Supporting Information of:

Modulation of cellular uptake of DNA origami through control over mass and shape

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Contents:

Supporting Information 1 | caDNAno designs and sequences of all nine structure

All designs were performed in caDNAno and structures were folded in a thermocycler and purified using standard techniques.

DNA sequences for all eleven structures, all with 3 Cy5 dyes on the core available in Supporting Table 1.

1. **p3024 scaffold sequence**

CCCGGTACCCAATTCGCCCTATAGTGAGTCGTATTACGCGCGCTCACTGGCCGTCGTTTTACAACGTCG
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2. p7308 scaffold sequence

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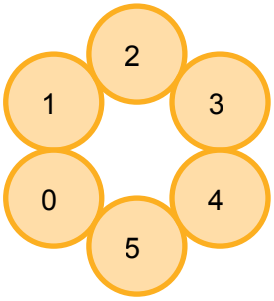
Illustration of L-thin_rod cadnano diagram. Arrows indicate 3’-end of DNA. Blue strand is scaffold strand; gray and red strands are staple strands.



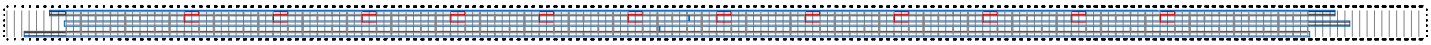
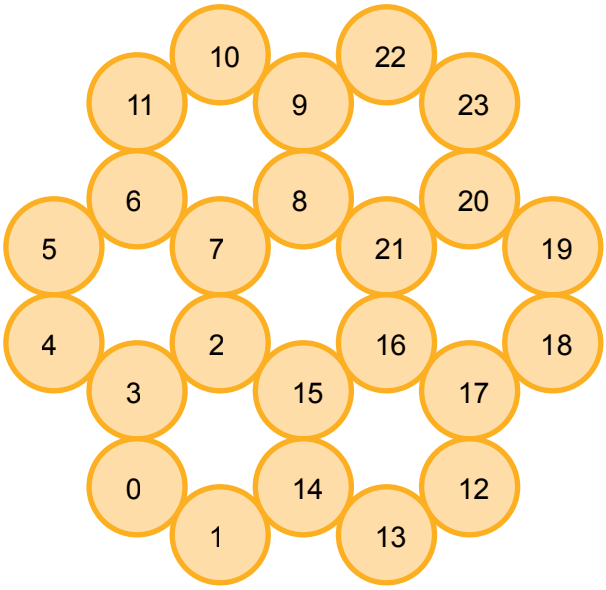


Illustration of L-thick_rod cadnano diagram. Arrows indicate 3'-end of DNA. Blue strand is scaffold strand; green and red strands are staple strands.



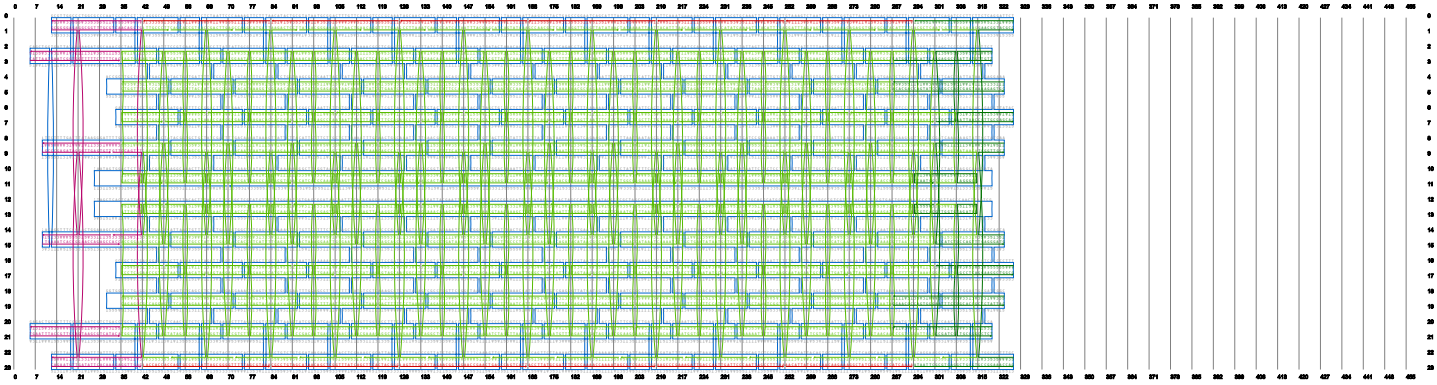
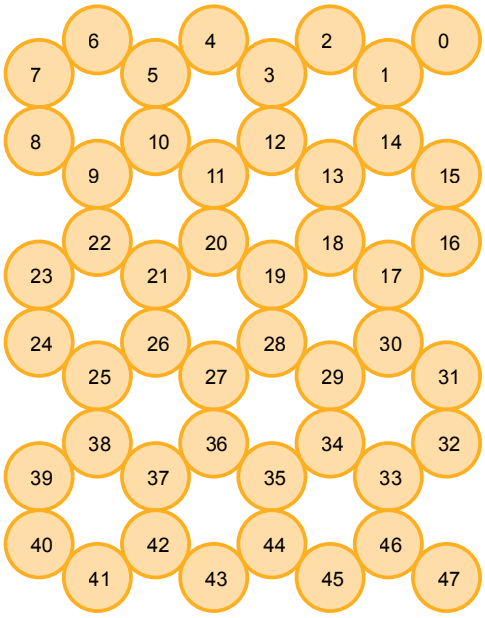


Illustration of L-block cadnano diagram. Arrows indicate 3’-end of DNA. Blue strand is scaffold strand; orange and red strands are staple strands.



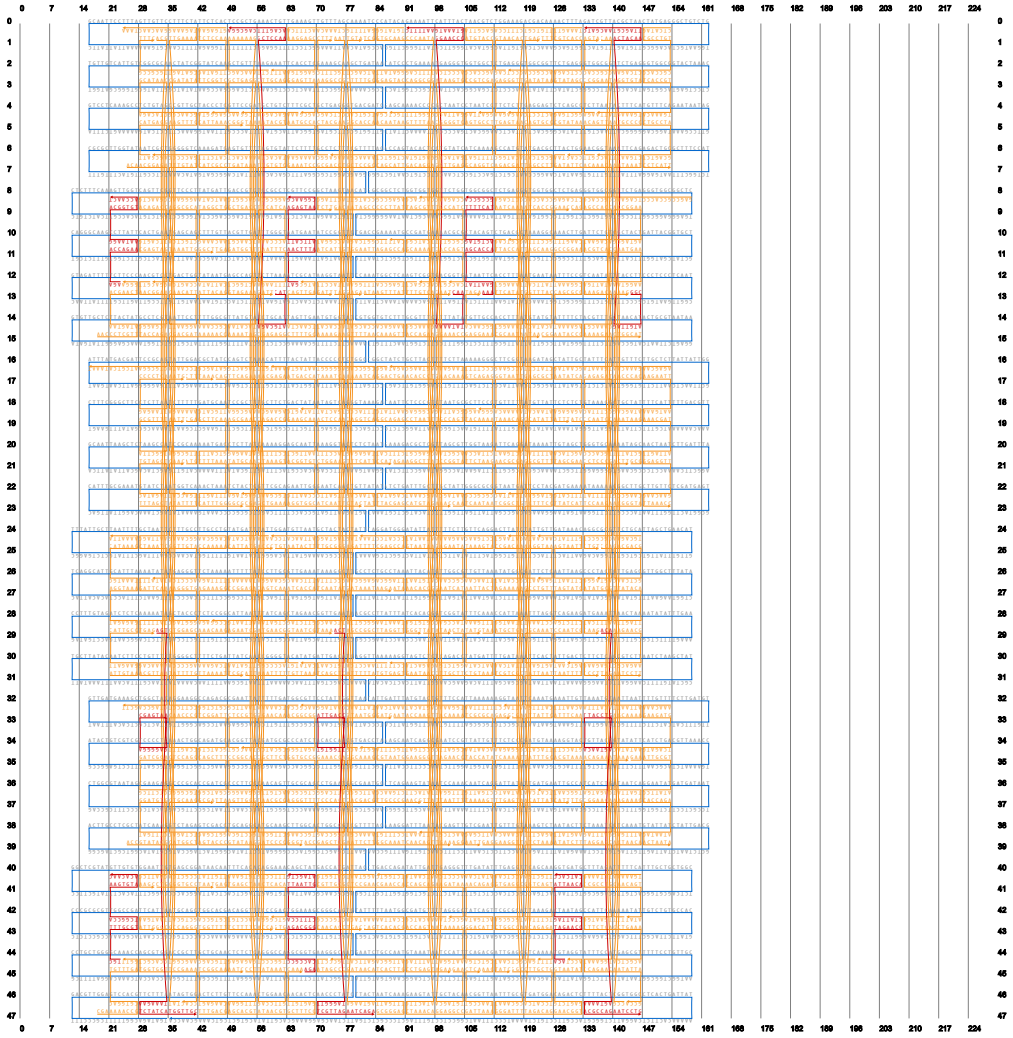
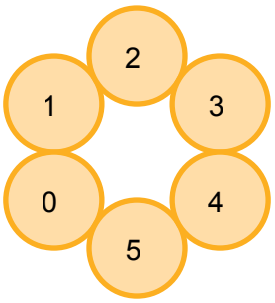


Illustration of L-ring cadnano diagram. Arrows indicate 3′-end of DNA. Blue strand is scaffold strand; grey and red strands are staple strands.



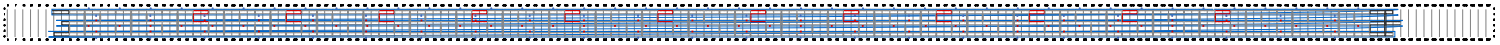
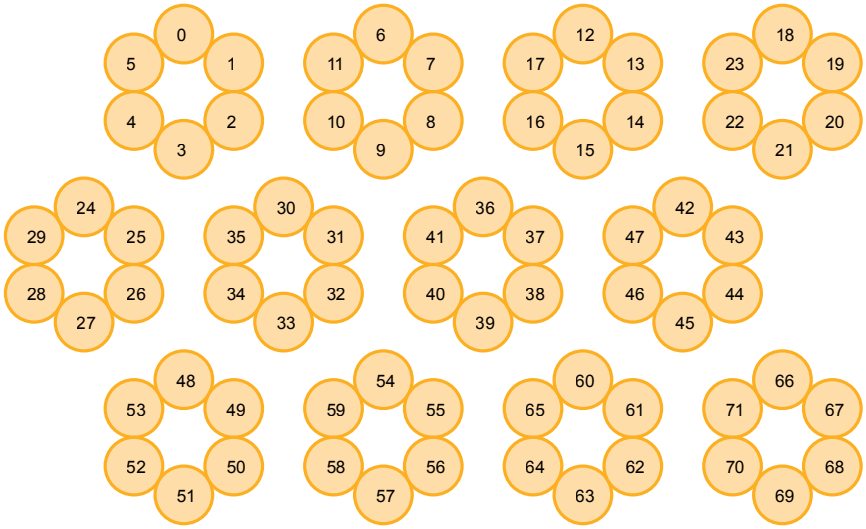


Illustration of L-octahedron cadnano diagram. Arrows indicate 3’-end of DNA. Blue strand is scaffold strand; green, orange and red strands are staple strands.



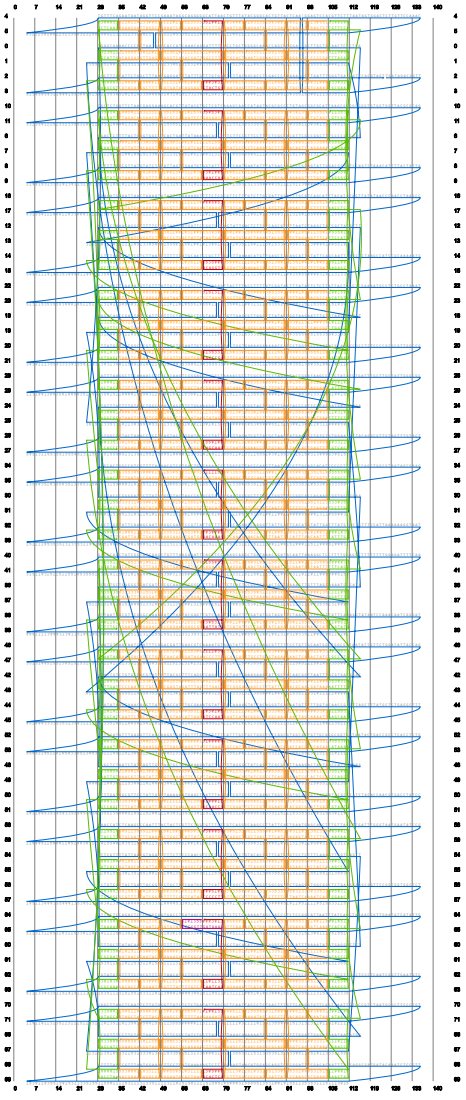
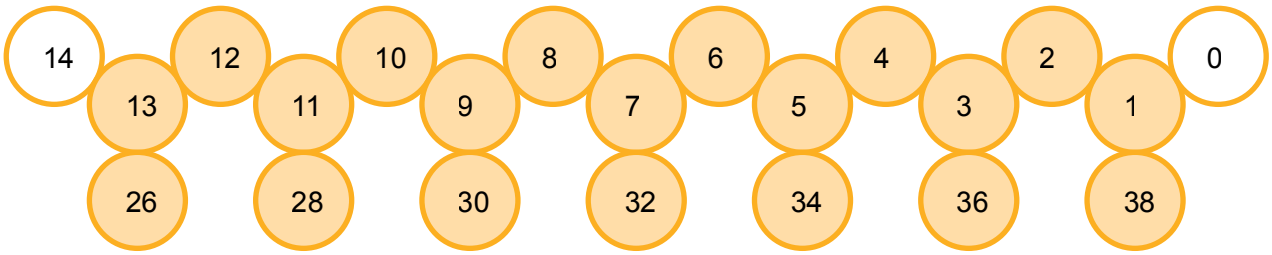


Illustration of L-barrel cadnano diagram. Arrows indicate 3’-end of DNA. Blue strand is scaffold strand and miniscaffold strands; green and orange strands are staple strands.



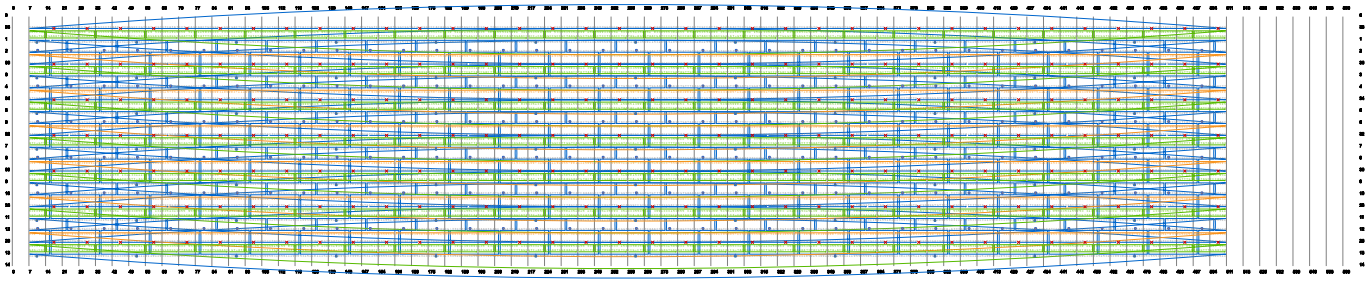
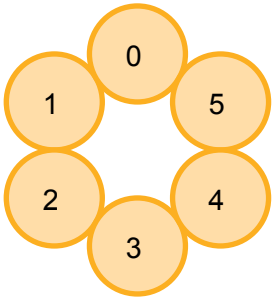


Illustration of S-thin_rod cadnano diagram. Arrows indicate 3’-end of DNA. Blue strand is scaffold strand; green, grey and red strands are staple strands.



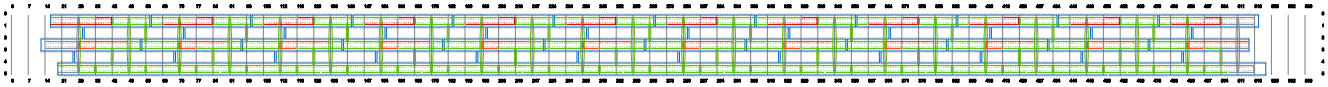
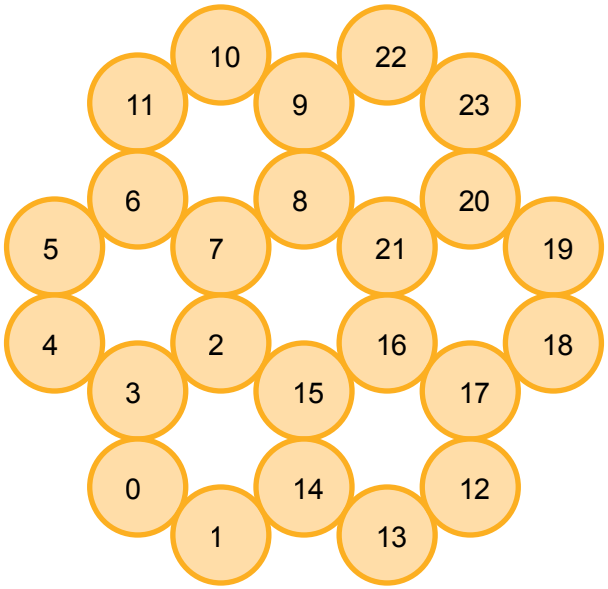


Illustration of S-thick_rod cadnano diagram. Arrows indicate 3’-end of DNA. Blue strand is scaffold strand; green and red strands are staple strands.



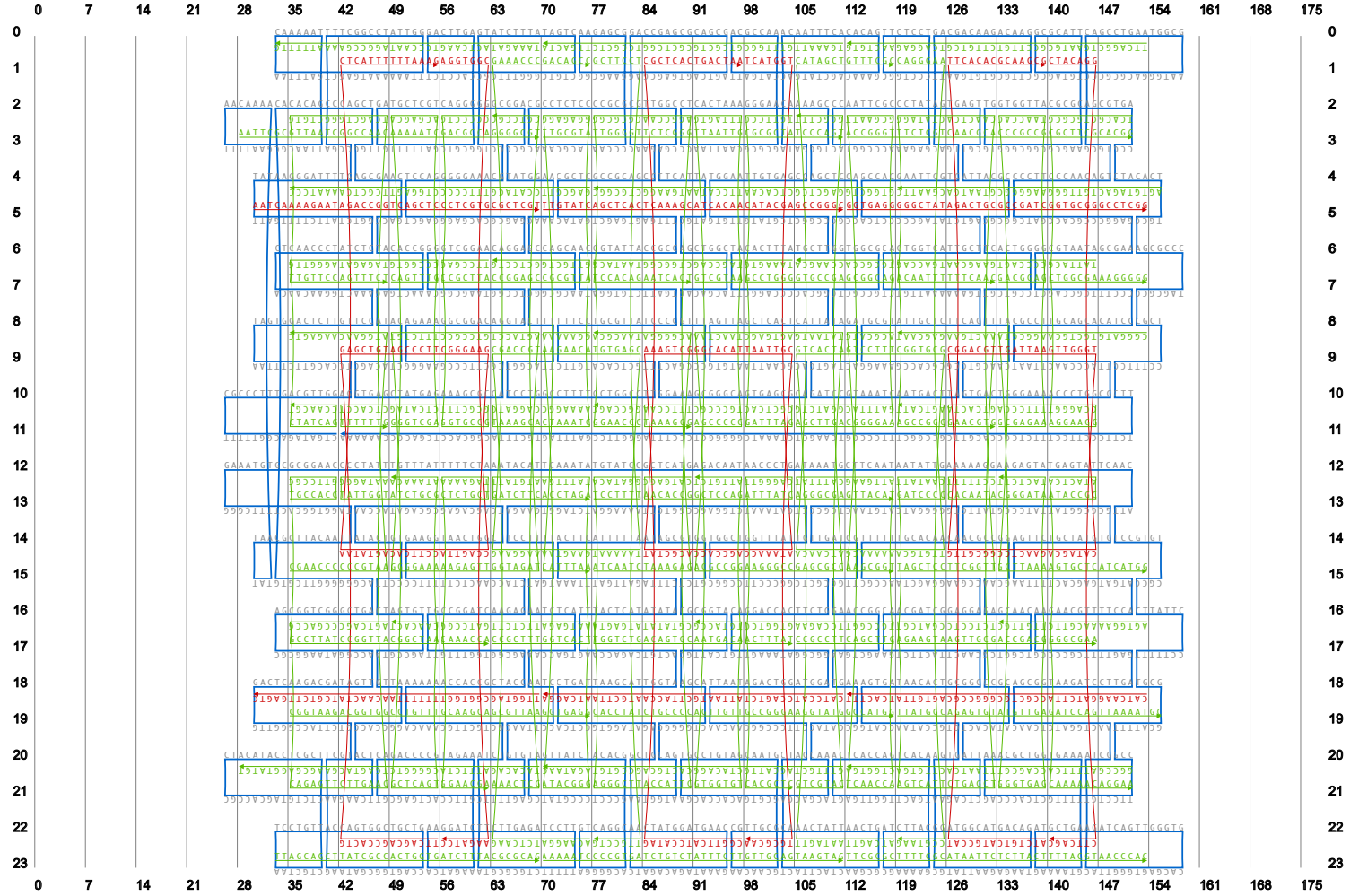
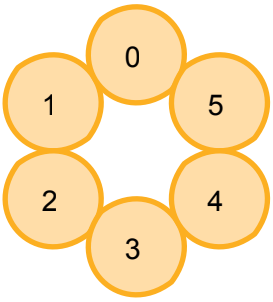


Illustration of S-ring cadnano diagram. Arrows indicate 3’-end of DNA. Blue strand is scaffold strand; green, grey and red strands are staple strands.



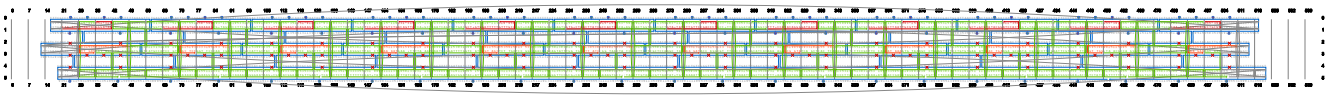


Table S1 | Staple listing of nine DNA nanostructures

L-octahedron
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GCAGCGATAGATAATATTAATGCAAATCATCAAACAGCCAGC
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L-block
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L-thin_rod
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Figure S1 | Presence of ssDNA or dsDNA handles diminishes cellular uptake of DNA origami structures in BMDCs.

In earlier designs of our shape library, we noticed the presence of single or double stranded DNA handles and handle-anti handle functionalized with fluorescent labels showed a negative correlation with cellular internalization. As this might be an important design parameter for general future studies that use labeled DNA origami to analyze cellular processes, we decided to perform a comparative analysis between all 4 presentations (core modified label (used in main paper) handle/anti handle labels, core with ssDNA handles, core with dsDNA handles). Indeed, for barrel, we see a clear negative impact of the presence of ss and dsDNA handles outside the structure.

(A) from top left to bottom right: core-modified structure with fluorophores conjugated directly to staple strands of the origami structure; fluorophores conjugated to antihandle ssDNA strand that is then hybridized to outstretched ssDNA; core-modified DNA origami with ssDNA modification (handles); core-modified DNA origami with dsDNA modification (handle-antihandle) **(B)** uptake of DNA origami nanostructures into murine BMDCs is diminished in a shape-dependent manner when ssDNA or dsDNA modifications are present.

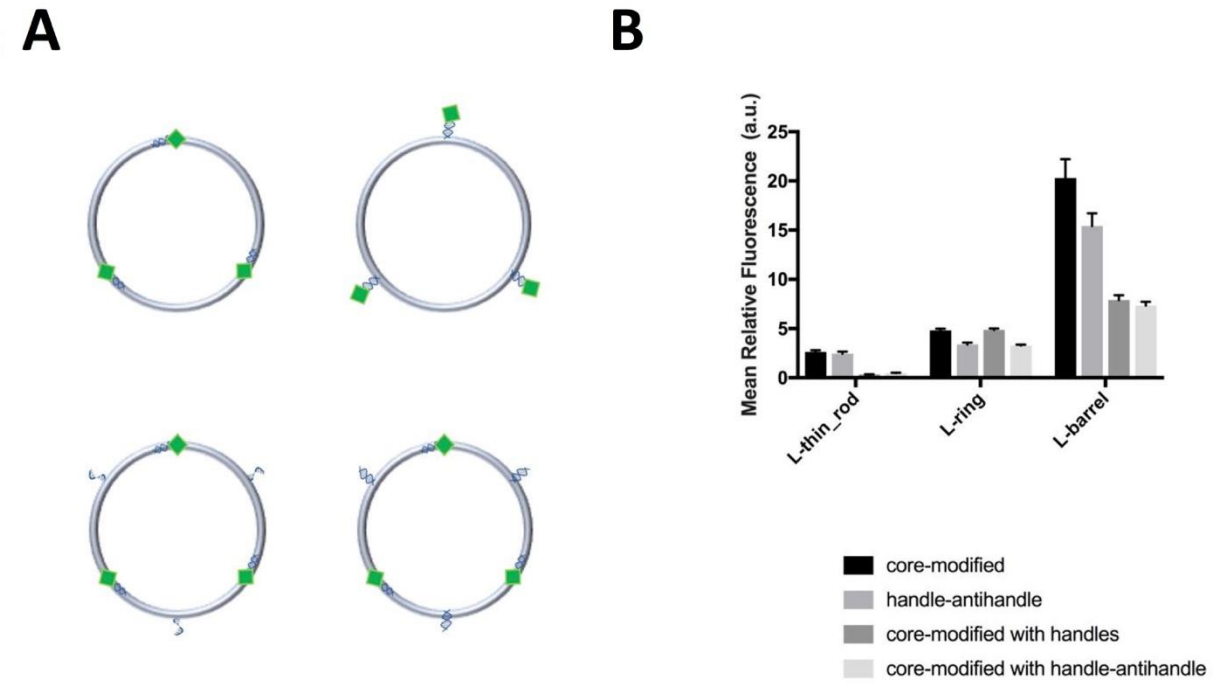


Figure S2 | AGE of nine DNA nanostructures and corresponding control dsDNA structures after purification. Structures were annealed as described in the methods and purified using the glycerol gradient ultracentrifugation method. Purified samples were individually loaded onto a 2% agarose gel and run in 0.5x TBE buffer with 11 mM $MgCl_2$ at 65V for 2 hours.

Panel **A** demonstrates EtBr scan of the purified S-shapes whilst **B** shows purified L-shapes. All samples are presented with reference to the p7308 or p3024 scaffold DNA.

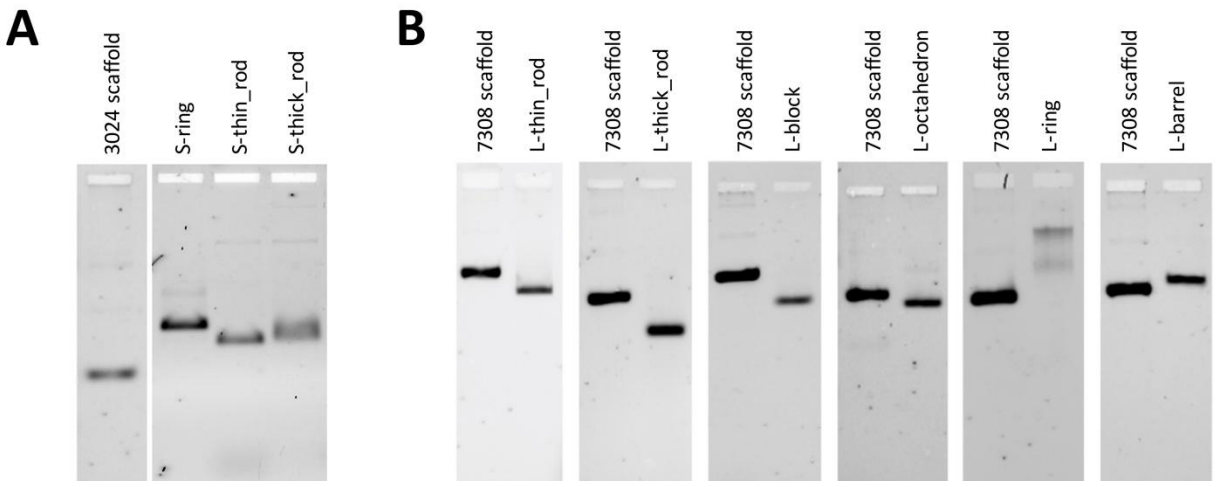


Figure S3 | **Negative stain TEM images of all nine structures after purification**. From top left to bottom right: L-thin_rod, L-thick_rod, L-block, L-ring, L-octahedron, L-barrel, S-thin_rod, S-thick_rod, S-ring. Structures were stained using 2% uranyl formate for 30 seconds. Scale bar is 100 nm in all images.

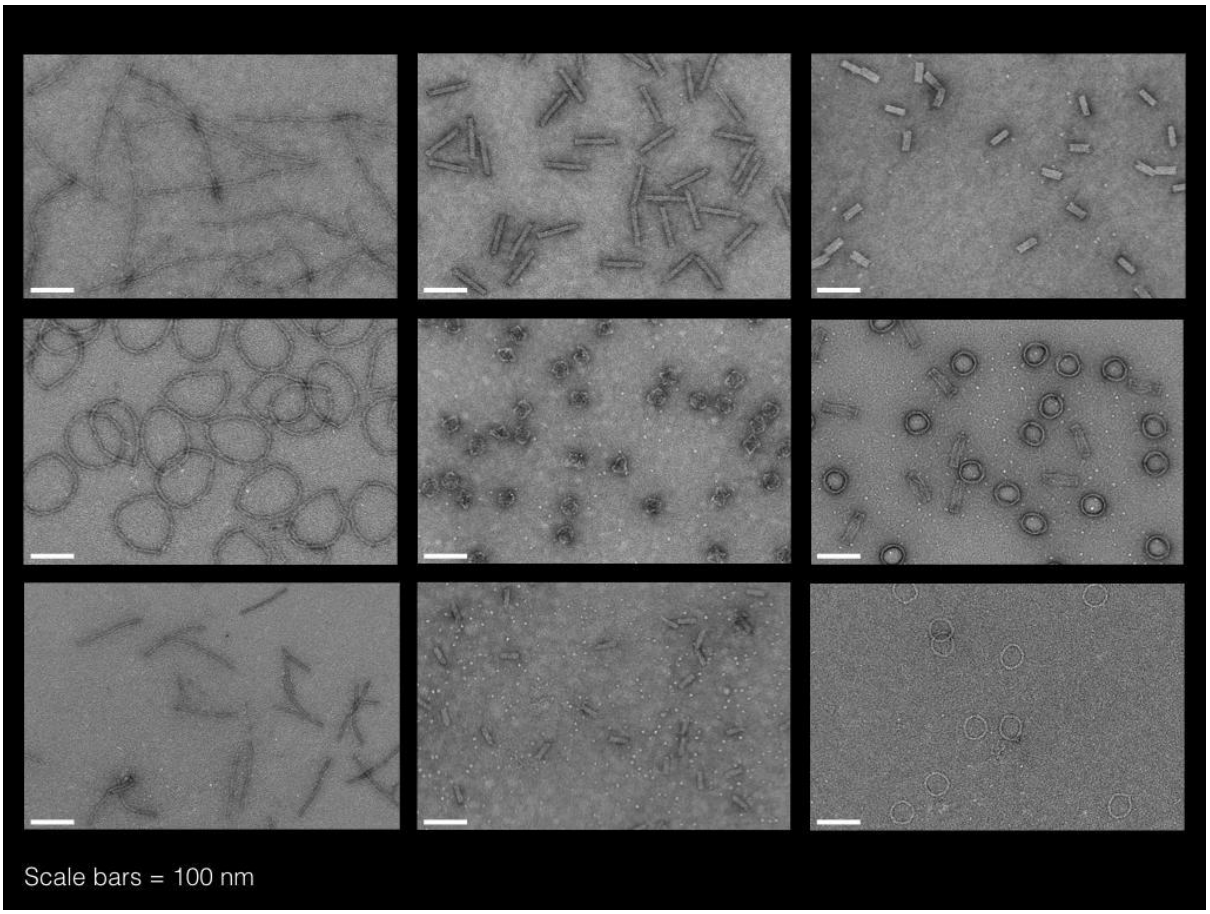


Figure S4 | K10-PEG5K coated barrel structures are incubated and uptaken by HUVE cells after 12 hours of incubation at various concentrations. No change in cell viability was observed.

K10PEG5K coated structures were incubated at 0.5-10 nM concentrations as described in the methods. Mammalian cells were imaged by confocal microscopy after 12 hours of incubation. Toxicity of our structures to the HUVE cells (BMDC confirmed before and previously published) we used the commercially available LIVE/DEAD® Viability/Cytotoxicity Kit (see **Materials and Methods**). We incubated cells with a concentration series of origami structures and measured if there would be more dead cells present, as a sign of toxicity. The kit discriminates live from dead cells by simultaneously staining with green-fluorescent calcein-AM to indicate intracellular esterase activity and red-fluorescent ethidium homodimer-1 to indicate loss of plasma membrane integrity. After fluorescence microscopy, we compared the various incubation conditions and concluded that no negative, toxic effects were resulting from any of the concentrations used. Panels show triplicates in vertical alignment.

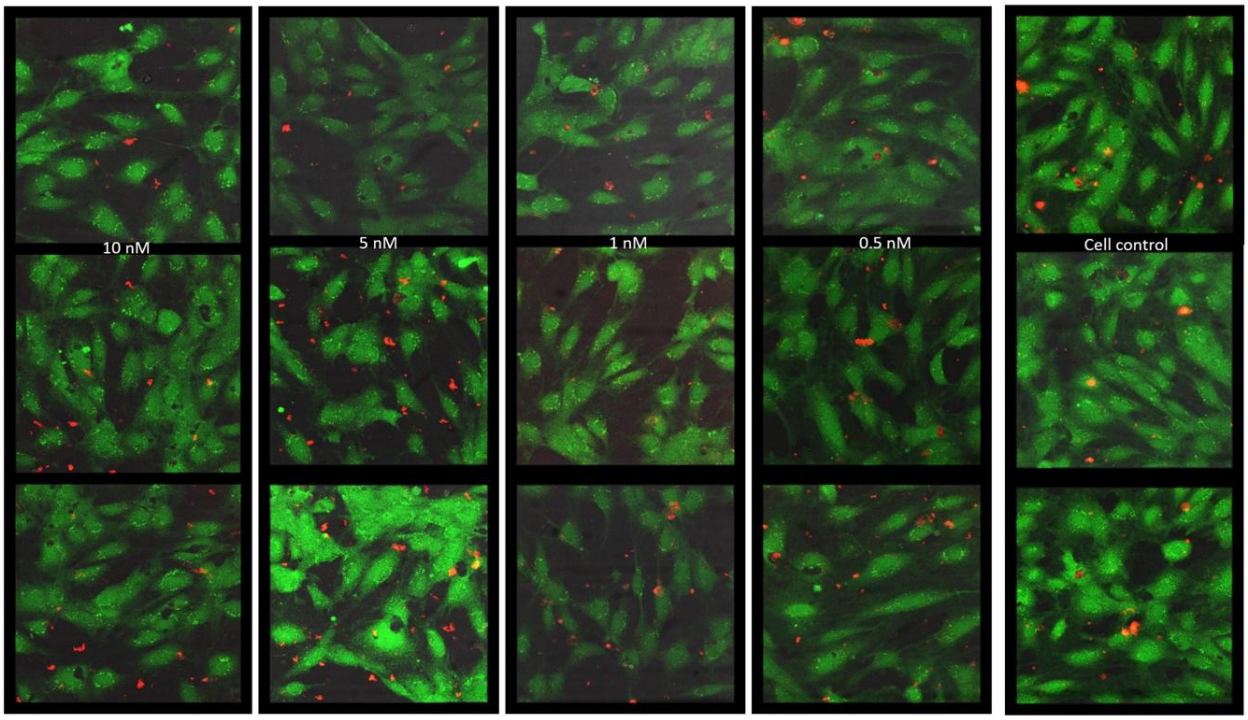
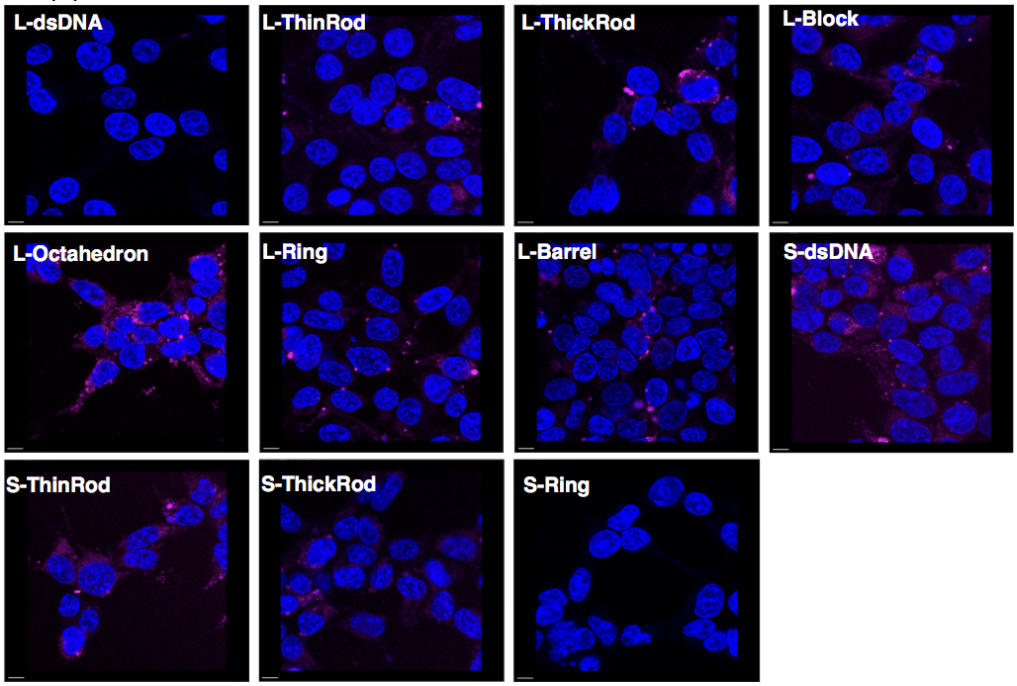


Figure S5 | K10-PEG5K coated DNA nanostructures were incubated with HEK293 (A) and HUVE (B) mammalian cells over 12 hours.

We visualized the uptake after overnight incubation of all shapes for HEK293 and HUVE. The cell nuclei are visible in blue (Hoechst 33342 excited at 350nm, added 30 minutes before imaging to the living cells), pink is Cy5 labeled DNA origami, excited at 645nm, after overnight incubation at standard 1nM concentration. All scale bars are 20 um.

(A) HEK293



(B) HUVEc

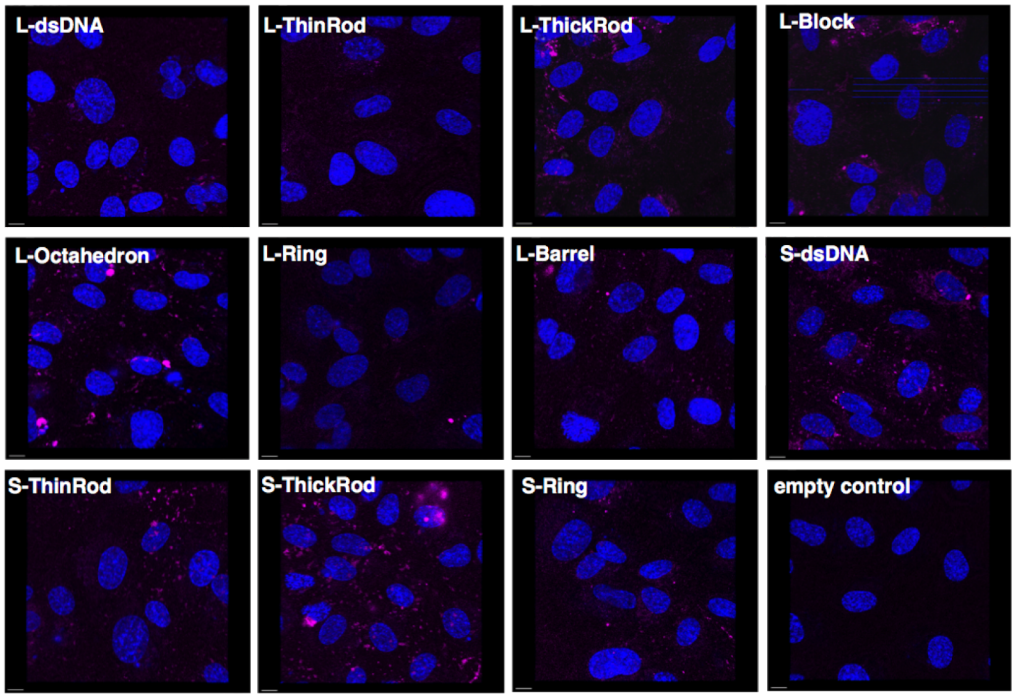


Figure S6 | DNAseI digestion of DNA origami using 20 U of DNAseI

To digest all DNA shapes that remained on the exterior of the cell after overnight incubation, we performed an incubation with 20U DNase1 at 37C for one hour. This figure demonstrates that even in the protective state, these conditions are indeed sufficiently harsh to digest the origami. The first lane (-) shows the reference (note, with the added positive charged shell, the sample now moves upward in the gel) and the (+) lane is the DNase treated sample in the conditions described above, demonstrating a full digestion of the DN.

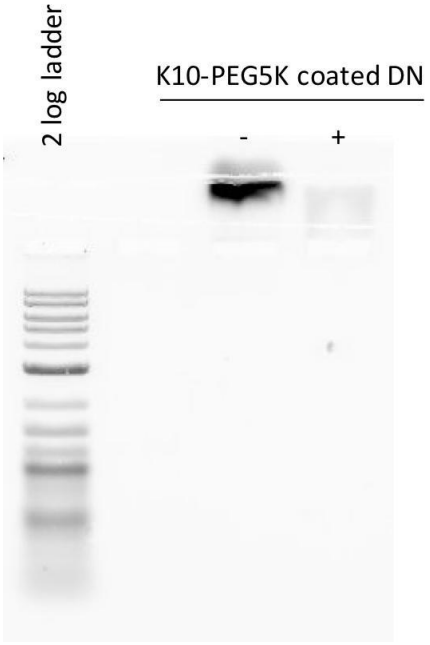


Figure S7 | Flow cytometry of all shapes uptake with 12 hour incubation for HEK293 and HUVEC. L-shapes in **(a)** BMDC, **(b)** HEK293**, (c)** HUVE cells. S-shapes in **(d)** BMDC, **(e)** HEK293**, (f)** HUVE cells For (a) to (f), all data is based of >6 replicates. **(g)** Linear correlation between structure compactness and uptake in HEK293 and HUVE cell lines for L-shapes. Error bars are standard deviations.

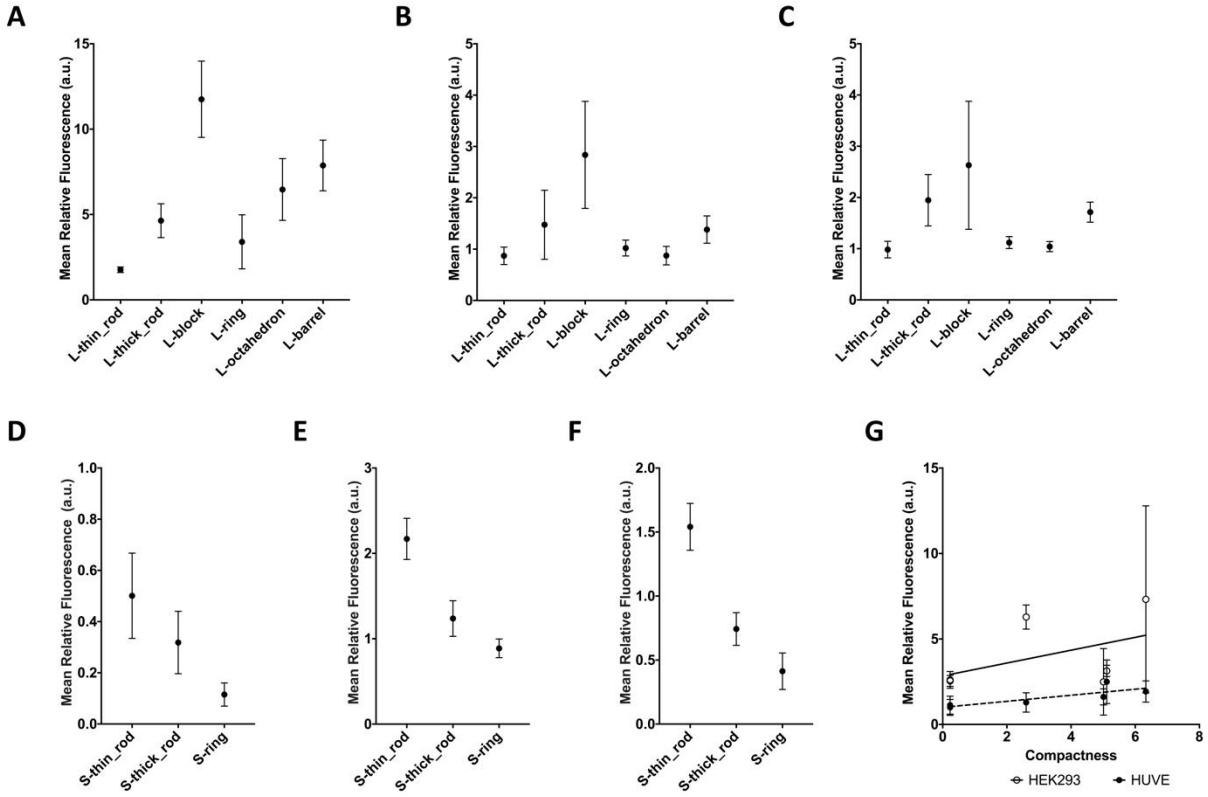


Table S2 | Fluorescence calibration of DN shape library

All our structures are labeled with 3 Cy5 labeled DNA oligos, that are part of the core of each structure. However, both the NHS-conjugation reaction with Cy5 and each oligo, as well as the incorporation yield per oligo in the final DNA origami product are not 100%. Therefore, to enable fair comparison of data between shapes, fluorescence intensity values within each of the data sets were normalized before calculating their mean across the eleven sets.

Normalization was performed by first measuring the final origami concentration by Nanodrop (Table 1) and creation of a sample series of equal volume (50 µL) and concentration (20 nM). We then determined the relative incorporation yield per structure. As each structure is designed to have 3 fluorescently labeled oligonucleotides, only if all 3 are present and the Cy5 ligation was 100% can they be directly compared. Accepting incomplete reaction and incorporation, this calibration step allows us to fairly compare flow-cytometry fluorescent data between shapes.

To do so, we ran an agarose gel using the normalized volumes to 20 nM to calculated the presence of free Cy5 that would influence the measured fluorescence intensity, and then measured fluorescence intensity of 15 µL triplicates in a 384 black well plate on a BioTek NEO fluorescent plate reader with excitation at 645 nm (Cy5 excitation).

To calculate the amount of Cy5 per origami structure, we divide the intensity of Cy5 fluorophore measured by plate reader by the percentage of origami as measured from gel. This correction factors are then calculated in relation to a chosen internal standard (here, L-barrel).

We observe that indeed most shapes result in close to 1 in correction factor, relative to L-barrel.

Structure	Concentration (nM)	Sample volume (uL)	Buffer volume (uL)	Final concentration (nM)	Final volume (uL)
L-dsDNA	147	6.8	43.2	20	50
L-thin_rod	173	5.8	44.2	20	50
L-thick_rod	94	10.7	39.3	20	50
L-block	182	5.5	44.5	20	50
L-ring	191	5.2	44.8	20	50
L-octahedron	188	5.3	44.7	20	50
L-barrel	259	3.9	46.1	20	50
S-dsDNA	83	12	38.0	20	50
S-thin_rod	83	12	38.0	20	50
S-thick_rod	85	11.8	38.2	20	50
S-ring	78	12.9	37.1	20	50

Structure	% origami	% Cy5	Fluor from Origami	Correction F
L-dsDNA	64	36	984	0.7
L-thin_rod	76	24	1554	1
L-thick_rod	58	42	1693	1.1
L-block	53	47	1718	1.1
L-ring	79	21	1522	1
L-octahedron	62	28	1586	1.1
L-barrel	48	52	1507	1
S-dsDNA	37	63	1029	0.7
S-thin_rod	42	58	965	0.6
S-thick_rod	40	60	1160	0.8
S-ring	45	55	1878	1.2

A calibration of Cy5 fluorescence over a concentration range of 10-200 nM was performed to confirm a linear relation could be assumed.

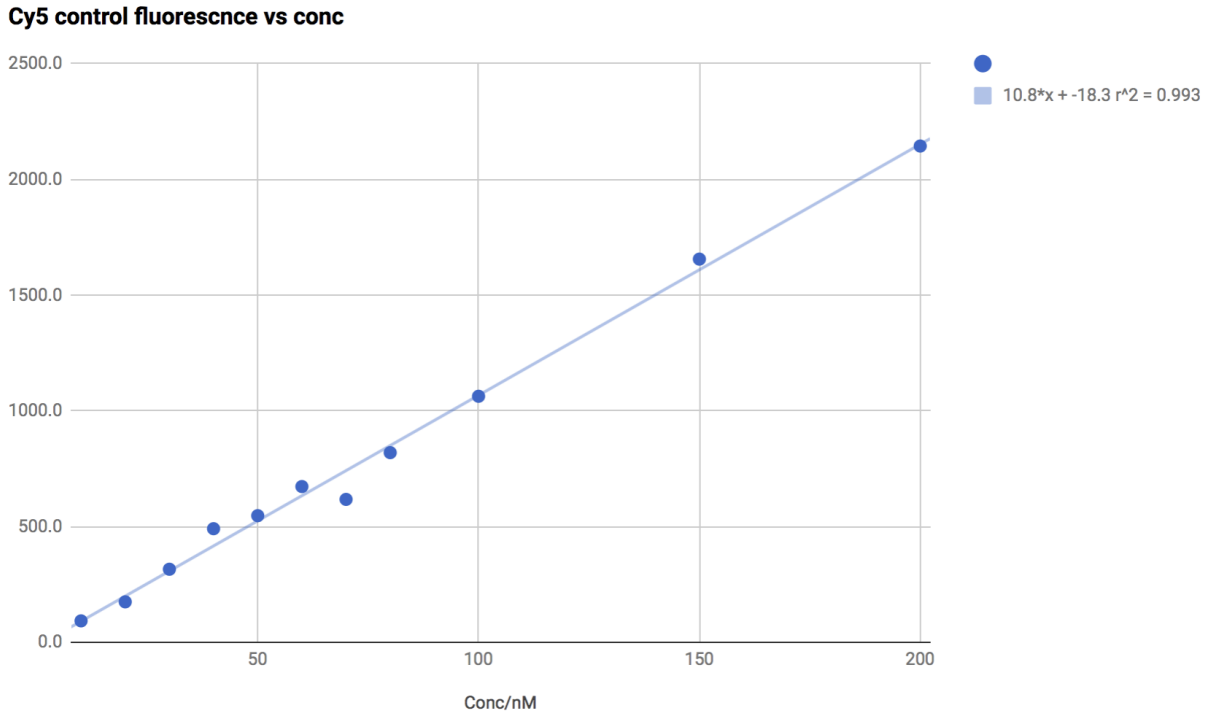
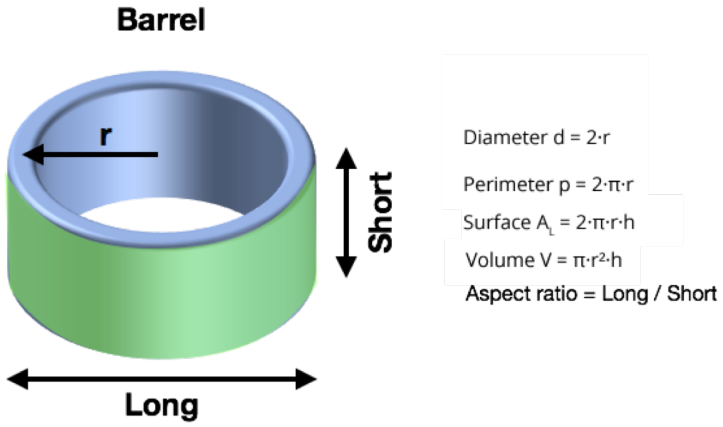


Table S3 | Calculation of volume, length and aspect ratio for individual nanostructures

For the mathematical analysis of correlation between shape and uptake, we need to define some parameters that can be used to quantitatively compare all structures. Given the many differences between designs, e.g. hollow / solid, 2D / 3D, closed / wireframe, we decided to present a complete geometrical calculation. The table below presents the calculation results per shape for accessible surface area, effective volume, compactness, aspect ratio and the measured internalized fraction.

Accessible surface area is defined here as the outside surface per structure the cell membrane can interact with. E.g. for barrel, only the outside cylindrical surface is calculated (green in cartoon). Effective volume is the volume body each structure would take up if it were solid. Compactness is used conform the definition in geometry taking the accessible surface area ^ 1.5 divided by the effective volume. Aspect ratio is given by the design and is the ratio of longest axis length divided by shortest axis as highlighted in the cartoon below.



	accessible surface area (ASA) [nm^2]	effective volume (EV) [nm^3]	"compactness" = $ASA^{1.5}/EV$	aspect ratio	internalized fraction [%] for BMDCs
L-thin_rod	4.7×10^3	140×10^4	0.23	57.1	57.66
L-thick_rod	5.1×10^3	14×10^4	2.60	6.7	48.49
L-block	3.8×10^3	3.7×10^4	6.33	2.4	51.48
L-octahedron	4.7×10^3	6.3×10^4	5.11	1.0	53.27
L-barrel	5.3×10^3	7.7×10^4	5.01	2.0	59.24
L-ring	4.7×10^3	140×10^4	0.23	57.1	59.92
S-thin_rod	2.0×10^3	11×10^4	0.81	24.3	71.18
S-thick_rod	2.2×10^3	2.5×10^4	4.13	2.7	60.12
S-ring	2.0×10^3	11×10^4	0.81	24.3	70.79

Figure S8 | Kinetic study of L-block and L-barrel for (A) HEK293 and (B) HUVE cells using flow cytometry. Data presented based on triplicate experiments. Error bars are standard deviations.

Plotted is the mean fluorescence of L-barrel and L-block relative to control (buffer addition only) after various incubation times in HEK293 and HUVE cells.