## Supplementary Materials

## Hairpin Forming DNA strands Promote Temperature Controlled Cargo Uptake in a Truncated Octahedral DNA Nanocage by Increasing the Flexibility of the Structure

Oskar Franch<sup>\*1</sup>, Federico Iacovelli<sup>\*2</sup>, Mattia Falconi<sup>2</sup>, Sissel Juul<sup>1</sup>, Alessio Ottaviani<sup>2</sup>, Claudia Benvenuti<sup>2</sup>, Silvia Biocca<sup>3</sup>, Yi-Ping Ho<sup>1</sup>, Birgitta R. Knudsen<sup>1#</sup>, Alessandro Desideri<sup>2#</sup>

<sup>1</sup>Department of Molecular Biology and Genetics and Interdisciplinary Nanoscience

Center (iNANO), Aarhus University, Aarhus, Denmark.

<sup>2</sup>Department of Biology, University of Rome "Tor Vergata", Rome, Italy.

<sup>3</sup>Department of Systems Medicine and Center of Biostatistics and Bioinformatics, University of Rome Tor Vergata, Via Montpellier 1, 00133, Rome, Italy.

\*shared first authors #shared corresponding authors; BRK (<u>brk@mbg.au.dk</u>); AD (desideri@uniroma2.it)



**Fig. S1.** Uses the design of Cagehp0 to illustrate the points of ligation and pattern of hybridization between the oligonucleotides that make up the cages. **A**, illustrates how the single stranded oligonucleotides that makes up one face of the structure hybridize in a triangular-pattern using the "blue"-oligonucleotide as a scaffold. The "needles" indicate where the oligoes are ligated. **B**, illustrates how the oligonucleotides shown in A fit into the 3D structure of Cagehp0.



**Fig. S2.** Schematic illustration of the spot assay used to detect encapsulation of HRP in the cage structures. A, Following native gelelectrophoresis at 4°C and EtBr staining of the band representing the cage, the cage with its potential HRP cargo was cut out of the gel. B, the gel piece was transferred to an Eppendorf tube where it was washed before it was incubated with (enhanced chemiluminescence) ECL which are oxidize to a chemiluminescence product if HRP is present. The Eppendorf tube was placed on an X-ray film in the dark to detect HRP acitvity. C, shows the dot shaped exposure, which is a typical result of a spot assay.