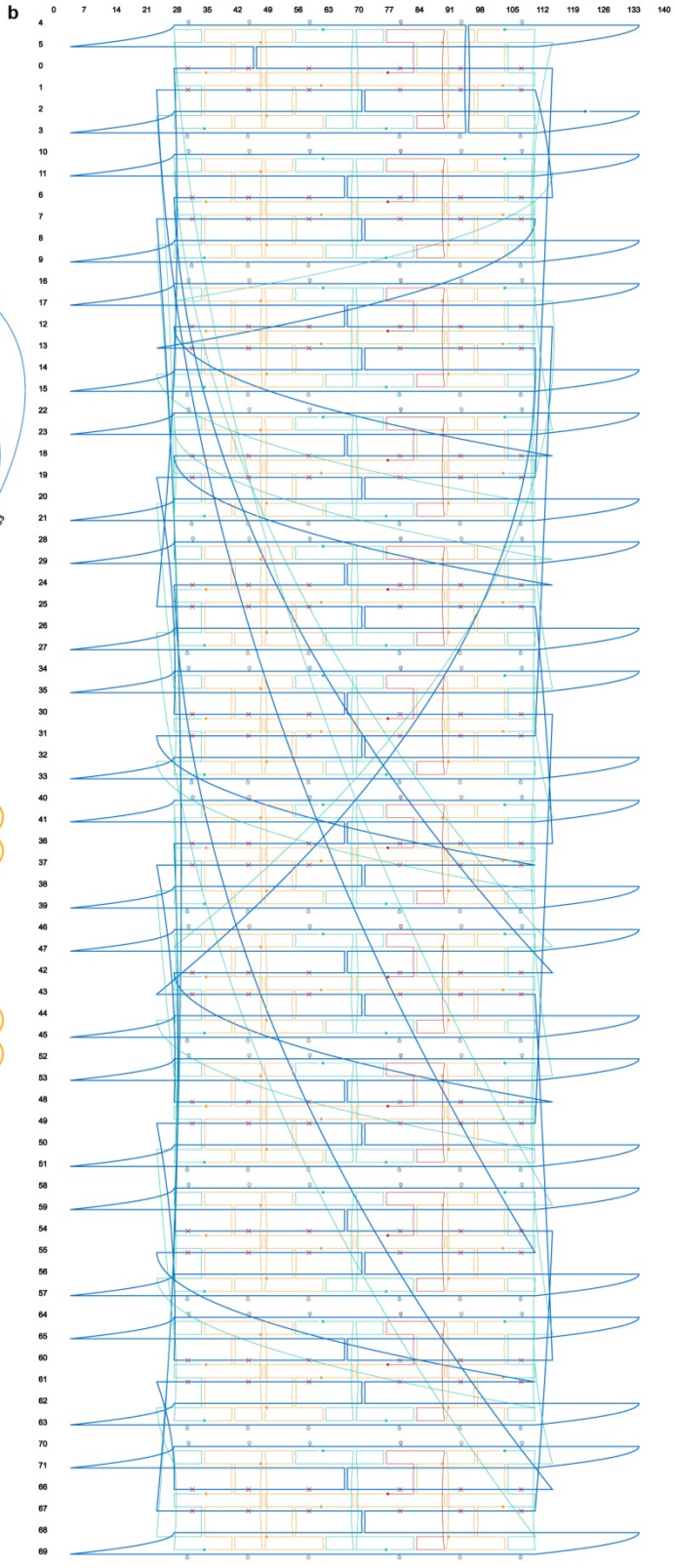
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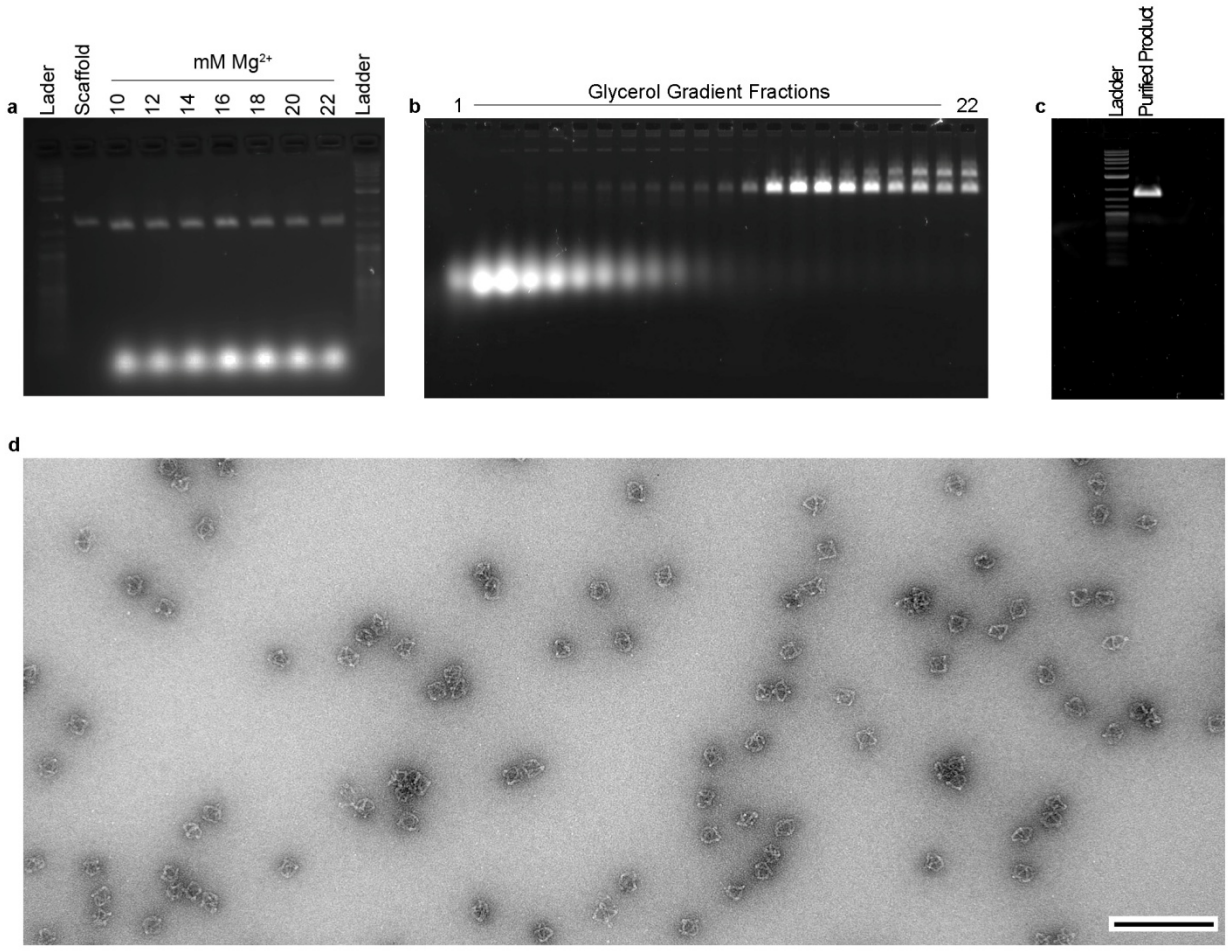
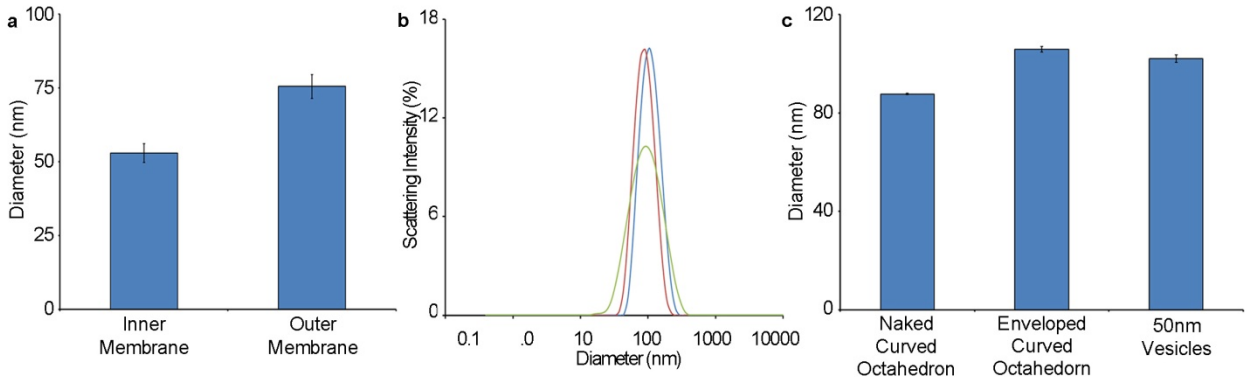
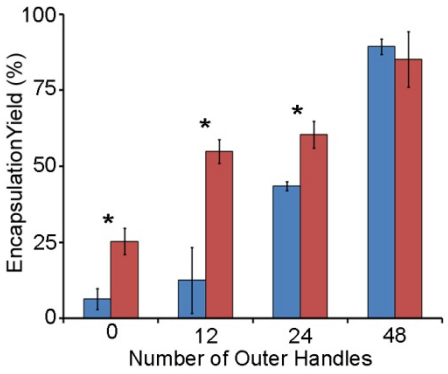
Supporting Information Fig. 1. Schematic of the DNO design and scaffold routing. (a) Schlegel diagram illustrating the routing of the p7308 scaffold through the 12 struts. **(b)** Staple oligonucleotide strand and scaffold organization from caDNAno. **(c)** Numerical **o**rganization of helices into six-helix bundles for the 12 struts. **Note**: the aspect ratio of the strut width (estimated to be 7.5nm based on known helical properties) to the DNO diameter shown illustrated in **Figure 1** is not meant to be to scale. Discrepancies between the illustrated model and TEM data could be due to less actual curvature in struts than anticipated from design, expansion of the DNO due to the presence of 5T linkers between struts, and errors in experimental measurement of DNO diameter.



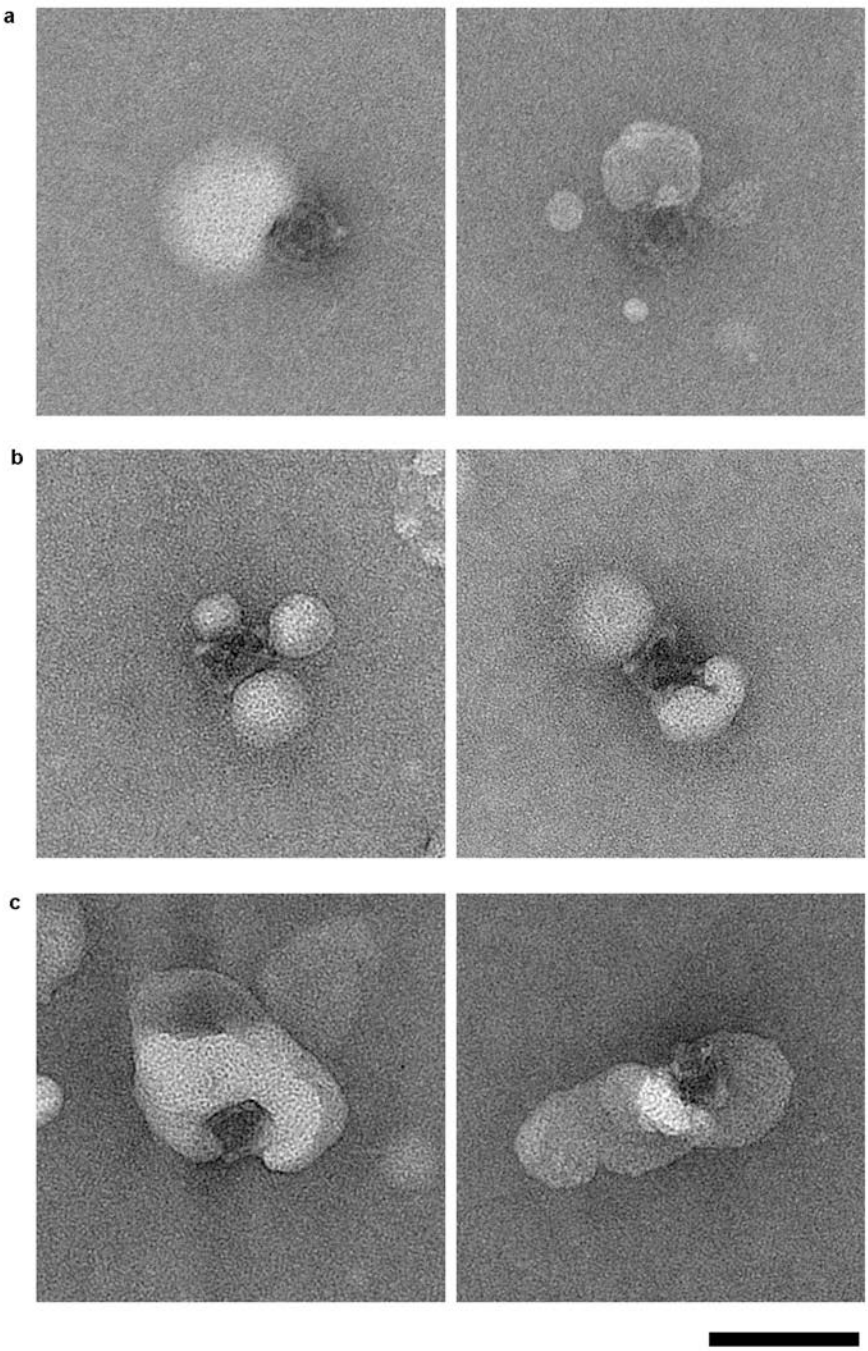
Supporting Information Fig. 2. Synthesis and purification of the DNO. (a) Folding of the DNO was tested over a range of 10-22mM Mg^{2+}, and 14mM was chosen as the best condition based on analysis from agarose gel electrophoresis and negative stain TEM. **(b)** Large quantities of structures were folded, concentrated, and separated *via* ultracentrifugation through glycerol gradients. The gradients were fractionated and an aliquot of each fraction was loaded into an agarose gel for analysis. This representative gel shows the excess staples remained at the top (fractions 1 ~ 12), and folded product migrated into the lower half of the gradient. Appropriate fractions were combined and run through a second glycerol gradient to achieve a high purity. **(c)** The final product was analyzed by agarose gel electrophoresis, showing a single product band, **(d)** as well as by TEM, which revealed a homogenous population of nanostructures. Scale bar = 200nm.



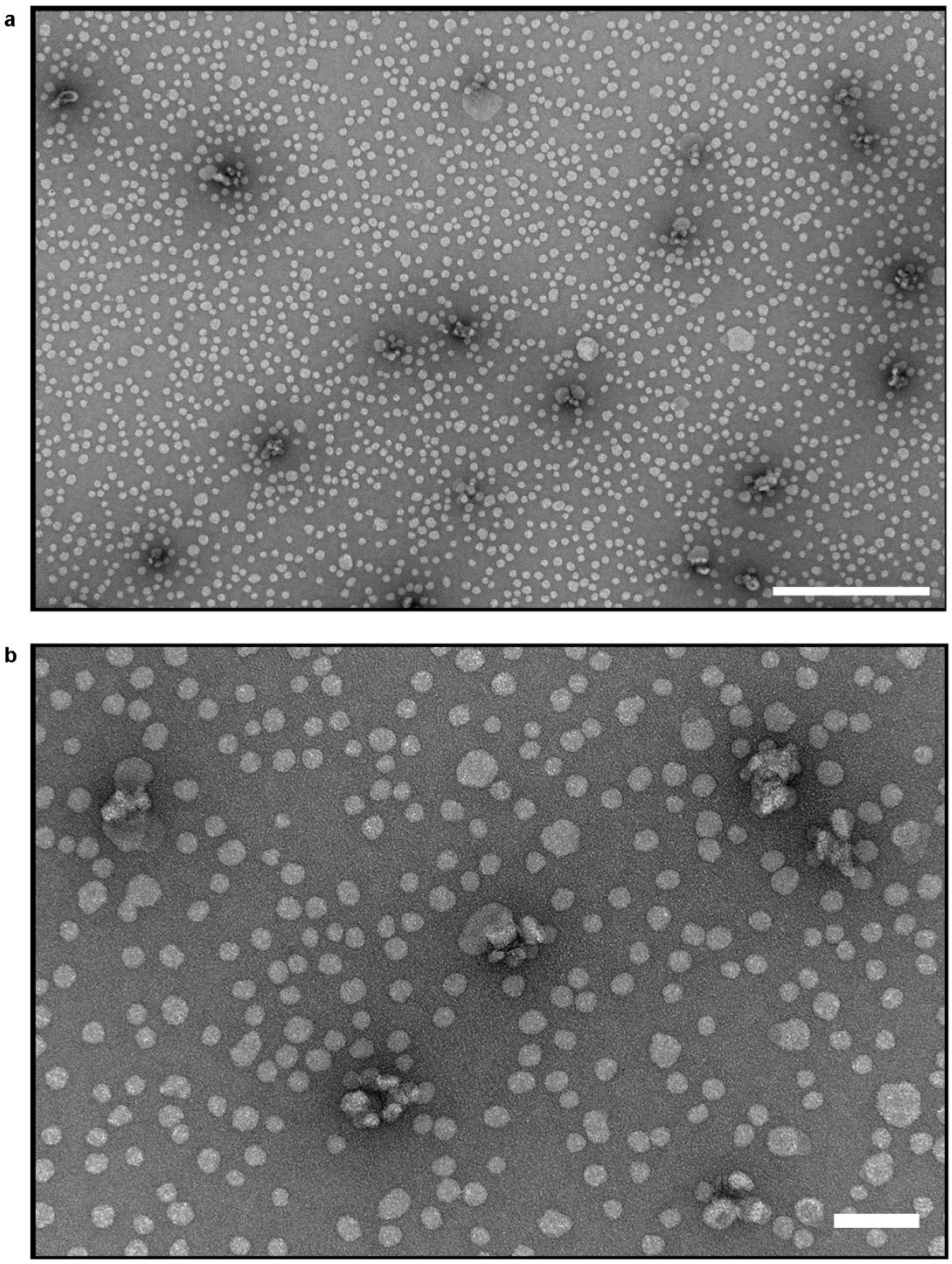
Supporting Information Fig. 3. Encapsulated-DNO sizing data. **(a)** Measurements of the inner and outer membrane diameters from TEM images. **(b)** Dynamic light scattering data of 50nm vesicles (—), the naked 48x-outer handle curved octahedron (—), and the enveloped, purified nanostructures (—). **(c)** Dynamic light scattering measured the N-DNO hydrodynamic diameter to be 93 ± 0.3nm, slightly smaller than for E-DNO and 50nm vesicles at 113 ± 1.3 and 115 ± 1.0nm, respectively (Error bars indicate SEM).



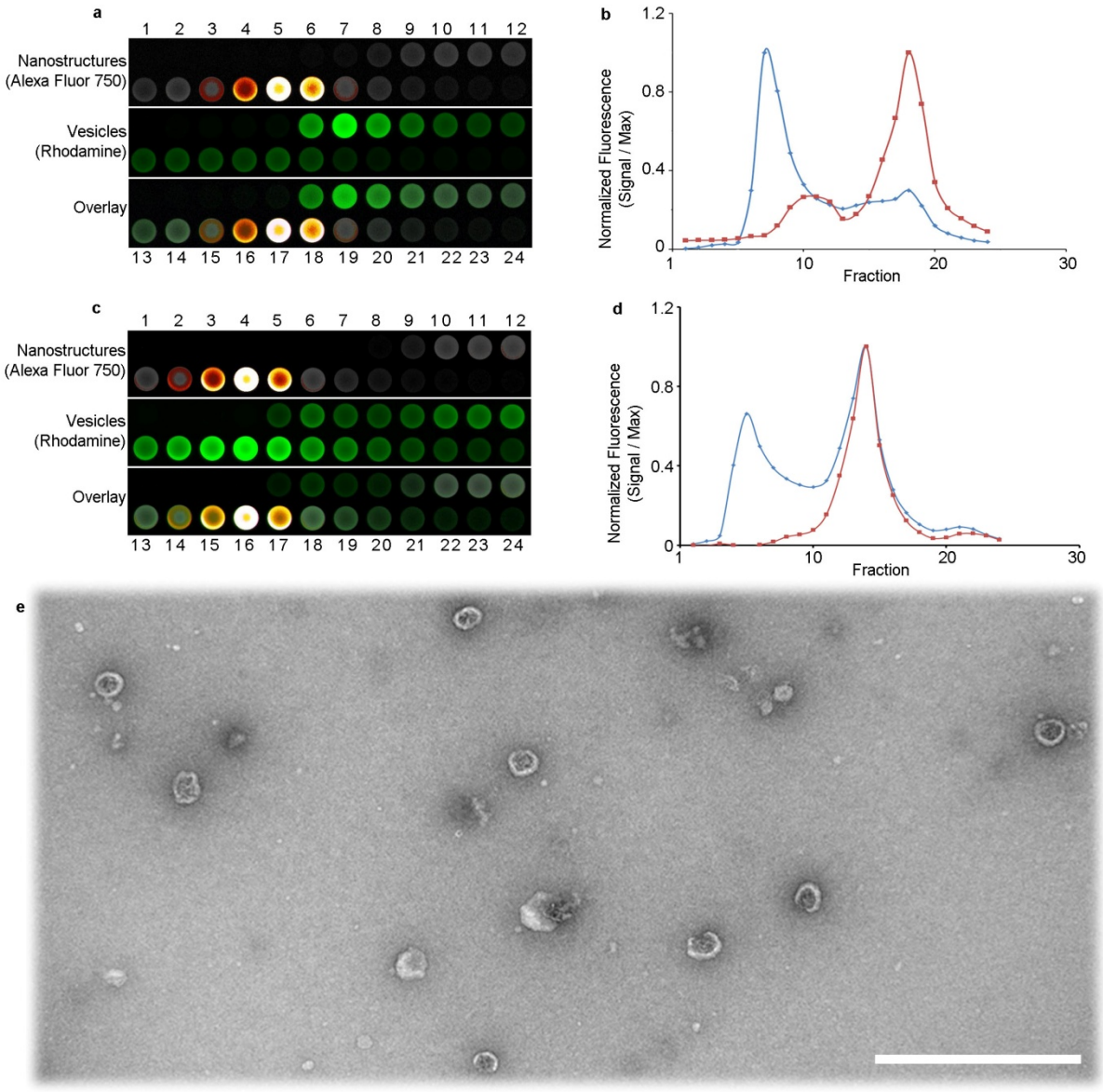
Supporting Information Fig. 4. Encapsulation yield dependence on membrane formulation. Two membrane formulations were compared for encapsulation yield using the PicoGreen dye exclusion assay; PC/PS/PEG-PE/Rhodamine-PE (84.1, 15.0, 5.0, 0.9%) **(■)** and PC/PEG-PE/Rhodamine-PE (94.1, 5.0, 0.9%) **(■)**. Inclusion of negatively-charged PS reduced encapsulation yield with 0, 12 and 24 handles ($p<0.05$), but did not have an effect on the 48-handle version. (*Student's t-test, $p<0.05$, error bars indicate SEM).



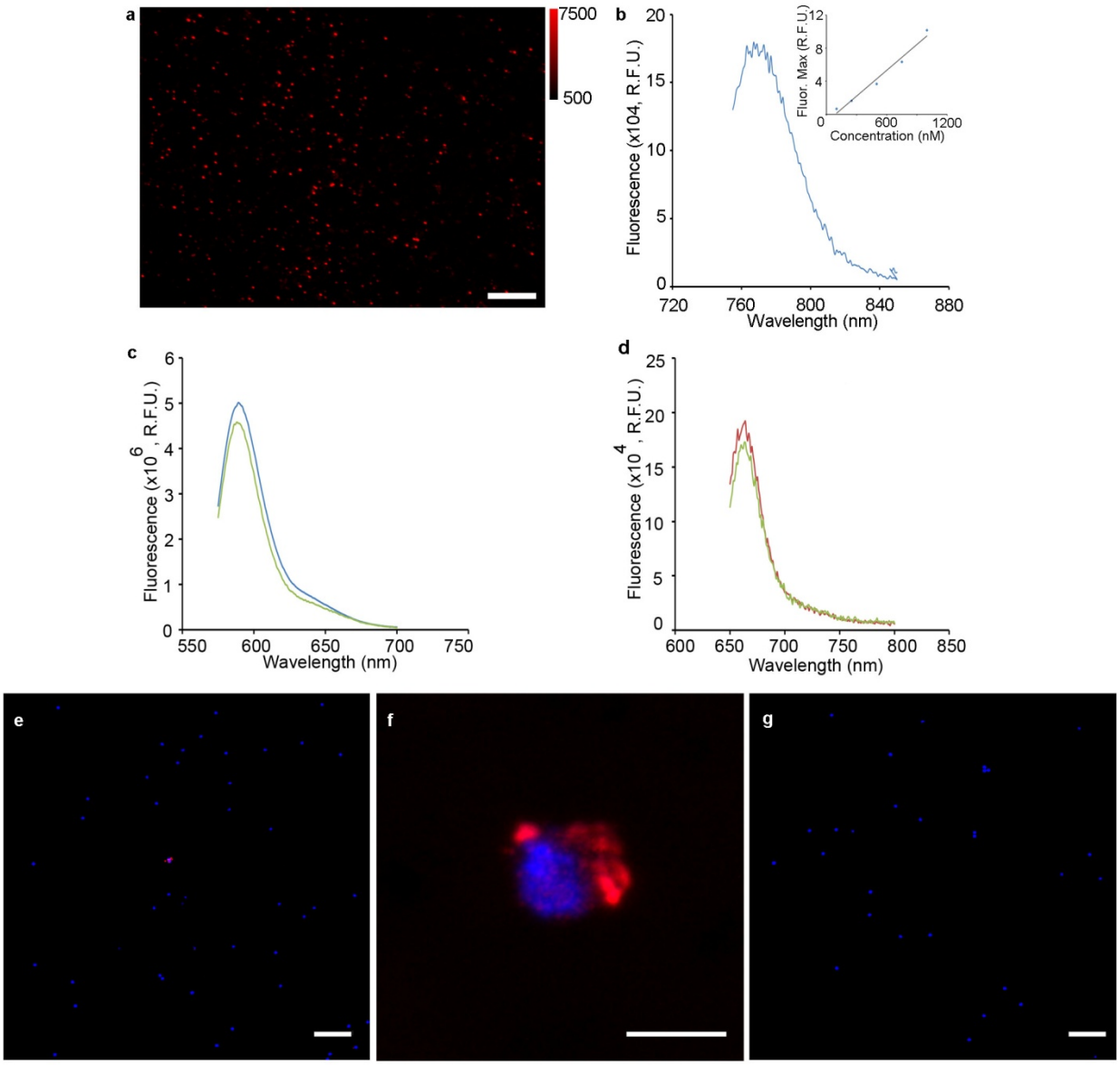
Supporting Information Fig. 5. Negative stain TEM images of DNO 0-24 outer handle variants after lipid treatment. (a) The 0-handle variant displayed some non-specific interaction with liposomes. **(b)** The 12-handle variant displayed a stronger interaction overall, but liposomes formed primarily away from the DNA surface, rather than around it. **(c)** The 24-handle variant showed greater capacity for encapsulation in membranes, but most cases were within larger liposome bodies, rather than tight-wrapping of the nanostructure.



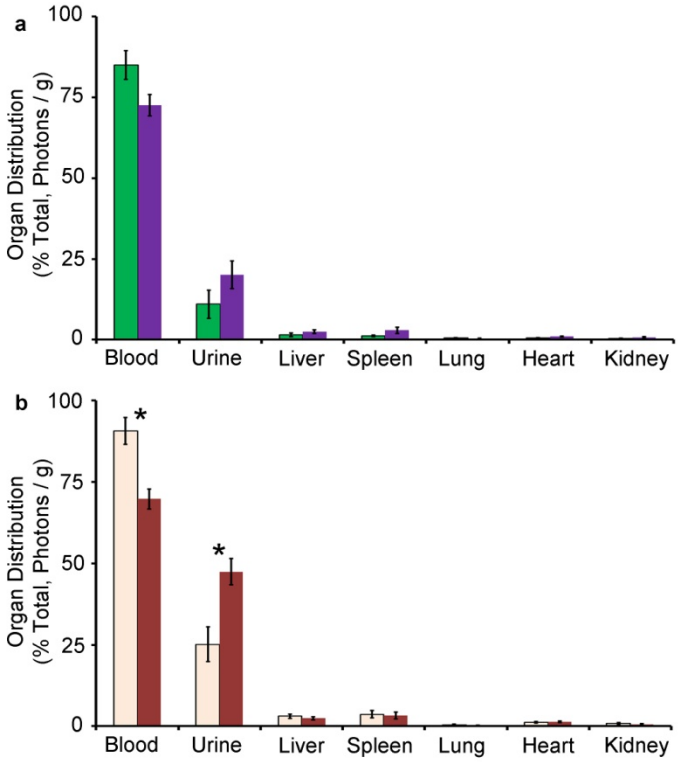
Supporting Information Fig. 6. Negative stain TEM Imaging of vesicle fusion. Low magnification **(a)** and high magnification **(b)** images of the post-encapsulation DNO after altering the lipid formulation to include 15% cholesterol. This resulted in a population of reconstituted vesicles of ~20nm, smaller than in the absence of cholesterol. Neighboring vesicles on the DNO were inhibited from fusing and fully encapsulating the nanostructures. Scale bars = 500nm (a) and 100nm (b).



Supporting Information Fig. 7. Float-up purification of E-DNO. **(a)** An iodixanol gradient with encapsulation product is prepared, centrifuged overnight, and fractionated. 50µL of each fraction is transferred into a 96-well fluorescence plate, which is imaged for fluorescence in the Alexa Fluor 750 (DNO) and rhodamine (vesicles) channels. **(b)** Analysis of rhodamine and **(—)** and Alexa Fluor 750 (—) fluorescence in the fractions shows separation of the excess vesicles higher and DNO lower in the gradient. **(c)** A second float-up is prepared with appropriate fractions from the first float-up, and measured by fluorescence in a 96-well plate. **(d)** Analysis of the second float-up shows further enrichment for E-DNO into lower fractions, which are collected for use in *in vitro* and *in vivo* experiments. **(e)** Negative stain TEM of purified E-DNO. Scale bar = 500nm.



Supporting Information Fig. 8. Imaging, measurement of DNO fluorescence and confocal microscopy of splenocytes. (a) Total internal reflectance fluorescence microscopy was used to image Cy5-labeled E-DNO. **(b)** Measurement of E-DNO Alexa Fluor 750 emission intensity, relative to a standard curve (upper right) of fluor-labelled oligonucleotides in encapsulation buffer. **(c)** The rhodamine emission profile was measured for the E-DNO (—) and a prepared solution of 50nm vesicles (—) used for *in vitro* and *in vivo* experiments. **(d)** The Cy5 emission profile was measured for N-DNO (—) and E-DNO (—) samples prepared for *in vitro* experiments. Scale bar = 10µm. **(e)** Splenocytes stained with Hoechst 33342 (blue, nucleus) and incubated with Cy5-labeled N-DNO (red) show rare, bright phagocytic cells (scale bar = 50µm), **(f)** higher magnification of same (scale bar = 5µm). **(g)** Splenocytes incubated with E-DNO showed no highly bright cells (scale bar = 50µm).



Supporting Information Fig. 9. Biodistribution profiling of the E-DNO membrane and nanostructure, and of the E-DNO *vs.* 50nm liposomes. (a) Organs were harvested 120 minutes post-injection of EDNO, and were imaged for Alexa Fluor 750 **(■)** and rhodamine **(■)** to profile nanostructure and membrane distribution, respectively. No significant differences were observed in distribution. **(b)** Organs were harvested 120 minutes post-injection of EDNO **(■)** and 50 nm liposomes **(■)** and imaged for rhodamine fluorescence to profile distribution (*Student's t-test, $p<0.05$, error bars indicate SEM).

Supporting Information Table 1. DNA NanoOctahedron Staple Sequence List. Lipid handle sequences are in red, fluorophore handle sequences in blue. The "Start" and "End" columns correspond to the helix number and [nucleotide position] along the helix (see Supporting Fig. 1).

Start	End	Sequence	Length
69[78]	70[62]	CCAGCGAGTTACTTAGCCGACTAAAGACACTCATCAGCGCTAA**TTTTTTCTTCACACCACACTCCATCTA**	70
40[61]	39[77]	TTCTTAACAGGGAGTTAAATAGAAAGGAGCTTTCGATCATCAT**TTTTTTCTTCACACCACACTCCATCTA**	70
33[78]	34[62]	GAAAACACCTTGCTTCTGTCATCGGGAGTGAAACATTTTCCCA**TTTTTTCTTCACACCACACTCCATCTA**	70
4[61]	3[77]	CCTGCCTCGGCAAAATCCCTTATAAATCAAACAGTTGGTAATA**TTTTTTCTTCACACCACACTCCATCTA**	70
67[105]	58[104]	ACCGAACATATTGAATAACTTTTCTCAGAGCCGGAACCCGTAACAAA**TTTTTTCTTCACACCACACTCCATCTA**	74
0[34]	27[35]	CTCAGTGCCAGCAGAATGGTTTTAGCTACACTTAAATCCGCCACCCT**TTTTTTCTTCACACCACACTCCATCTA**	74
37[105]	28[104]	GTAGATATTTTGTTTTCACTTTTACAGACAACCAGTACATCAGATAT**TTTTTTCTTCACACCACACTCCATCTA**	74
66[34]	39[35]	GGAAACGCATCGGGTAAAATTTTAACCGATGCCGACAAATTATCATT**TTTTTTCTTCACACCACACTCCATCTA**	74
9[78]	10[62]	GGCTTTTTCATTGAATCCCTAGGAATACACCAAAATTTGACGA**TTTTTTCTTCACACCACACTCCATCTA**	70
16[61]	15[77]	AAACTTTAATAAGAATAAATAGTGAATTACAAAGAGATTAGAG**TTTTTTCTTCACACCACACTCCATCTA**	70
12[34]	22[104]	TTTAGTAAATCACCGAAAGTTTTGTATTGGAATCGGCCTCGAGCCAG**TTTTTTCTTCACACCACACTCCATCTA**	74
70[61]	69[77]	TATCAGATTTTTAAGAAAATTAACGTCAGTAATTGTTTGACCC**TTTTTTCTTCACACCACACTCCATCTA**	70
63[78]	64[62]	AATTCATTAAAGGTGAATTTTAAGACTCTCACAATACAAAGGC**TTTTTTCTTCACACCACACTCCATCTA**	70
13[105]	45[35]	CATATAAACATACTTTTTATTTTGTAATAATTACATTGGGTGGCATC**TTTTTTCTTCACACCACACTCCATCTA**	74
27[78]	28[62]	ACCCATGATCTAAAGTTTTCGGAATAGGGCAAGCCTTTAGCGA**TTTTTTCTTCACACCACACTCCATCTA**	70
15[78]	16[62]	AGTACCTTTTTAAATATGCAGCAAAGCGAGGTCAGACGCGAGA**TTTTTTCTTCACACCACACTCCATCTA**	70
61[105]	46[104]	TTAGAGCTCATAATCACCATTTTTGTACCAAATAAAGCATATTACCG**TTTTTTCTTCACACCACACTCCATCTA**	74
22[61]	21[77]	TCCAGACATCCCATCCTAAAACAGTAGGGGTAAAGTCCAGTCG**TTTTTTCTTCACACCACACTCCATCTA**	70
18[34]	33[35]	GGCTGTAATTCGAGCATAATTTTCCTTTGACCAAGCTTGAATTATTC**TTTTTTCTTCACACCACACTCCATCTA**	74
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19[105]	4[104]	GCCCTGTATGCGACGCCAATTTTATCACCCGGGCGAAATAAAGAACG**TTTTTTCTTCACACCACACTCCATCTA**	74
21[78]	22[62]	GGAAACCTCACCAGTGAGATTATCCGCTCCCGCTTTAATTCTG**TTTTTTCTTCACACCACACTCCATCTA**	70
28[61]	27[77]	ACCTCCCACGCTAACGAGCTCATCGAGAGAGGCGTCAATAGGA**TTTTTTCTTCACACCACACTCCATCTA**	70
64[61]	63[77]	TATCAGGTTGATAATCAGAAAGATTCAAGAGATCTCAATAGAA**TTTTTTCTTCACACCACACTCCATCTA**	70
57[78]	58[62]	CGTTTGCCCCTCAGAGCCAACGTCACCATAGCCCCAAACACCA**TTTTTTCTTCACACCACACTCCATCTA**	70
24[34]	70[104]	AATAAAGGCGGACCGCCACTTTTTTGTGTCTACAACGGCGGGAGAA**TTTTTTTCTTCACACCACACTCCATCTA**	74
31[105]	16[104]	AATCCTCACCGCGCGGGCCTTTTGCTGAATGGTCATTTTAACTATAT**TTTTTTCTTCACACCACACTCCATCTA**	74
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1[105]	10[104]	GATTTAAGTTGCGTTGTTCTTTTTCCAATAGGGTAATACCCGCCGCG**TTTTTTCTTCACACCACACTCCATCTA**	74
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Start	**End**	**Sequence**	**Length**

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3[78]	4[62]	AGTTTTAAGGTGCCGTAAACTTGATATTAGTGTACTAATGCCC**TTTTTTCTTCACACCACACTCCATCTA**	70
6[34]	57[35]	AAAGAGAACAACCCAAAAGTTTTTTATGCGTTTAATTTGTCAGACTG**TTTTTTCTTCACACCACACTCCATCTA**	74
39[78]	40[62]	ATTCCTGATATCAAAATTAACAAACAATCGGAATTAGGTGAAT**TTTTTTCTTCACACCACACTCCATCTA**	70
49[105]	34[104]	AGCTTTTTTGAAGCAGAAGTTTTACATAAATCATTTGAAAAGGGGGA**TTTTTTCTTCACACCACACTCCATCTA**	74
36[34]	51[35]	GCGAAACAATAGGAACGTTTTTTGCAAATCTATCAAACTAGCCAGCT**TTTTTTCTTCACACCACACTCCATCTA**	74
7[105]	15[35]	GAATGATTGACGTGTAGCGTTTTTACCGACTCATCTTCGAGCTTCAA**TTTTTTCTTCACACCACACTCCATCTA**	74
48[34]	63[35]	TTAGAGATTGTACATCAAATTTTACTAGCAAAACAAGAAAAGAAACG**TTTTTTCTTCACACCACACTCCATCTA**	74
42[34]	52[104]	AGACAATTGATTCTATATTTTTTATCGTAAATGGGATATATTAACAC**TTTTTTCTTCACACCACACTCCATCTA**	74
46[61]	45[77]	TTGACGCAGATAGAACCCTTAGTAATAAGGAAATAATCATACA**TTTTTTCTTCACACCACACTCCATCTA**	70
52[61]	51[77]	TCTAAAGAAGGTTATCTAATAAAACATCGCAGCAACGGATTCT**TTTTTTCTTCACACCACACTCCATCTA**	70
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34[103]	32[91]	TGTGCTGATCGGTTTCTGGTGCCGGAAATTTTATGTGAG	39
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35[91]	31[104]	GAAGGGCGCAAGGCGTTACATTTAACAATTTCAATATCCCTTAG	44
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43[63]	44[49]	GTTTGACATTCTGACCTGAAAGCGTAAACGAGTAAATGGTC	41
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Start	**End**	**Sequence**	**Length**

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53[91]	49[104]	CGAACGACAACAGTGGGCGGATTGACCGTACCGTGCATCCAGCC	44
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46[103]	44[91]	CCAGCCAACTCAAACGCAAATTAACCCAAGGATATGACC	39
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44[48]	42[35]	AATAACCACTAATAGTCTGAAATGGATTATAAGGGACGTGGCAC	44
56[48]	54[35]	AGCAGCAGTTTTCATTTGGGCTTGAGATGGATTTTAAATAAAAC	44
25[63]	26[49]	AGTATAGACGTCTTTCCAGAGCCTAATGAGGGTTCCGTACT	41
24[76]	29[90]	GTACCGCCCGTCGTCTTTCCAGACGTTGGTATTAAGCCGTT	41
57[36]	59[48]	TAGCGCCCGTAATCACCAGTAGCACCACGTTAGAACTGG	39
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23[91]	19[104]	CGCCATAGAGAATATCCAGCTGCATTAATGGCGCCAGCCGCCTG	44
23[49]	19[62]	AAATAATGACGACAGCGTTGCGCTCACTGCACAATTTCCTGT	42
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12[76]	17[90]	AAGAGTCGAAACTAAAGTACGGTGTCTTTAAGACATCATAG	41
70[103]	68[91]	TAACTGACAGAGAATTTATCCCAATCCAATCAGCGACCT	39
17[91]	13[104]	GTCTGAGGCTGATGCCTCCTTTTGATAAGAATAATGCTTCATTC	44
17[49]	13[62]	GGCGTTATTCAAATGAAGCAAACTCCAACGATTGCAACTATT	42
16[103]	14[91]	GTAAATAGACTACGCGATAGCTTAGAGGAAGTTGTAGCT	39
71[49]	67[62]	GAAGCCCGAGATAAAGAATACACTAAAACTTTTTCAACAGAG	42
Start	**End**	**Sequence**	**Length**

71[91]	67[104]	AGCCTTTAACACCCTAAGCGCGAAACAAAGGAAATCCTAAGGGA	44
9[36]	11[48]	TAACCCCATAACGATTATTACAGGTAAGGCCAGGAACGG	39
26[48]	24[35]	CAGGAGGCACCACCCGGGAGGTTTTGAAGCATTTTATGTTACAA	44
8[48]	6[35]	GCAGATATCGTTTACCTTTCCTCGTTAGAATTAGACACCGAGTA	44
37[63]	38[49]	TTAGACTAAGGCCGCTTTTGCGGGATCGGATTTACTCGTAT	41
7[63]	8[49]	GTTGAGAGAGTGTTTTTATAATCAGTGGAAAGATAACTAAT	41
6[76]	11[90]	AGGAAGGTTCCTCAAATGCTTTAAACAAACGTGGGGGCGCT	41
36[76]	41[90]	GAGTGAGTTTTTGCACGTAAAACAGAAAACAGTTAAGGAAT	41
11[91]	7[104]	AGGGCGCGCGCCGCTAAGTTTTGCCAGAGGCTGCGGAAAAACGA	44
11[49]	7[62]	TACGCCATAACGTGCAGACGACGATAAAACACATTCTCATCA	42
10[103]	8[91]	CTTAATTGGCAAGGGGAAAGCCGGCGGTTCAGATCGTCA	39
41[91]	37[104]	TGCGAATTAATTGTAGATGATGGCAATTCAATGGAAGAAATTGC	44
41[49]	37[62]	AGGCTTGACAGCTTAACCACCAGAAGGAGTCGACAAGAAGTA	42
3[36]	5[48]	GCGTCATAAAGCCATTGACAGGAGGTGGGCGGGATTATT	39
40[103]	38[91]	AGCCTTAATAATTCTAAACAACTTTCATAAAGGGTTAGA	39
2[48]	0[35]	TCCTCATTACATGGCAACCTATTATTCTGAGGATTAGGGTTTTG	44
68[48]	66[35]	GTTTCCAGAGGCAAACCCACAAGAATTGAGATAGCTAACCAGAA	44
1[63]	2[49]	ACGATTGCGAAAATCCTGTTTGATGGTTGAGGCAAAATAAA	41
0[76]	5[90]	CAGCAGGGCGCACTAAATCGGAACCCTCGCTGGTTAGCCCG	41
69[36]	71[48]	ACGAAATTAAACGGAACGAGGGTAGCAAAGTTTCTTACC	39
5[91]	1[104]	AGATAGGCCCTTGAGCAGTGCAACGTCAAAAAATCAAAGCCCCC	44
5[49]	1[62]	CCGAAATATTTCGGTTTTGATGATACAGGCACAAACGGTCAG	42
4[103]	2[91]	TGGACTGTTGAGTAGCAAGCGGTCCAAAAGGGGTTTTTT	39
33[36]	35[48]	ATTTCAATTGCTTAACGTCAGATGAAAGGATCGCAAGTC	39
67[63]	68[49]	GCTTTGAGTGTAAGCAGATAGCCGAACAACGGCTTGAGGAA	41
58[103]	56[91]	GCTGCTAATCTTGTTGAAAGAGGACACCAGAACCTCAGA	39

Supporting Information Note 1: Prediction of DNO Six Helix Bundle Curvature Analytical Derivation

Approximation of Energy for bending DNA bundle:

Please see Dietz *et al.*, Supporting Figure 1 for additional data[1].

$$E_{total} = E_{stretch/compression} + E_{bend}$$

$$E_{stretch/compression} = \frac{1}{2}\frac{S}{d_{eq}}\sum_i n_i\left(d_i - d_{eq}\right)^2$$

$$E_{bend} = \frac{1}{2}Bd_{eq}\sum_i \frac{n_i}{r_i^2}$$

$$where\ r_i = r_{ref}\left(\frac{delta_i}{r_{ref}} + 1\right)$$

$$\theta_i = \frac{n_i d_{eq}}{r_i}$$

$$E_{bend} = \frac{1}{2}\frac{B}{d_{eq}}\sum_i \theta_i^2 \frac{1}{n_i}$$

$$but \sum_i \theta_i^2 \frac{1}{n_i} \approx \theta^2 \sum_i \frac{1}{n_i}$$

$$\therefore E_{bend} \approx \frac{1}{2}\frac{B}{d_{eq}}\theta^2\sum_i \frac{1}{n_i}$$

The rationale for the above approximation is based on the idea that the underestimate in bending energy for the convex-face helices will be mostly offset by the overestimate in bending energy for the concave-face helices.

$$E_{total} = \frac{1}{2}\frac{S}{d_{eq}}\sum_i n_i\left(d_i - d_{eq}\right)^2 + \frac{1}{2}\frac{B}{d_{eq}}\theta^2\sum_i \frac{1}{n_i}$$

Please note that for this approximation of energy, the following relations hold:

At fixed values of d_i

$$\frac{\partial E_{stretch/compression}}{\partial \theta} = 0$$

At fixed values of θ

$$\frac{\partial E_{bend}}{\partial d_i} = 0$$

Step 0: Obtain an expression for the length $n_{ref}d_{ref}$ of a reference double helix in the curved bundle as a function of angle, assuming the bundle is at the equilibrium length for that angle. Do this by letting $\partial E_{stretch/compression}/\partial n_{ref}d_{ref} = 0$ and solving for $n_{ref}d_{ref}$ (recall from the previous page that $\partial E_{bend}/\partial d_{ref} = 0$). The position in the bundle of the reference double helix is encoded in the $delta_i$ offset values. The reference helix could be imaginary, the position within the bundle is arbitrary, and the value of n_{ref} never has to be specified (it cancels out during Step 1).

$$E_{stretch/compression} = \frac{1}{2}\frac{S}{d_{eq}}\sum_i n_i\left(d_i - d_{eq}\right)^2$$

$$d_i = \frac{n_{ref}d_{ref}}{n_i}\left(\frac{delta_i}{r_{ref}} + 1\right)$$

$$r_{ref} = n_{ref}d_{ref}/\theta$$

$$E_{stretch/compression} = \frac{1}{2}\frac{S}{d_{eq}}\sum_i n_i\left(\frac{n_{ref}d_{ref}}{n_i}\left(\frac{delta_i}{n_{ref}d_{ref}/\theta} + 1\right) - d_{eq}\right)^2$$

$$E_{stretch/compression} = \frac{1}{2}\frac{S}{d_{eq}}\sum_i n_i\left(\frac{1}{n_i}\left(n_{ref}d_{ref} + \theta\cdot delta_i\right) - d_{eq}\right)^2$$

$$\frac{\partial E_{stretch/compression}}{\partial(n_{ref}d_{ref})} = \frac{S}{d_{eq}}\sum_i n_i\left(\frac{1}{n_i}\left(n_{ref}d_{ref} + \theta\cdot delta_i\right) - d_{eq}\right)\frac{1}{n_i}$$

$$= \frac{S}{d_{eq}}\sum_i\left(\frac{1}{n_i}\left(n_{ref}d_{ref} + \theta\cdot delta_i\right) - d_{eq}\right)$$

$$0 = \frac{S}{d_{eq}}\sum_i\left(\frac{1}{n_i}\left(n_{ref}d_{ref} + \theta\cdot delta_i\right) - d_{eq}\right)$$

$$= n_{ref}d_{ref}\sum_i\frac{1}{n_i} + \theta\sum_i\frac{delta_i}{n_i} - d_{eq}\sum_i 1$$

$$n_{ref}d_{ref} = \frac{1}{\sum_i\frac{1}{n_i}}\left(d_{eq}\sum_i 1 - \theta\sum_i\frac{delta_i}{n_i}\right)$$

Step 1: Use the expression for the length $n_{ref}d_{ref}$ from Step 0 to obtain an expression for the length d_i of double helix$_i$ in the curved bundle as a function of angle, assuming the bundle is at its equilibrium length for that angle.

$$n_{ref}d_{ref} = \frac{1}{\sum_i\frac{1}{n_i}}\left(d_{eq}\sum_i 1 - \theta\sum_i\frac{delta_i}{n_i}\right)$$

$$d_i = \frac{n_{ref} d_{ref}}{n_i}\left(\frac{delta_i}{r_{ref}} + 1\right)$$

$$\theta = \frac{n_{ref} d_{ref}}{r_{ref}}$$

$$d_i = \frac{1}{n_i}\left(\theta \cdot delta_i + n_{ref} d_{ref}\right)$$

$$= \frac{1}{n_i \sum_i \frac{1}{n_i}}\left(\theta \cdot delta_i \sum_i \frac{1}{n_i} + d_{eq} \sum_i 1 - \theta \sum_i \frac{delta_i}{n_i}\right)$$

$$d_i = \frac{1}{n_i \sum_i \frac{1}{n_i}}\left(\theta\left(delta_i \sum_i \frac{1}{n_i} - \sum_i \frac{delta_i}{n_i}\right) + d_{eq} \sum_i 1\right)$$

Python Script:

```
deq = 0.335
S = float(660)
B = float(230)
num_helices = 6

 n is the number of basepairs installed in region with
#   a default of nref base pairs
# e.g. nref = 105
n = [0 for i in range(num_helices)]
n[0]  = 96
n[1]  = 99
n[2]  = 104
n[3]  = 106
n[4]  = 111
n[5]  = 114
#190 degrees
#n[0]  = 59
#n[1]  = 70
#n[2]  = 92
#n[3]  = 106
#n[4]  = 129
#n[5]  = 140

# delta is the distance in nm from an arbitrary axis
delta = [0 for i in range(num_helices)]
delta[0]  = 3.5
delta[1]  = 2.5
delta[2]  = 0.5
delta[3]  = -0.5
delta[4]  = -2.5
delta[5]  = -3.5
```

```
for i in range(num_helices):
        delta[i] *= -1.125

total_n = 0
for i in range(num_helices):
        total_n += n[i]
average_n = float(total_n)/num_helices
print "average n is", average_n, "\n"

# Calculate delta_div_n_sum, one_div_n_sum, and beta values
delta_div_n_sum = 0
one_div_n_sum = 0
for i in range(num_helices):
        delta_div_n_sum += delta[i]/n[i]
        one_div_n_sum += float(1)/n[i]

beta = [0 for i in range(num_helices)]
for i in range(num_helices):
        beta[i] = (delta[i]-delta_div_n_sum/one_div_n_sum)/n[i]

# Calculate theta
beta_n_sum = 0
beta_delta_sum = 0
for i in range(num_helices):
        beta_n_sum += beta[i]*n[i]
        beta_delta_sum += beta[i]*delta[i]
numerator = deq*beta_n_sum
denominator_term_0 = beta_delta_sum
denominator_term_1 = (B/S)*(one_div_n_sum)
denominator = denominator_term_0 + denominator_term_1
theta = numerator/denominator

theta_in_degrees = theta*180/3.1416
```

```
print "theta is", theta_in_degrees,"degrees or ", theta, "radians"

# Calculate d
d = [0 for i in range(num_helices)]
for i in range(num_helices):
        factor_0 = theta*(delta[i] - delta_div_n_sum/one_div_n_sum)
        factor_1 = deq*num_helices/one_div_n_sum
        d[i] = (factor_0 + factor_1)/n[i]

for i in range(num_helices):
        print "helix", i, "\tlength per bp is", d[i]

print
print "delta_div_n_sum is", delta_div_n_sum
print "one_div_n_sum is", one_div_n_sum
print "beta_n_sum is", beta_n_sum
print "beta_delta_sum is", beta_delta_sum
print "numerator is", numerator
print "denominator_term_0 is", denominator_term_0
print "denominator_term_1 is", denominator_term_1
print "denominator is", denominator
```