Supporting Information

Receptor-Mediated Entry of Pristine Octahedral DNA Nanocages in Mammalian Cells.

Giulia Vindigni[†#], Sofia Raniolo[†#], Alessio Ottaviani[‡], Mattia Falconi[‡], Oskar Franch[§⊥], Birgitta R. Knudsen[§⊥], Alessandro Desideri[‡*] and Silvia Biocca[†*]

†Department of Systems Medicine, University of Rome Tor Vergata, Via Montpellier 1, 00133, Rome, Italy.

‡Department of Biology, Interuniversity Consortium, National Institute Biostructure and Biosystem (INBB), University of Rome Tor Vergata, Via della Ricerca Scientifica 1, 00133, Rome, Italy.

§ Department of Molecular Biology and Genetics, Aarhus University, Aarhus 8000, Denmark;

⊥ Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Aarhus 8000, Denmark.

#These two authors contributed equally.

*_Correspondence to:_ Silvia Biocca - biocca@med.uniroma2.it; Alessandro Desideri - desideri@uniroma2.it

S1 Oligonucleotides.

All oligonucleotides were HPLC purified and purchased from DNA Technology with the exception of the biotinylated oligo ($OL8_{BIO}$) which was purchased from Sigma Aldrich. The sequences of the oligos are reported in Table S1. The 5' of each oligo is phosphorylated. The TTTTT represent a short non pairing spacer inserted within the strands as DNA junction at each vertex of the assembled 3D structure. The eighth oligo is modified by adding a biotin tetraethylenglycol molecule (BtndT).

Table S1. Sequence of the oligonucleotides used for the assembly of DNA octahedra.

Name	Sequence (5'→3')
OL1	**Phos-**GCCACCAGG**TTTTT**CGATGTCTAAGCTGACCG**TTTTT**GGACCGTGATTCCATGAC**TTTTT**CTTAGAGTT
OL2	**Phos-**TGGCTACAG**TTTTT**CGGTCAGCTTAGACATCG**TTTTT**GAATCCTATGCTCGGACG**TTTTT**GGCTCACAT
OL3	**Phos-**TCACGGTCC**TTTTT**CTATCCGATCGAGGCATG**TTTTT**CATACTGAGAGCGTTCCG**TTTTT**GTCATGGAA
OL4	**Phos-**CAGATACGC**TTTTT**CATGCCTCGATCGGATAG**TTTTT**CTGTAGCCAATGTGAGCC**TTTTT**GTCGCAGTT
OL5	**Phos-**CTCAGTATG**TTTTT**CGGTTACGGTACAATGCC**TTTTT**CGCAAGACGTTAGTGTCC**TTTTT**CGGAACGCT
OL6	**Phos-**GGTGTATCG**TTTTT**GGCATTGTACCGTAACCG**TTTTT**GCGTATCTGAACTGCGAC**TTTTT**CCACCGAAT
OL7	**Phos-**CGTCTTGCG**TTTTT**GTATGACGCAGCACTTGC**TTTTT**CCTGGTGGCAACTCTAAG**TTTTT**GGACACTAA
$OL8_{BIO}$	**Phos-**ATAGGATTC**TTTTT**GCAAGTG[**BtndT**]GCGTCATAC**TTTTT**CGATACACCATTCGGTGG**TTTTT**CGTCCGAGC

The biotin is linked to the oligonucleotide ($OL8_{BIO}$) through an additional valerate chain to give a 15Å spacer (Figure S1).

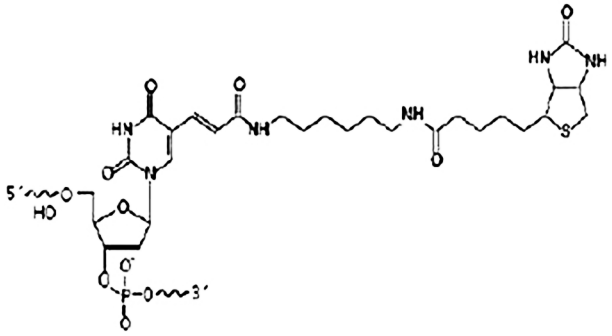

Figure S1. Schematic representation of biotin molecule linked to a DNA strand.

S2 Stability of DNA nanocages in fetal bovine serum (FBS) at 4°C.

Biotinylated cages (20 ng) were incubated in 10% FBS at 4°C for different times (5, 24, 48 and 72 hours). After incubation, each sample was treated with proteinase K (100 μg/ml) for 1 hours at 37°C for digesting the bound proteins. Protein digestion was stopped by adding PMSF to a final concentration of 5 mM. Samples were run in 4% polyacrylamide gels, blotted and visualized by the streptavidin (HRP)-biotin reaction. Figure S2 shows that DNA nanocages are fully stable up to 72 hours at 4°C. The differences in electrophoretic migration between input (lane 1) and FBS incubated samples (lanes 2-5) are due to partial digestion of proteinase K treatment.

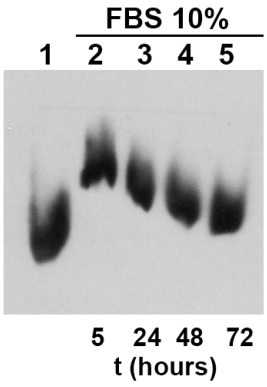

Figure S2. Time-course incubation of DNA nanocages in 10% FBS at 4°C. 20 ng of purified DNA nanocages before incubation with FBS (input) is shown in lane 1. Incubation times are indicated under the gel.

S3 Lifetime and half-life analysis of DNA octahedra in FBS at 37°C

For densitometric analysis of Figure 3C, the relative intensity of each band was quantified using the Image J software and normalized to the intensity of the band corresponding to 20 ng of purified biotinylated DNA nanocages not incubated with FBS (input). The mean lifetime and the half-life have been calculated as follows:

$$N(t) = N_0\, e^{-\lambda t}$$

$$\tau = \frac{1}{\lambda}$$

$$t_{\frac{1}{2}} = \tau\, ln2$$

where *No* is the initial band intensity, *N* is the band intensity at the time *t*, λ is the decay constant and τ is the lifetime and $t_{1/2}$ is the half-life.

Table S3. Summary of exponential decay analysis of DNA octahedra

λ (hrs^{-1})	R^2	τ (hrs)	$t_{1/2}$ (hrs)
0.026	0.932	38.46	26.65

S4 COS cell transfection efficiency.

COS cells were grown on poly-L-lysine coated glass slides and then transfected for 5 hours with 1, 1.5 and 3 μg/ml of plasmid pEF/V5-LOX-1 and JetPEI reagent maintaining a DNA/transfectant reagent ratio of 1:2 (w/v). After 24 hours from transfection cells were washed in PBS, fixed in 4% paraformaldehyde and neutralized with $NaBH_4$. LOX-1-V5 receptors expressed at the cell surface were visualized by using Mab anti-V5 and Rhodamine Red-X-conjugated AffiniPure donkey anti-mouse IgG as the secondary antibody and nuclei stained with Hoechst 33342 (Pierce) for detecting apoptotic cells (Figure S4A). The percentage of LOX-1 expressing cells (red fluorescent) is calculated as the ratio between the number of red fluorescent cells and total number of blue nuclei (at least 200) in different optical fields. Histogram (Figure S4B) shows the correlation between the transfection efficiency and the level of apoptosis for each pEF/V5-LOX-1 concentration used. Since 1.5 μg/ml DNA is the condition with lower level of apoptosis (6% ±2) related to transfection efficiency (40% ±5), we have chosen this plasmid concentration for all experiments reported in this work.

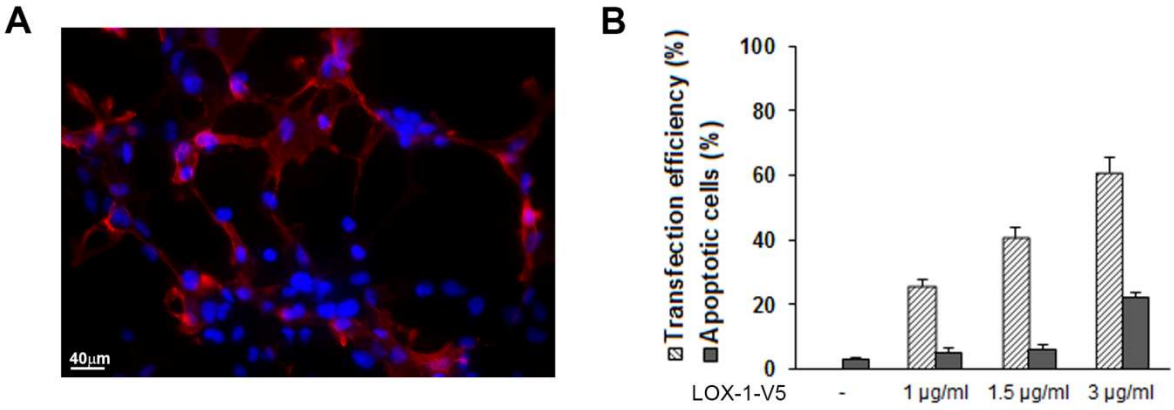

Figure S4. (A) Representative fluorescence image of LOX-1-V5 transfected COS cells in one optical field. Scale bar, 40 μm (B) Histogram shows the correlation between the transfection efficiency and the apoptotic level for each pEF/V5-LOX-1 concentration used. Values are expressed as a mean ± S.E.M.

S5 Uptake experiments with the single biotinylated oligonucleotide $OL8_{BIO}$.

In order to demonstrate a specific binding between assembled DNA nanocages and LOX-1 receptors, we have performed uptake experiments with the single biotinylated oligonucleotide $OL8_{BIO}$. In detail, different concentrations (ranging from 2.6 to 13 μg/ml) of $OL8_{BIO}$ were incubated for 2 and 24 hours at 37°C with LOX-1 transfected and not transfected COS cells (COS nt). $OL8_{BIO}$ was detected with streptavidin-FITC. No fluorescence was detectable inside the cells in any of the above described experimental conditions, notwithstanding the fact that concentrations of $OL8_{BIO}$ were from 8 to 40 times higher than the assembled DNA nanocages (Figure 4).

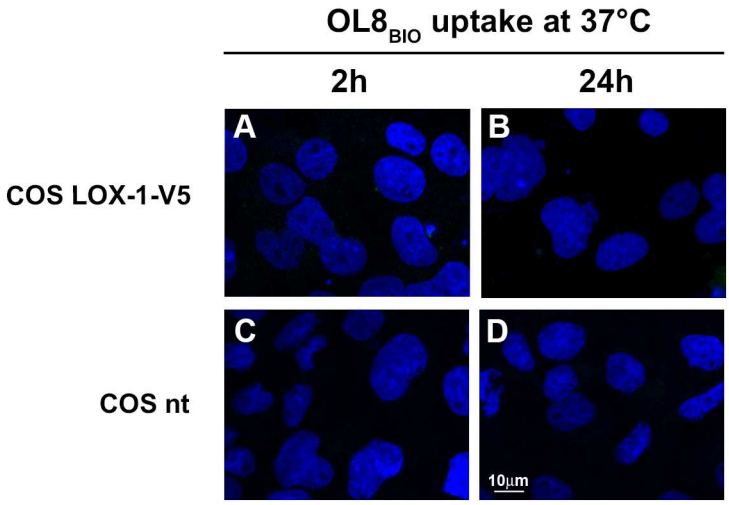

Figure S5. Representative fluorescence images of LOX-1 transfected were incubated with 2.6 μg/ml biotinylated oligonucleotides for 2 and 24 hours at 37 °C (panels A and B). The same experimental conditions were used for COS nt (panels C and D). Biotinylated oligonucleotides were detected with streptavidin-FITC and nuclei were blue stained with DAPI. Scale bar, 10 μm.

S6 Effect of serum proteins on DNA nanocages-LOX-1 recognition.

In order to verify whether the proteins surrounding the cages (protein corona) play an important role in LOX-1 receptor recognition or receptors directly interact to the DNA strands, we have performed a binding experiments at 4°C by adding the pristine cages to cell culture previously depleted of serum proteins. Figure S6 show green fluorescent membrane dots indicative of specific DNA nanocage binding to two LOX-1 transfected cells in the presence of bovine serum (panels B and C) and in one cell in the absence of serum (panels E and F). It is worth noting that within transfected LOX-1 cells some cells are not expressing LOX-1 according to the transfection efficiency. Interestingly, the binding efficiency of DNA nanocages to LOX-1 receptors is not affected by the presence of serum proteins.

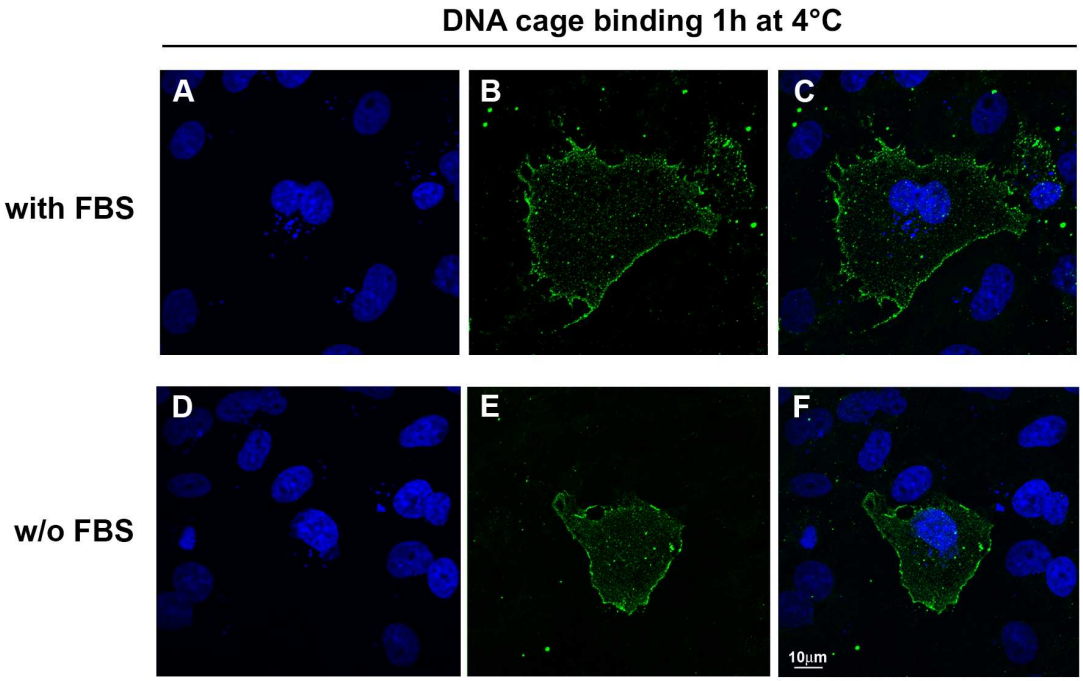

Figure S6. Representative fluorescence images of LOX-1 transfected COS cells incubated with 2,6 µg/ml pristine DNA nanocages in the presence (A) or in the absence (B) of serum proteins. Biotinylated DNA cages were detected with streptavidin-FITC and nuclei are blue stained with DAPI. Scale bar, 20 µm.

S7 DNA nanocage stability in cell extracts.

COS extracts were prepared by incubating cells in lysis buffer (20 mM Tris/Cl pH 7.8, 10 mM EDTA pH 8, 100 mM NaCl, 0.5% NP40, 0.5% NAD), followed by centrifugation (12,000 rpm for 20 min) to remove nuclear DNA and insoluble cell membrane debris. Biotinylated DNA nanocages (20 ng) were then incubated with cell extracts for various times (2, 5, 24 hours) at 4° and 25°C, treated with proteinase K and visualized with streptavidin-HRP in DNA blot assay (Figure S7).

After incubation with cell extracts, DNA cages appear as intact bands and show the same intensity of purified DNA nanocages used as input, demonstrating that DNA nanocages are stable for the times and temperatures analyzed.

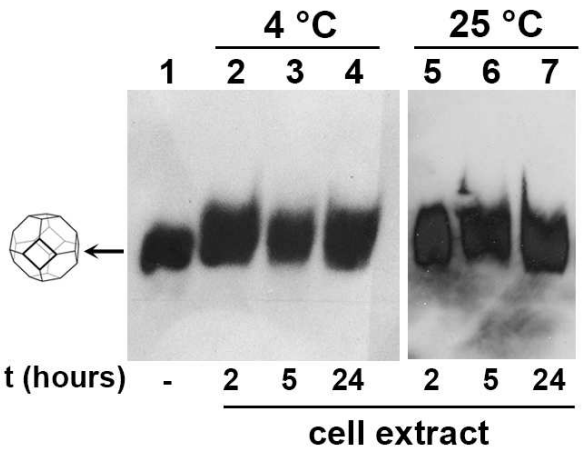

Figure S7. DNA blot analysis of nanocage stability in cell extract. Lane 1 shows 20 ng of purified DNA nanocages before incubation with cell extracts (input). DNA nanocages after incubation with cell extracts at 4°C (lanes 2-4) and 25°C (lanes 5-7). Incubation times were indicated under the gel.