DNA nanotechnology

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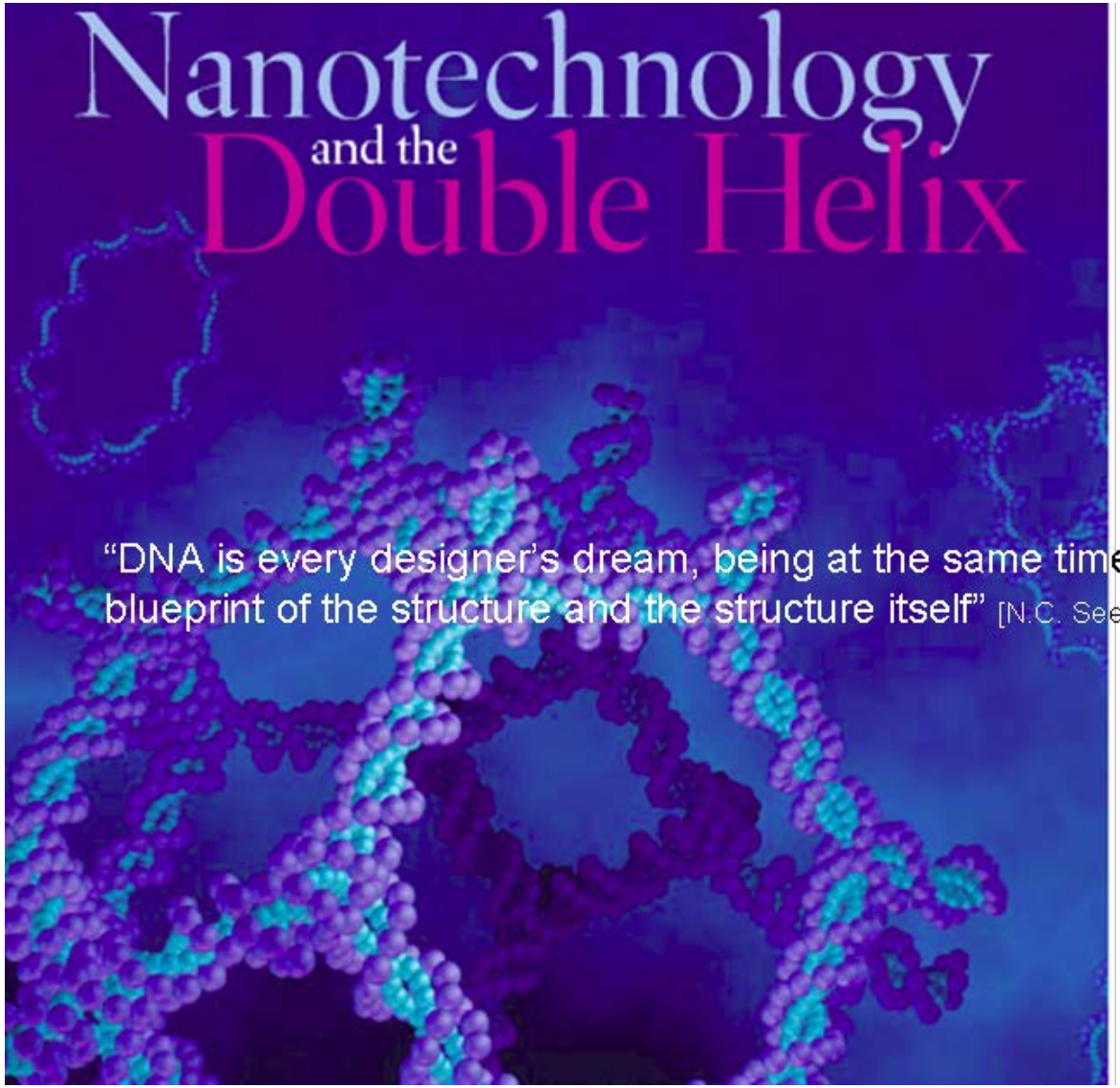
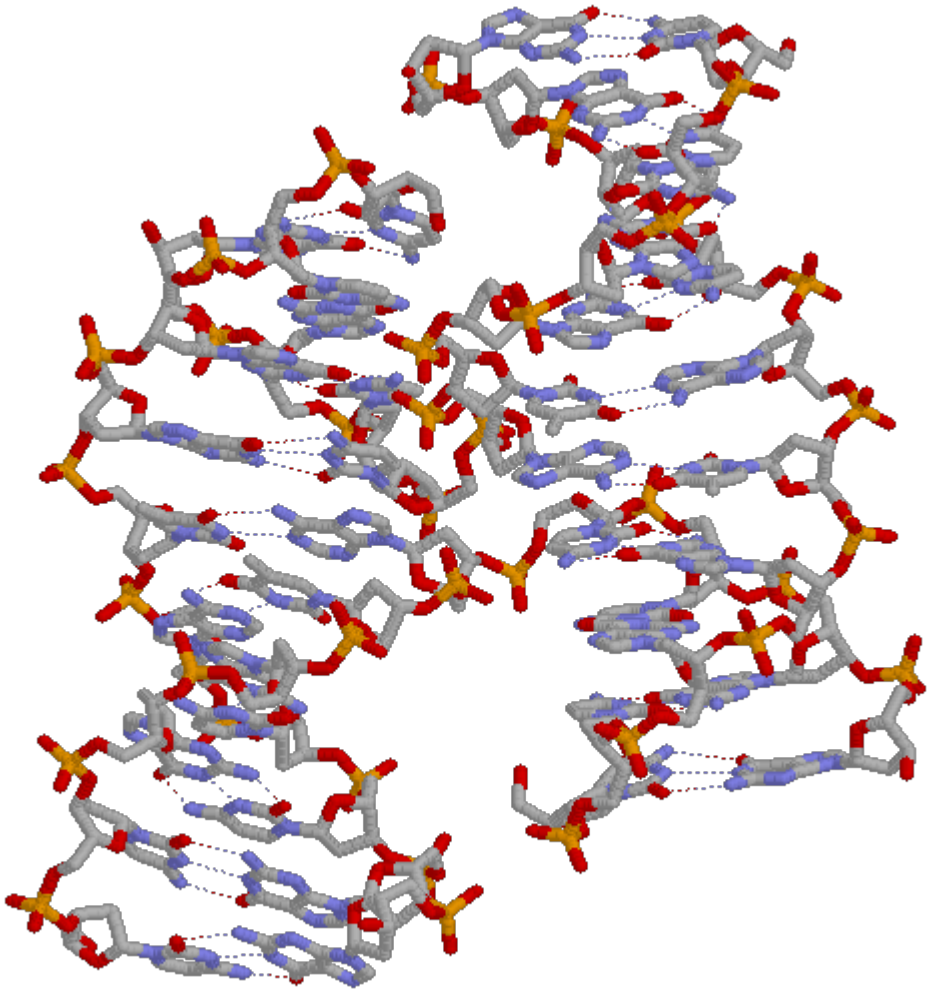
Costruire con il DNA? Costruire con il DNA!

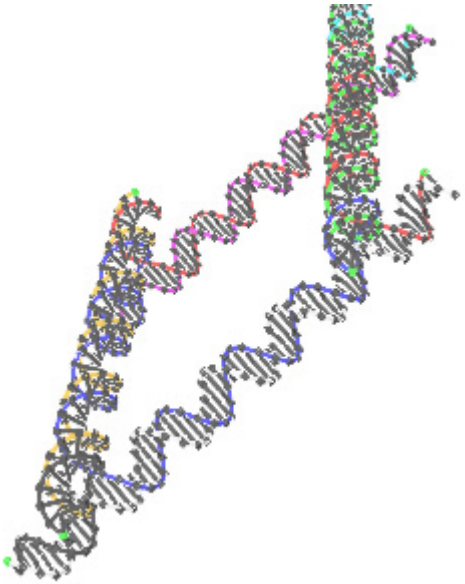


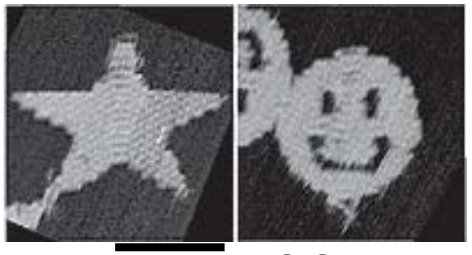
La struttura della giunzione di Holliday

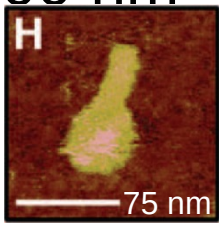


DNA self-assembled nanostructures

- Nanocarriers for functional units
- Small nanostructures: high functionality “per mass”
- Many units can possibly enter a cell
- Possible to polymerize them
- Simpler and cheaper



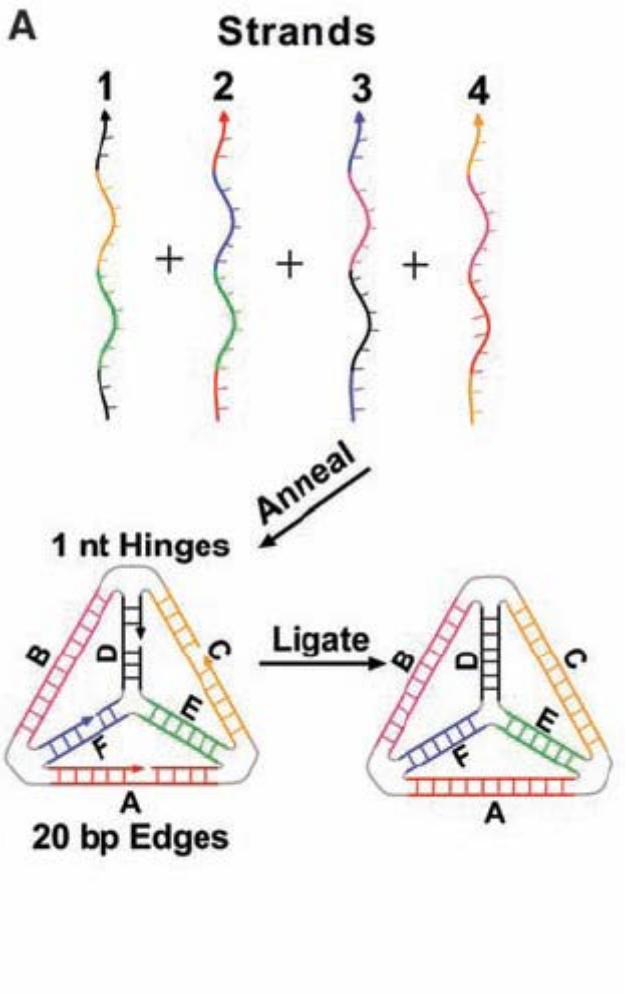


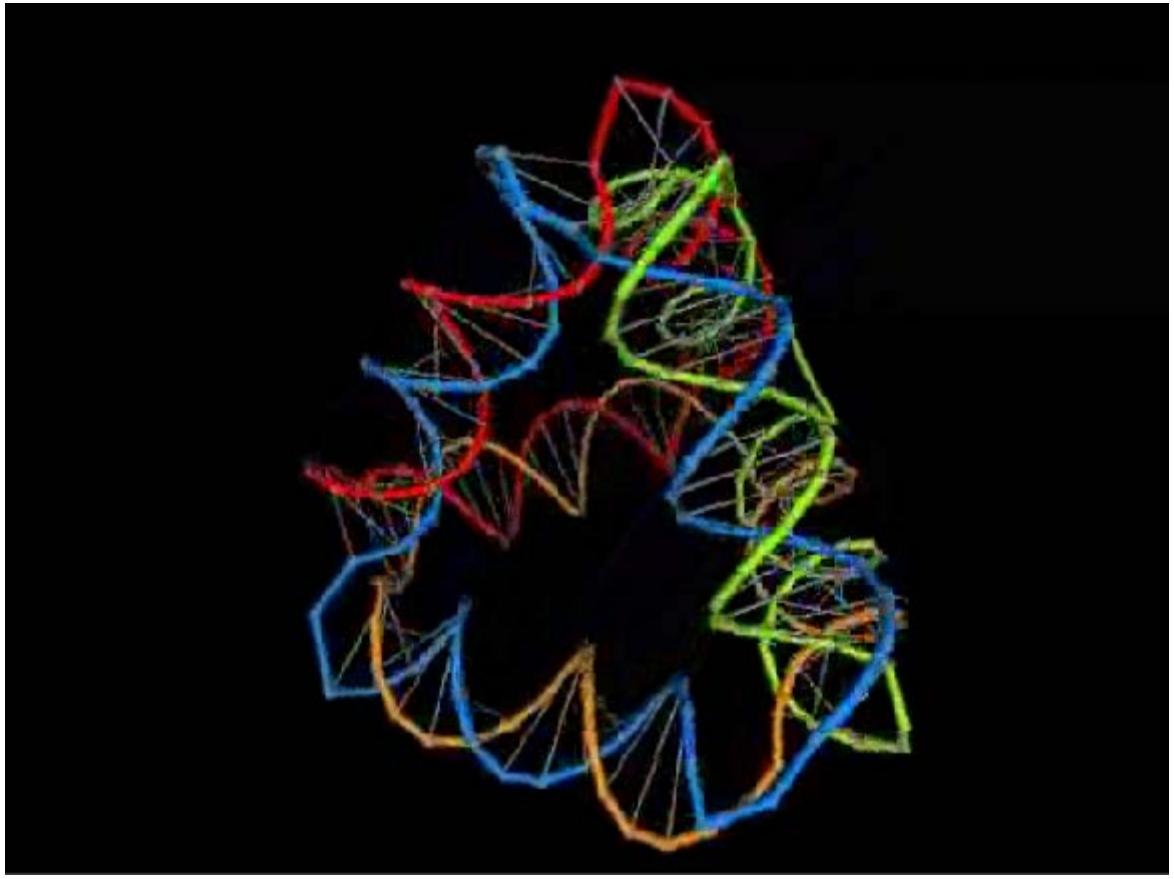


Beautiful examples of **large nanostructures** from Paul Rothemund (Nature, 2006) and from Hao Yan (Science, 2011).

Tetraedri di DNA

Una struttura semplice ed elegante

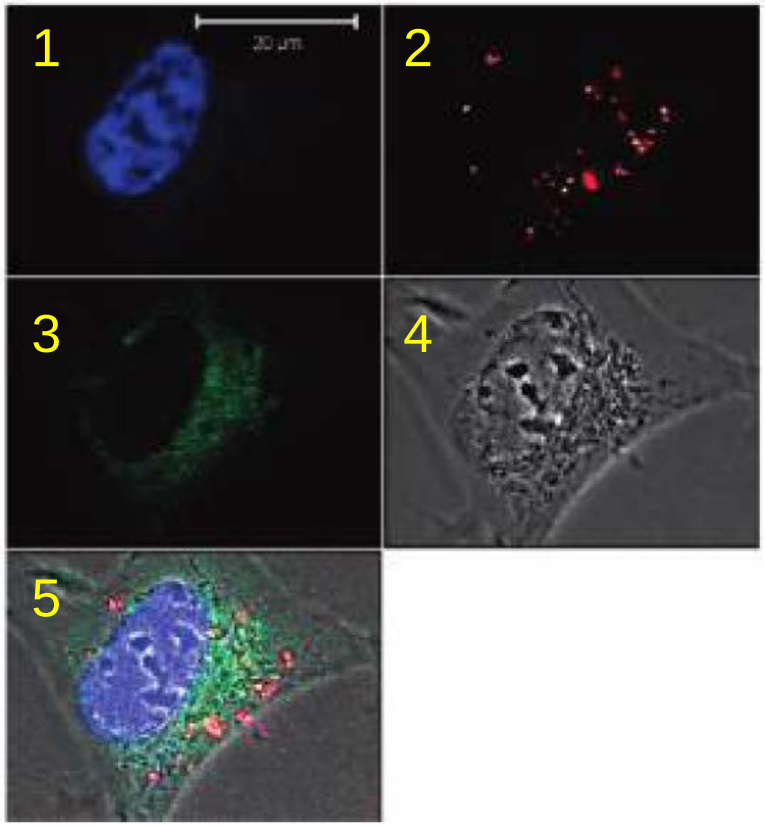




I tetraedri di DNA come vettori o biosensori intracellulari

Si riesce a fare internalizzare tetraedri autoassemblati di DNA nelle cellule.

Questo possono contenere sequenze in grado di funzionare da sensori intracellulari, in modo da poter dare segnali i) su una singola cellula, ii) in tempo reale.



Microscopia confocale di cellule trasfettate (dopo 24 ore):

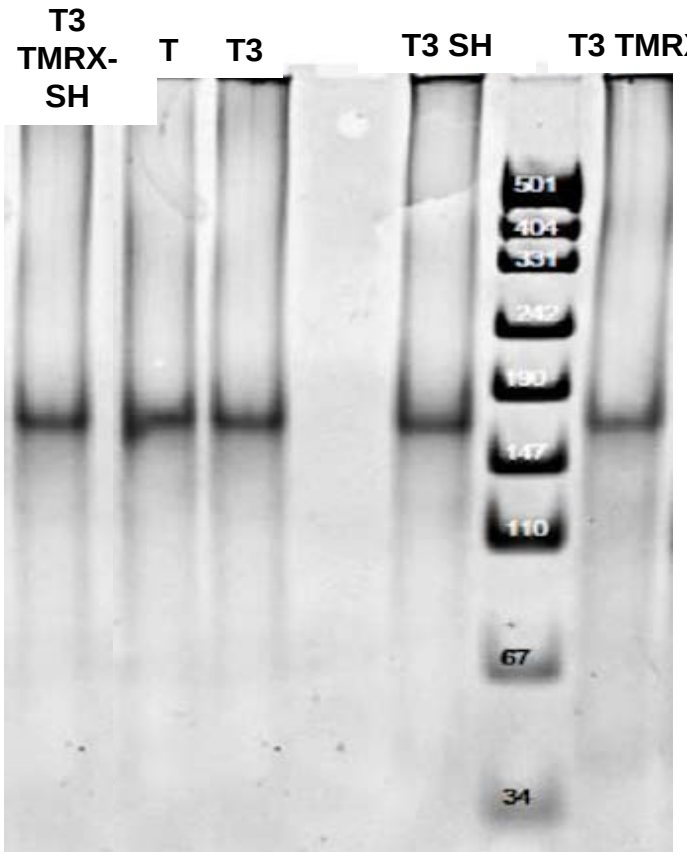
1) In blu un colorante nucleare
2) In rosso, il tetraedro marcato con Cy5
3) In verde, un colorante dei lisosomi
4) Immagine in contrasto di fase
5) Sovrapposizione delle immagini

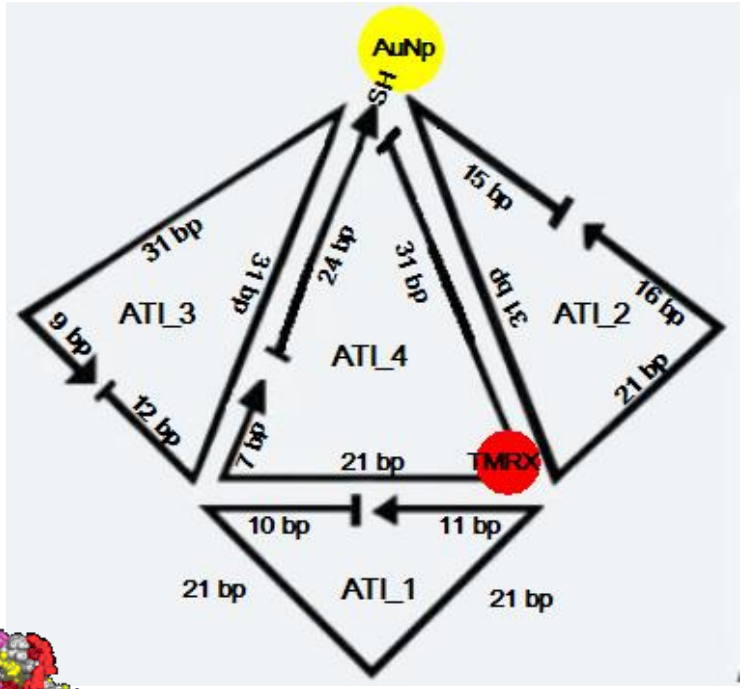
(barra= 20 µm)

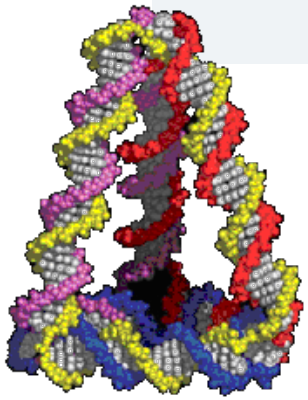
Walsh et al., ACS Nano, 2011.

Bergamini et al.. Methods, 2014.

DNA tetrahedra: a versatile tool for carrying functional units

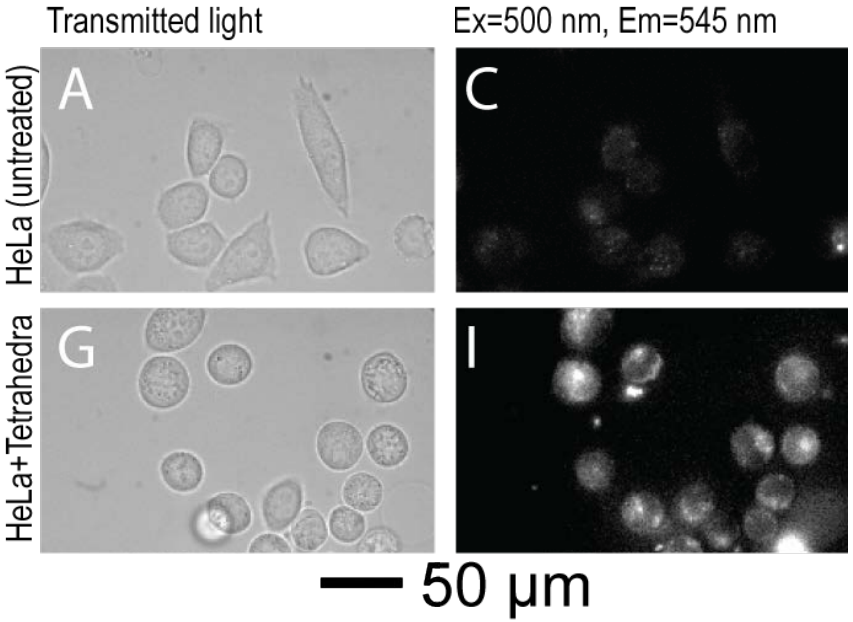


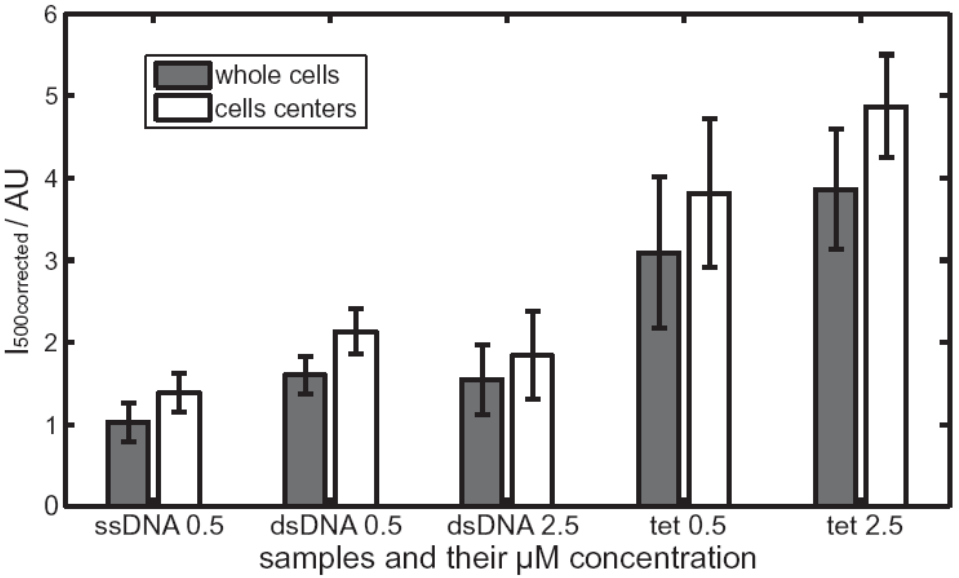




Tetrahedra can carry functional units, such as nanoparticles or fluorophores

Fluorescence after correction for autofluorescence



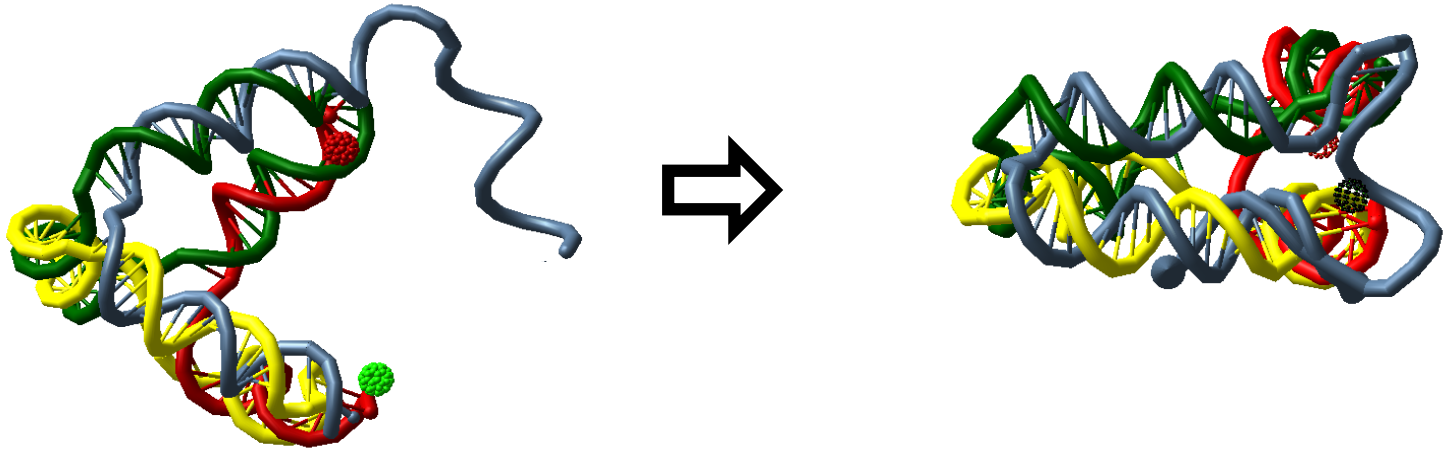


The increase of fluorescence can be safely attributed to the **increase of fluorophore-induced fluorescence**

Increase of cell fluorescence on treating with tetrahedra (larger increase than treating with ssDNA or dsDNA)

Some apparent localization of fluorescence

pH sensing tetrahedron

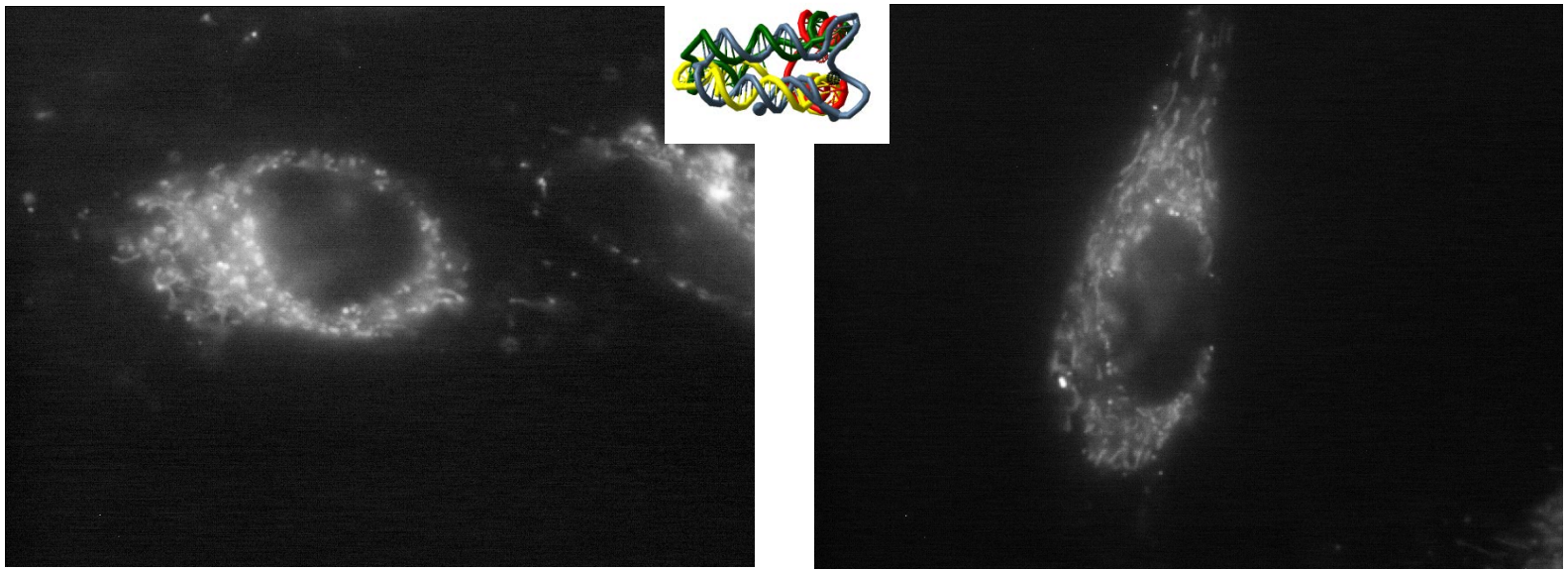


The association of the loose end of a flexible nanostructure with its target sequence on another portion of the structure leads to a large conformational change.

The conformational change can be detected via fluorescence.

These nanostructures are uptaken efficiently by live cells

Glioblastoma cells treated for 6 hrs with 100 nM nanostructures (labelled with Cy3 and Cy5)



Cy3 (donor) fluorescence

Treatment with nanostructures leads to their uptake by cells and partitioning between internal structures: it appears that some end up in lysosomes and some in mitochondria/Golgi apparatus.

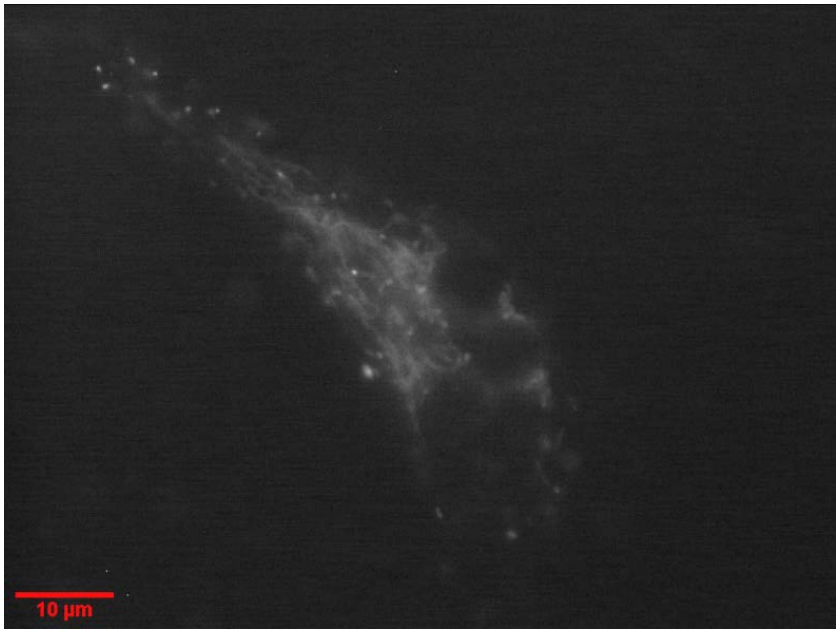
Cy3 and Cy5 signals are co-localized. Some FRET emission is seen.

pH responsive nanostructure inside live cells (fluorescence microscopy)

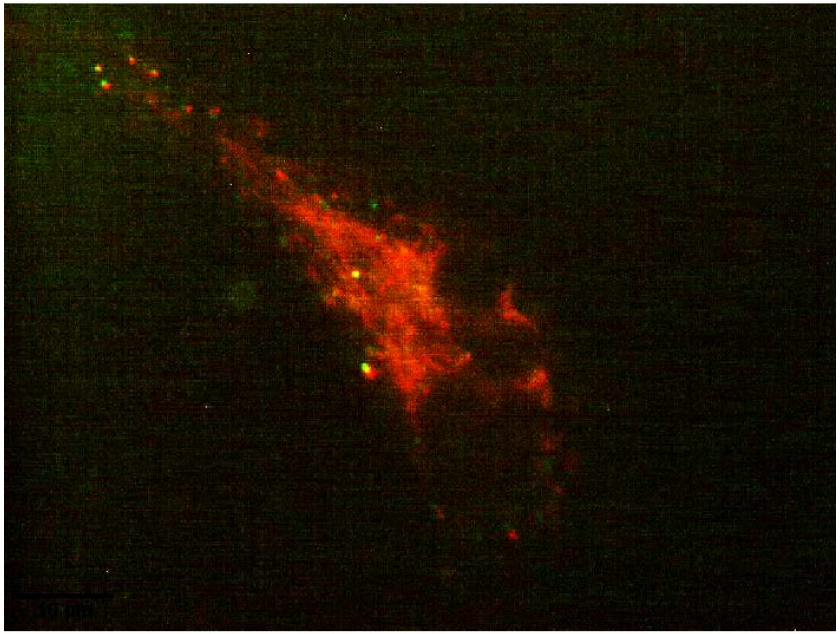
The pH responsive nanostructure is uptaken by live cells.

It localizes in lysosomes and mitochondria/Golgi, with some diffused signal from the cytoplasm, No nuclear localization.

The Cy3->Cy5 FRET signal appears localized in the lysosomes

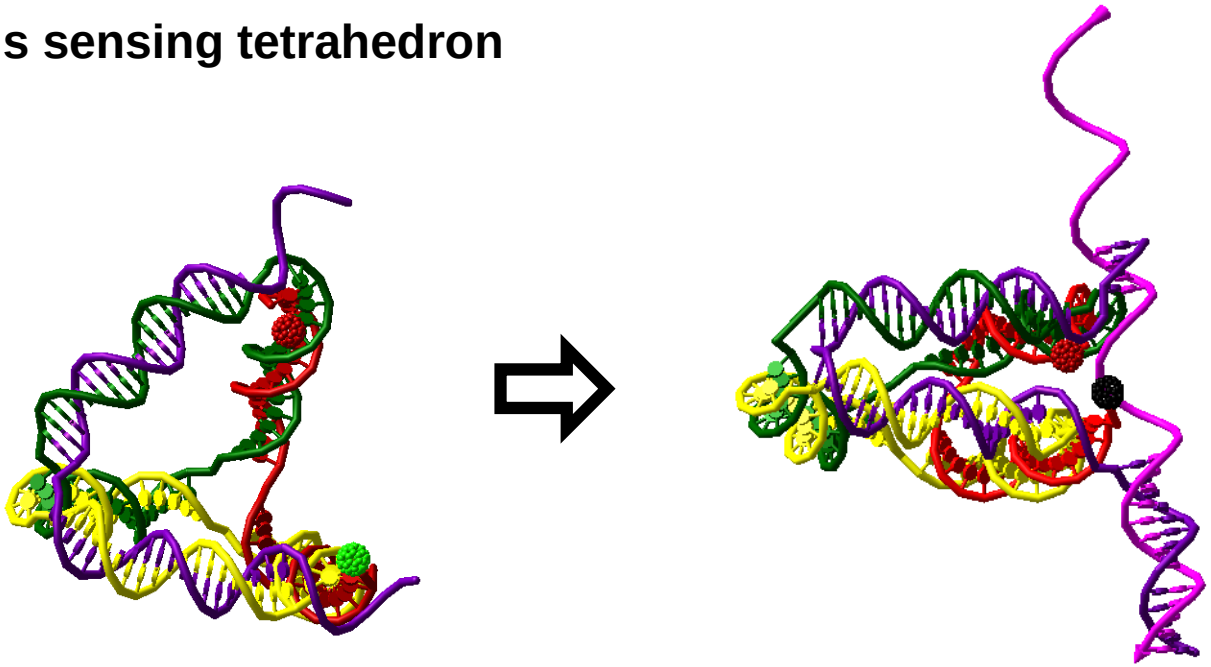


Cy3 (donor fluorescence)



Overlay (Cy3 red, FRET green)

Nucleic acids sensing tetrahedron



The association of the loose ends of a flexible nanostructure upon binding with a target DNA/RNA sequence leads to a large conformational change.

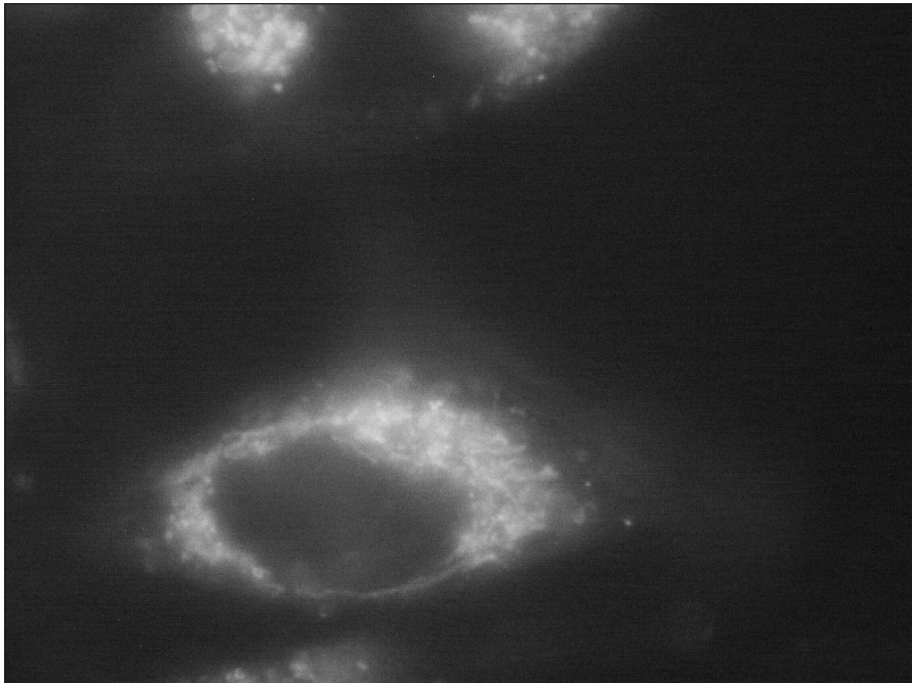
The conformational change can be detected via fluorescence.

Recognition is with two short DNA probes, thus with higher specificity than if done with a longer single one

NA binding nanostructure inside live cells (fluorescence microscopy)

The NA responsive nanostructure is uptaken by live cells too.
Analogously, it localizes in lisosomes and mitochondria/Golgi, possibly some diffused signal from the cytoplasm. No nuclear localization.

Cy3 (donor) signal after 6 hrs incubation of Glioblastoma (T67) cells with a 100 nM solution of nucleic acids responsive nanostructure



Need to improve cytoplasmatic localization to be useful