Electronic Supplementary Information

(ESI)

Entry, fate and degradation of DNA nanoparticles in mammalian cells: a matter of receptors

Sofia Raniolo[1], Giulia Vindigni[1], Valeria Unida[2], Alessio Ottaviani[2], Elena Romano[2], Alessandro Desideri[2] and Silvia Biocca[1*]

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

[2]Department of Biology, University of Rome Tor Vergata, Via della Ricerca Scientifica 1, 00133, Rome, Italy.

**Correspondence to:* biocca@med.uniroma2.it

S1. Sequences of oligonucleotides used for octahedral Bio-Fol-DNA nanocages assembly

Unmodified oligonucleotides were HPLC purified and purchased from Integrated DNA Technologies (IDT), biotinylated oligos (OL2) were purchased from Sigma Aldrich. The sequences of the oligos for the assembly of octahedral Bio-Fol-DNA nanocages are reported in Table S1. The 5' of each oligo is phosphorylated. TTTTT represents a short non-pairing spacer inserted within the strands as a DNA junction at each vertex of the assembled 3D structure. OL2 has a biotin tetra-ethylen-glycol molecule (BtndT) at the T represented in blue. The OL8 oligo has been functionalized with folic acid at the bases represented in red.

Oligo	Sequence
OL1	5'-P-GCCACCAGGTTTTTCGATGTCTAAGCTGACCGTTTTTGGACCGTGATTCCATGACTTTTTCTTAGAGTT-3'
OL2	5'-P-TGGCTACAGTTTTTCGGTCAGC**T**TAGACATCGTTTTTGAATCCTATGCTCGGACGTTTTTGGCTCACAT-3'
OL3	5'-P-TCACGGTCCTTTTTCTATCCGATCGAGGCATGTTTTTCATACTGAGAGCGTTCCGTTTTTGTCATGGAA-3'
OL4	5'-P-CAGATACGCTTTTTCATGCCTCGATCGGATAGTTTTTCTGTAGCCAATGTGAGCCTTTTTGTCGCAGTT-3'
OL5	5'-P-CTCAGTATGTTTTTCGGTTACGGTACAATGCCTTTTTCGCAAGACGTTAGTGTCCTTTTTCGGAACGCT-3'
OL6	5'-P-GGTGTATCGTTTTTGGCATTGTACCGTAACCGTTTTTGCGTATCTGAACTGCGACTTTTTCCACCGAAT-3'
OL7	5'-P-CGTCTTGCGTTTTTGTATGACGCAGCACTTGCTTTTTCCTGGTGGCAACTCTAAGTTTTTGGACACTAA-3'
OL8	5'-P-ATAGGATTCTTTTTGCAAGTGC**T**GCGTCATACTT**T**TTCGATACACCATTCGGTGGTTTTTCGTCCGAGC-3'

Table S1. Sequences of the oligonucleotides used for the assembly of Bio-Fol-DNA cages. In red is depicted the biotinylated T in OL2 and in blue are shown the two T in OL8 used for folate conjugation.

S2 Biotin conjugation of the oligonucleotide OL2

An additional valerate chain to give a 15Å spacer is added to oligonucleotide 2 (Figure S2).

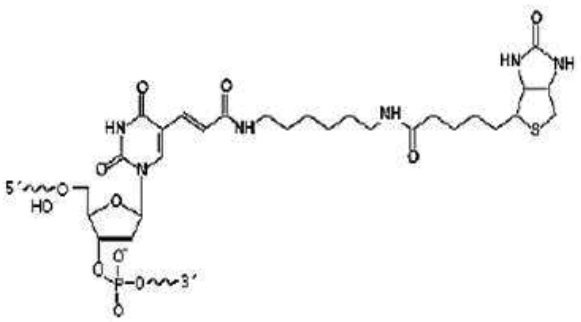
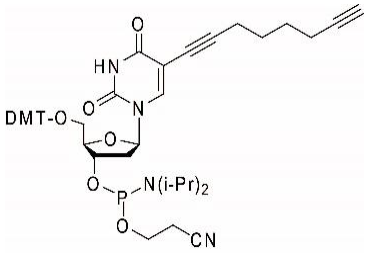


Figure S2. Schematic representation of biotin molecule linked to a DNA strand.

S3 Folate conjugation of the oligonucleotide OL8



C8-Alkyne-dU-phosphoramidite used for the synthesis of the double alkyne modified oligonucleotide OL8.

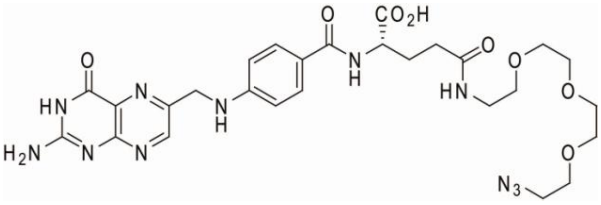


Figure S3. Folate-PEG3-azide used to label the double-alkyne modified oligonucleotide (OL8). The click reaction was carried out as described.[1]

S4. Pristine and folate modified nanocages internalization in LOX-1-expressing COS cells

In order to verify whether folate-modification of DNA nanocages interfered with LOX-1 receptors recognition, we analysed and compared the internalization of biotinylated (Bio-) and biotinylated and folate-modified (Bio-Fol-) DNA cages in transfected COS cells, that do not express the folate receptors.[2] Figure S4 shows representative fluorescence images of LOX-1-expressing COS cells incubated with Bio-DNA and Bio-Fol-DNA cages, as indicated, for 4 h at 37°C. The cages appear in many small fluorescent dots in the cytoplasm. From analysis of the confocal images and counting the number of positive cells in different experiments, we concluded that pristine and folate modified cages are uptaken with an identical efficiency and the presence of folate molecules does not interfere with DNA cage interaction with LOX-1 receptors in transfected COS cells.

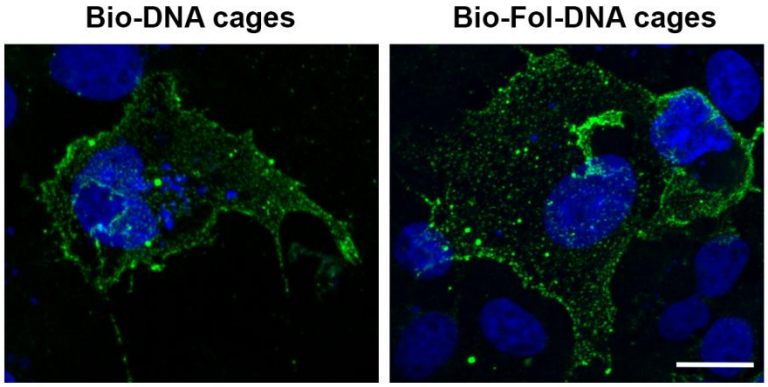


Figure S4. Confocal analysis of DNA nanocages internalized in cells. Representative fluorescence images of COS cells, transiently transfected with LOX-1-V5 plasmid, incubated with Bio- and Bio-Fol-DNA nanocages for 4 h at 37°C. Biotinylated cages were detected with streptavidin-FITC and nuclei are blue stained with DAPI. Scale bar: 20μm.

S5. Effect of serum proteins on DNA nanocage-receptor recognition

For LOX-1 receptors the binding efficiency of DNA nanocages to LOX-1 is not affected by the presence of serum proteins.[3] In order to verify whether the proteins surrounding the cages (protein corona) interfere with α-folate receptor (αFR) recognition, we have performed a binding experiment at 4°C by adding Bio-Fol-DNA nanocages to cell cultures previously depleted of serum proteins. Figure S5 shows green fluorescent membrane dots indicative of specific DNA nanocage binding to HeLa cells in the absence (panels A and B) or in the presence (panels C and D) of 10% fetal bovine serum (FBS). Interestingly, the binding efficiency of DNA nanocages is not affected by the presence of serum proteins.

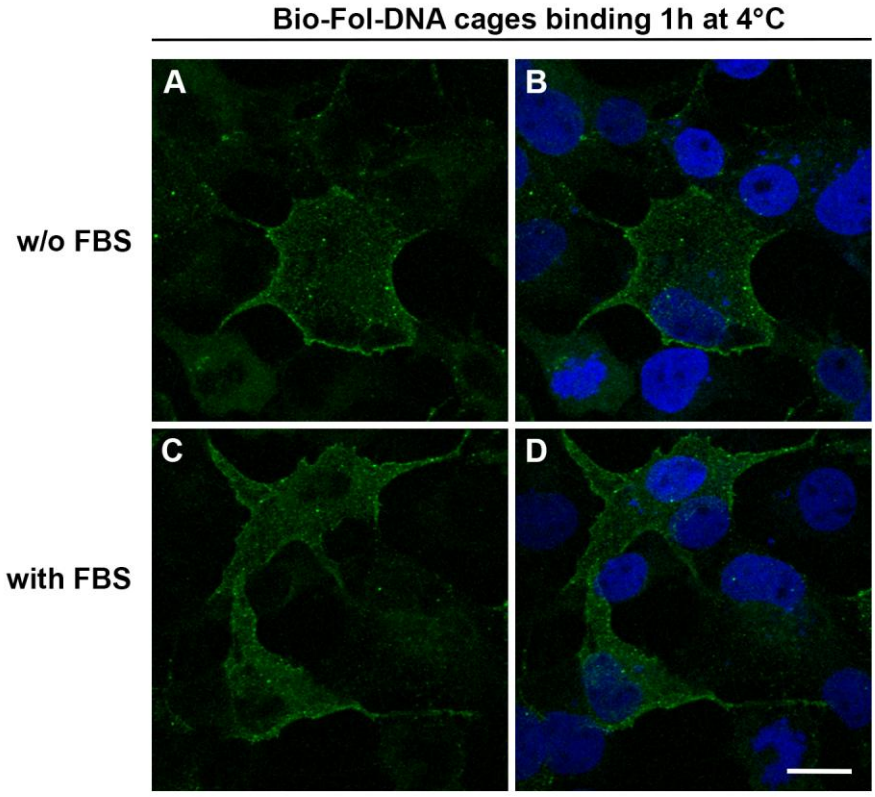


Figure S5. Effect of serum proteins on Bio-Fol-DNA nanocage recognition. Representative fluorescence images of HeLa cells incubated with Bio-Fol-DNA nanocages in the absence (A and B) or in the presence (C and D) of 10% FBS proteins. Biotinylated DNA cages were detected with streptavidin-FITC and nuclei are blue stained with DAPI. Scale bar: 20μm.

S6. Confocal analysis of internalized Bio-Fol-DNA nanocages in HeLa cells.

As further evidence that DNA nanostructures are internalized, we performed confocal analysis of cells incubated with DNA nanostructures for 4 h a 37°C.

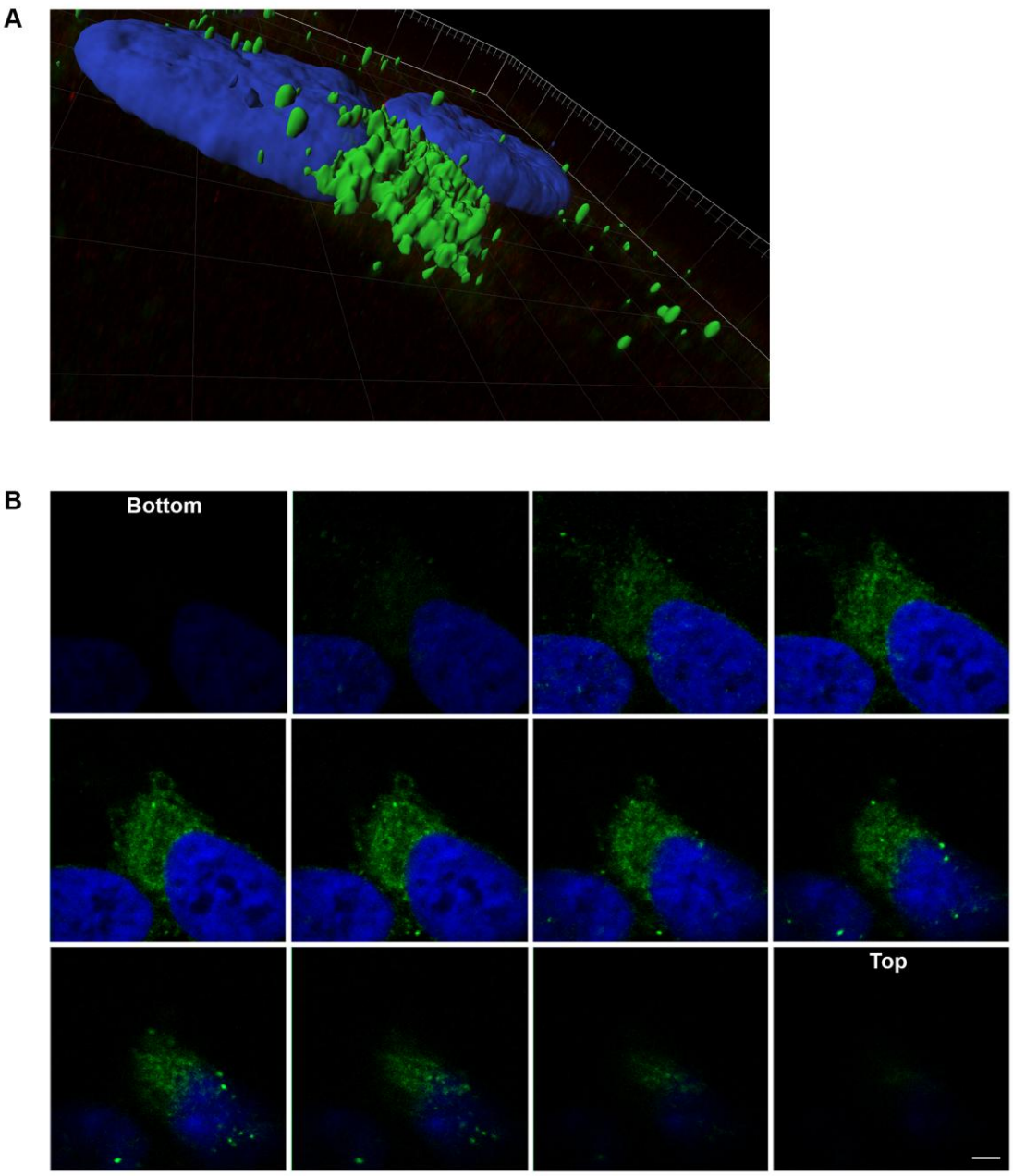


Figure S6 Confocal detection of internalized Bio-Fol-DNA nanocages. HeLa cells were incubated with Bio-Fol-DNA nanocages for 4 h at 37°C. (A) 3D volume render from 12–micrometer serial optical sections with isosurface for blue and green channels. Green fluorescence indicates DNA nanocages accumulated in the cytoplasm and the blue fluorescence shows the cell nuclei. (B) Gallery of 12 serial optical sections from bottom to top of the cells. Scale bar: 3μm.

S7. Co-detection of Bio-Fol-DNA nanocages and αFR receptors.

As further evidence that folate-modified DNA nanocages are internalized in HeLa cells by αFR-mediated mechanism, we performed co-localization experiments on the cell membrane (Figure S7). Bio-Fol-DNA cages and MoV19 have been incubated singularly (panels A and C) or simultaneously (panels D-F) to address if they co-localize or if they impair the binding of each other to αFR. Figure S7 shows representative images in which the membrane binding of Bio-Fol-DNA cages (green fluorescence) to HeLa cells is strongly impaired when DNA cages and MoV19 (red fluorescence) are simultaneously incubated (compare A with D and C with F).

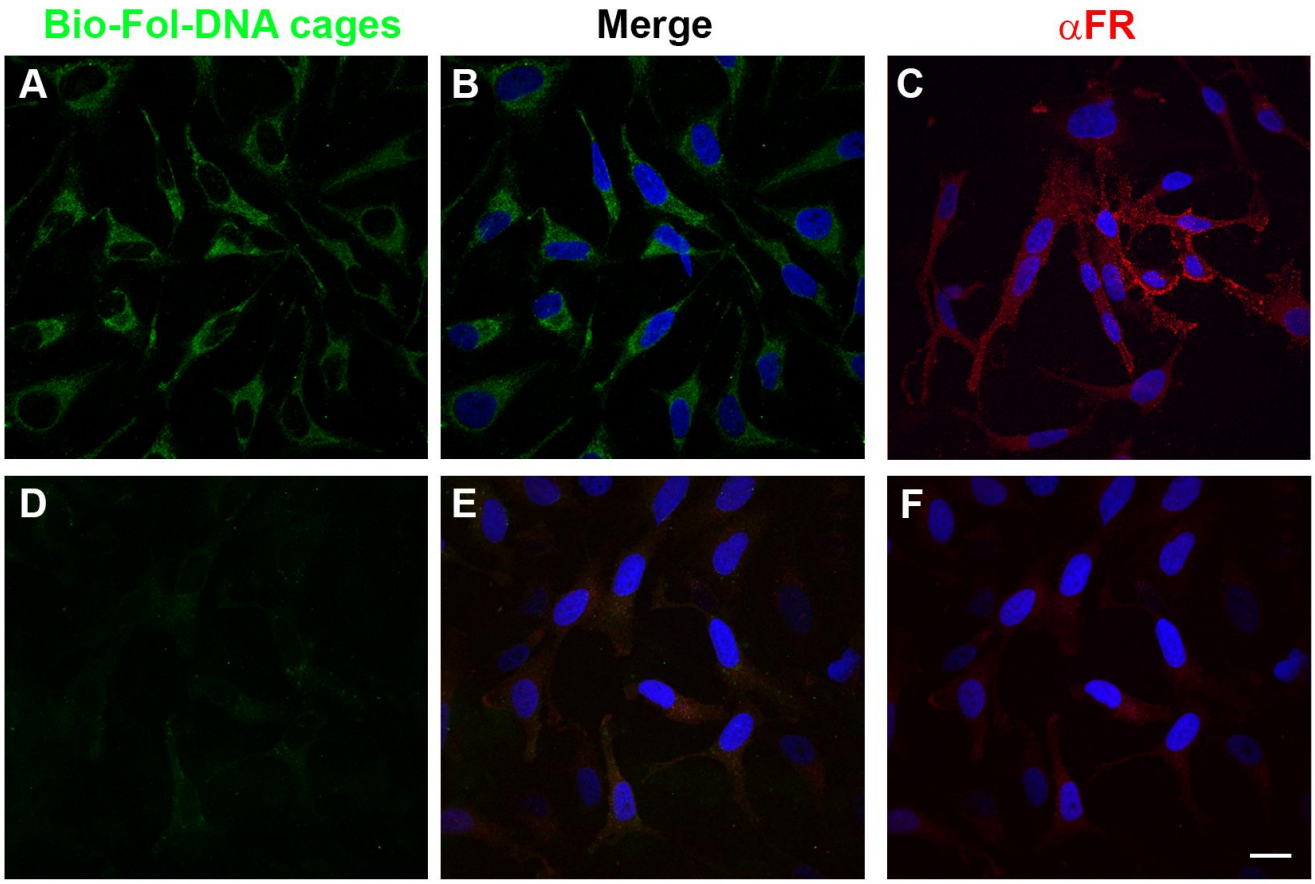


Figure S7. Co-localization analysis of Bio-Fol-DNA nanocages and αFR receptors.
Double fluorescence of HeLa cells incubated with DNA nanocages at 4°C for 1 h. Membrane αFR receptors were visualized using mouse monoclonal MoV19 as primary antibody and Rhodamine Red-X-conjugated donkey anti-mouse IgG as secondary antibody (red), and biotinylated cages were detected by using streptavidin−FITC (green). The nuclei were stained with DAPI. Scale bar: 10μm.

References

1. S. Raniolo, G. Vindigni, A. Ottaviani, V. Unida, F. Iacovelli, A. Manetto, M. Figini, L. Stella, A. Desideri, S. Biocca. *Nanomedicine*, 2018, pii: S1549-9634(18)30034-0.

2. P. Yu, X. M. Xia, M. Wu, C. Cui, Y. Zhang, L. Liu, B. Wu, C. X. Wang, L. J. Zhang, X. Zhou, R. X. Zhuo, S. W. Huang, *Colloids Surf B Biointerfaces*. 2014, **120**, 142-51.

3. G. Vindigni, S. Raniolo, A. Ottaviani, M. Falconi, O. Franch, B. R. Knudsen, A. Desideri, S. Biocca, *ACS Nano*. 2016, **10**, 5971-9.