Synthesis and characterization of an octahedral DNA nanocage having a pH dependent opening/closing switching mechanism and analysis of its entry in mammamalian cells

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DNA is an extremely suitable polymer for the generation of nanocapsules to be used for biomedical applications for its intrinsic properties of high stability, biocompatibility and versatility. In the last years DNA nanotechnology has been extensively used to build an impressive variety of DNA cage structures . Recently we have designed synthesized and characterized an octahedral DNA nanocage having a diameter of about 15 nm, showing how changing the structural dynamical properties are strictly determined by the length of the side and to a lesser extent by the kind of the sequences composing it. Here we have inserted two DNA triple helices at two corners of the octahedral scaffold to permit a pH dependent opening and closing swithching mechanism. We created our switches by taking advantage of the well characterized pH sensitivity of the parallel Hoogsteen (T,C)- motif in triplex DNA.To do so, we designed a DNA-based triplex pH-triggered nanoswitch that consists in a double intramolecular hairpin stabilized with both Watson−Crick (W−C) and parallel Hoogsteen interactions. One hairpin of the triplex nanoswitch is formed by the W−C hybridization of two complementary portions separated by a 5 base loop. This duplex DNA is then able to form a triplex structure via the formation of a second hairpin through Hoogsteen parallel interactions with the other extremity of the switch. Importantly while W−C base pairings are almost insensitive to pH Hoogsteen interactions show a strong and variable pH dependence, since the CGC parallel triplet requires the protonation of the N3 of cytosine in the third strand in order to form (average pKa of protonated cytosines in triplex structure is ≈6.535,38) giving rise to a close conformation at pH 5 and an open one at pH 8. We provide evidence that the cage can switch from an open to a close conformation and that can be efficiently uptaken in mammalian cellular systems representing an useful nanovectors to be used for useful biomedical applications.