Università di Roma Tor Vergata

DIPARTIMENTO DI BIOLOGIA

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Temperature dependent protein encapsulation in self-assembled DNA nanocages

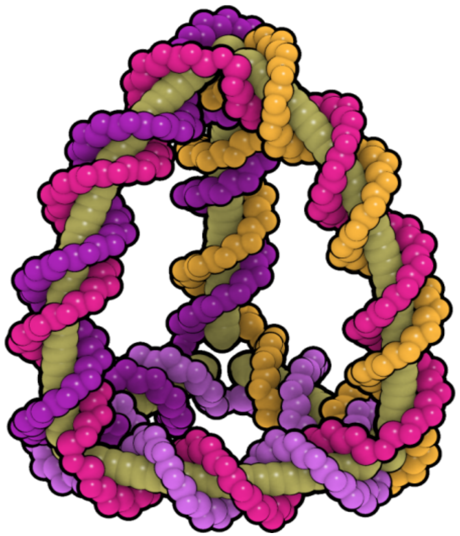
BIOSYSTEMS, ENERGY, AND CULTURAL HERITAGE: MATERIALS ENHANCEMENT FOR TECHNOLOGICAL APPLICATION

July3rd, 2013 –Università di Roma Tor Vergata

DNA nanostructure

DNA advantage:
- The code is composed by only four monomers and hence is easy to program
- The resulting structure is easily predictable

DNA nanostructure use:
- rigid building blocks
- molecular cages
- DNA motors and fuels
- application of DNA lattices to protein structure determination.
- drugs delivery
- replicas of natural structures



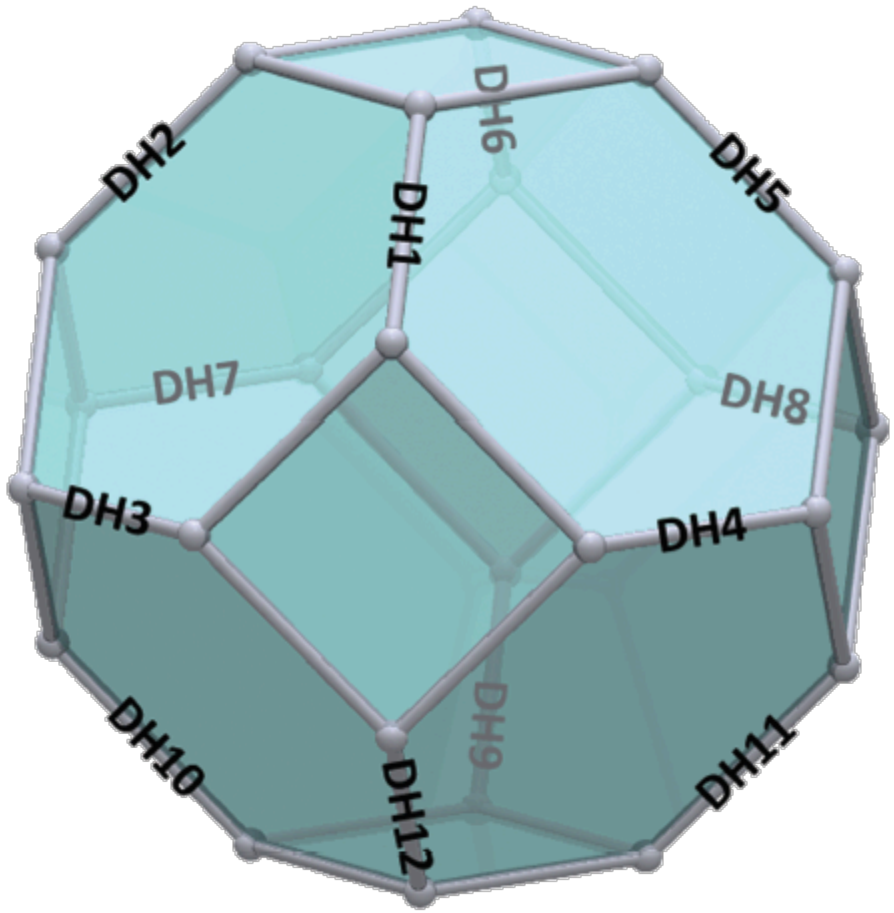
Reference:

- R. P. Goodman, R. M. Berry, A. J. Turberfield, The single-step synthesis of a DNA tetrahedron, Chem. Comm. 1372-1373 (2004)
- J. Bath, S. J. Green, A. J. Turberfield, A free-running DNA motor powered by a nicking enzyme, Angew. Chem. Int. Ed. 44, 4358-4361 (2005)

Predicted Structure

12 Double Helices

24 Seven Thymidine Single Strand



Design of a Truncated Octahedral DNA cage

O1	**GCCACCAGGTTTCGATGTCTAAGCTGACCGTTTGGACCGTGATTCCATGACTTTCTTAGAGTT**
O2	**TGGCTACAGTTTCGGTCAGCTTAGACATCGTTTGAATCCTATGCTCGGACGTTTGGCTCACAT**
O3	**TCACGGTCCTTTCTATCCGATCGAGGCATGTTTCATACTGAGAGCGTTCCGTTTGTCATGGAA**
O4	**CAGATACGCTTTCATGCCTCGATCGGATAGTTTCTGTAGCCAATGTGAGCCTTTGTCGCAGTT**
O5	**CTCAGTATGTTTCGGTTACGGTACAATGCCTTTCGCAAGACGTTAGTGTCCTTTCGGAACGCT**
O6	**GGTGTATCGTTTGGCATTGTACCGTAACCGTTTGCGTATCTGAACTGCGACTTTCCACCGAAT**
O7	**CGTCTTGCGTTTGTATGACGCAGCACTTGCTTTCCTGGTGGCAACTCTAAGTTTGGACACTAA**
O8	**ATAGGATTCTTTGCAAGTGCTGCGTCATACTTTCGATACACCATTCGGTGGTTTCGTCCGAGC**

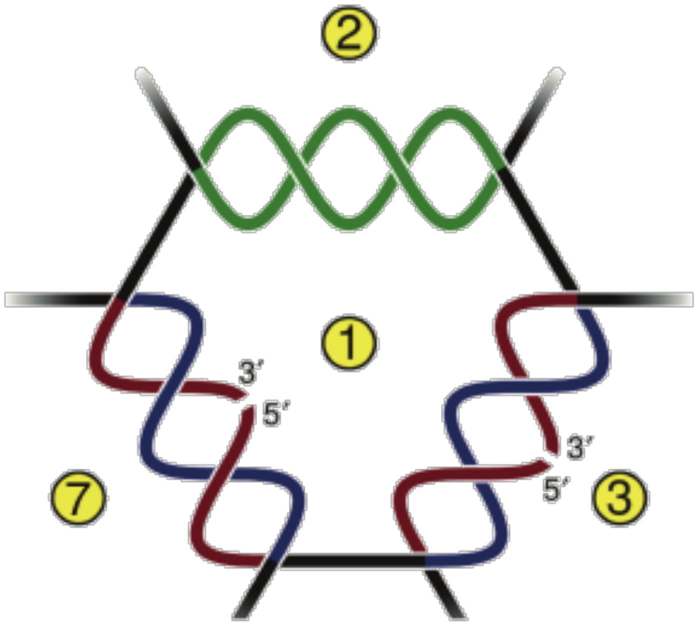
Theoretical complementary green region annealing temperature = **56°C**

Theoretical complementary red-blue region annealing temperature = **28°C**

G1-G2 G3-G4 G5-G6 G7-G8

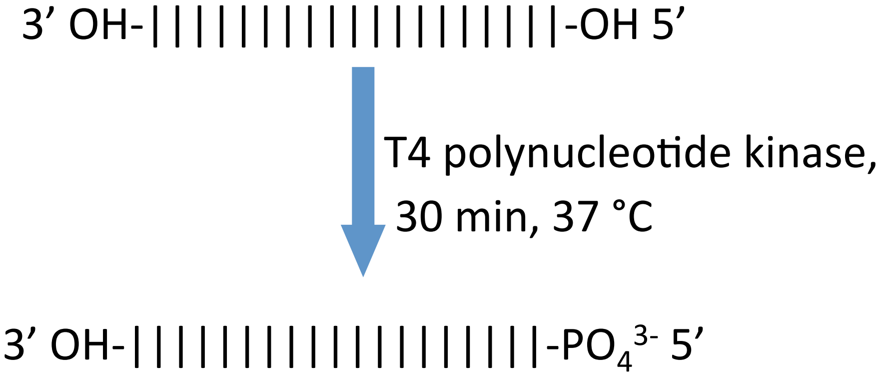
B1-R3 B2-R8 B3-R5 B4-R2

B5-R7 B6-R4 B7-R1 B8-R6



Assembling Procedure

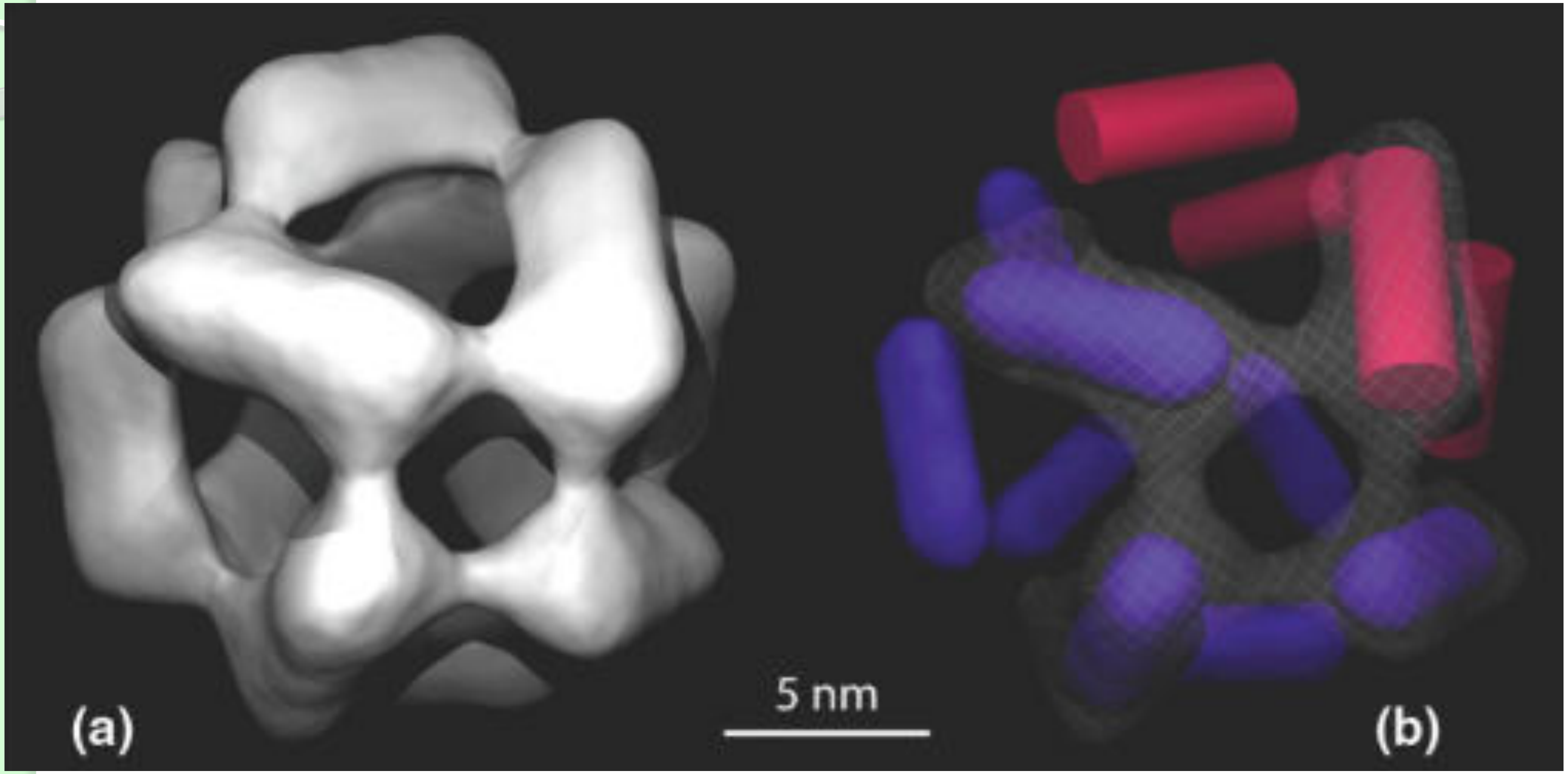
- 5’ extremities activation

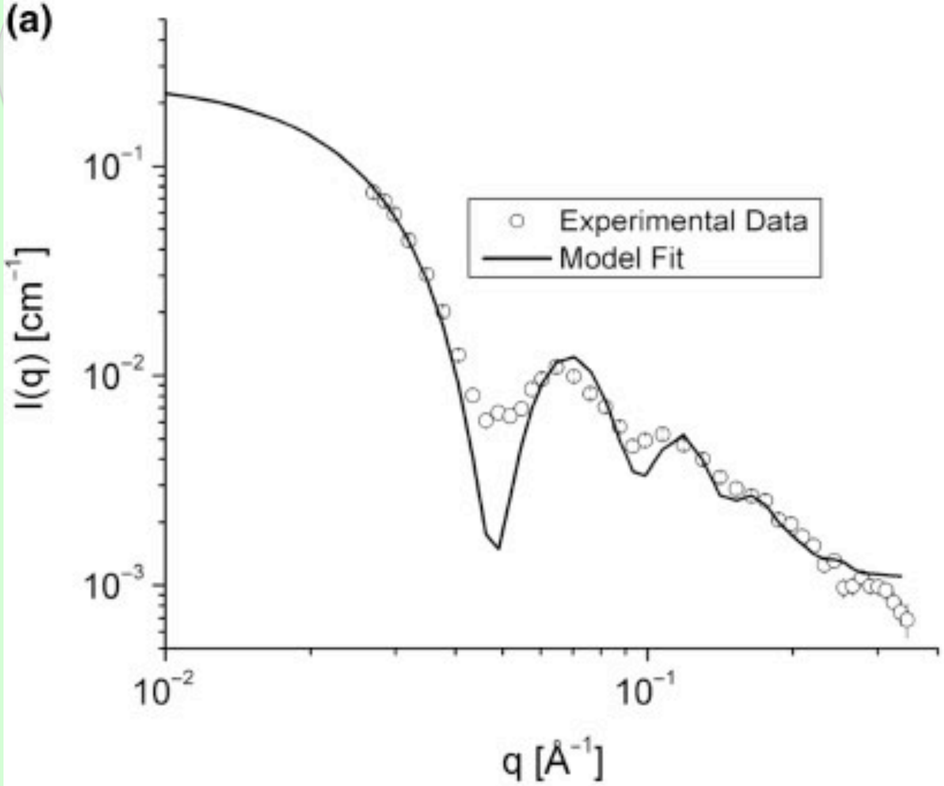


- Heating to 65°C for 10 min
- Cooling by 0.25°C/min until 30°C
- T4 DNA ligase addition
- Cooling by 0.25°C/min until 16°C

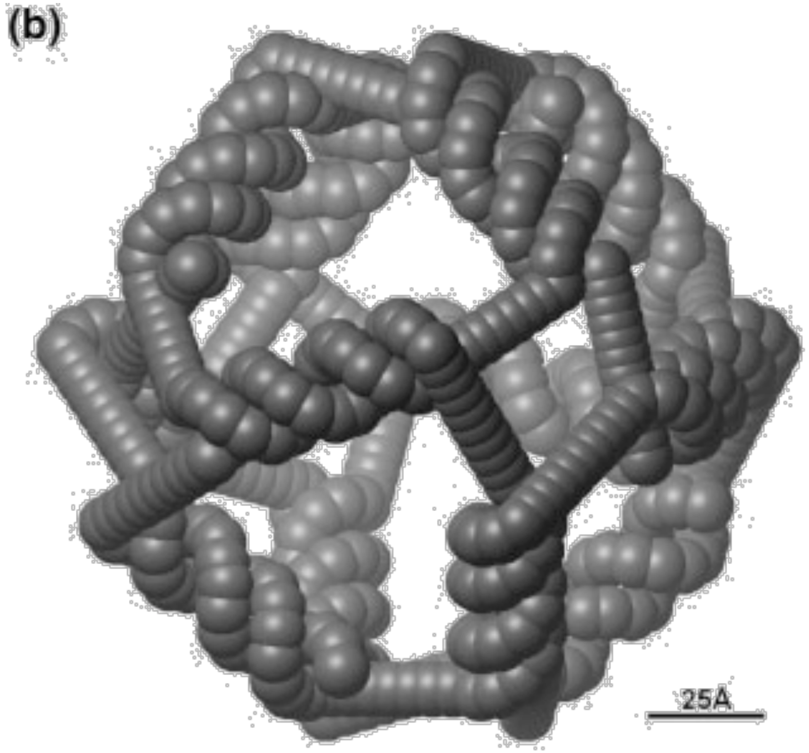
The Nanocage

cryo-TEM



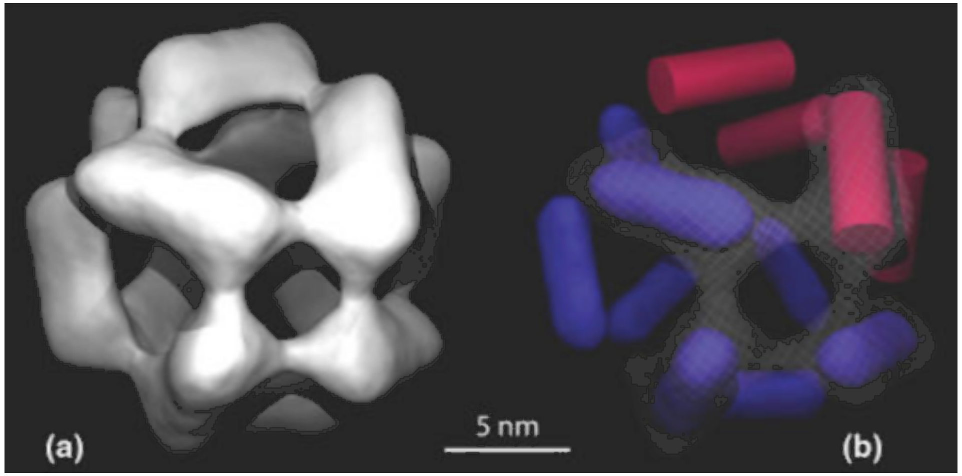


(a)
I(q) [cm⁻¹]
Experimental Data
Model Fit
q [Å⁻¹]



(b)
25Å

Predicted Structure



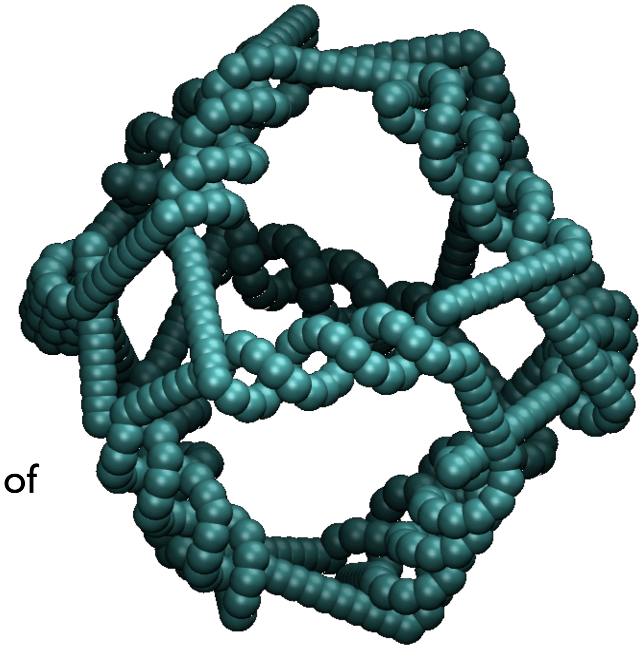
Correct assembly due to the careful design of the sequences

cryo-TEM reconstruction

12 Double Helices

24 Seven Thymidine base-pair Single Strand

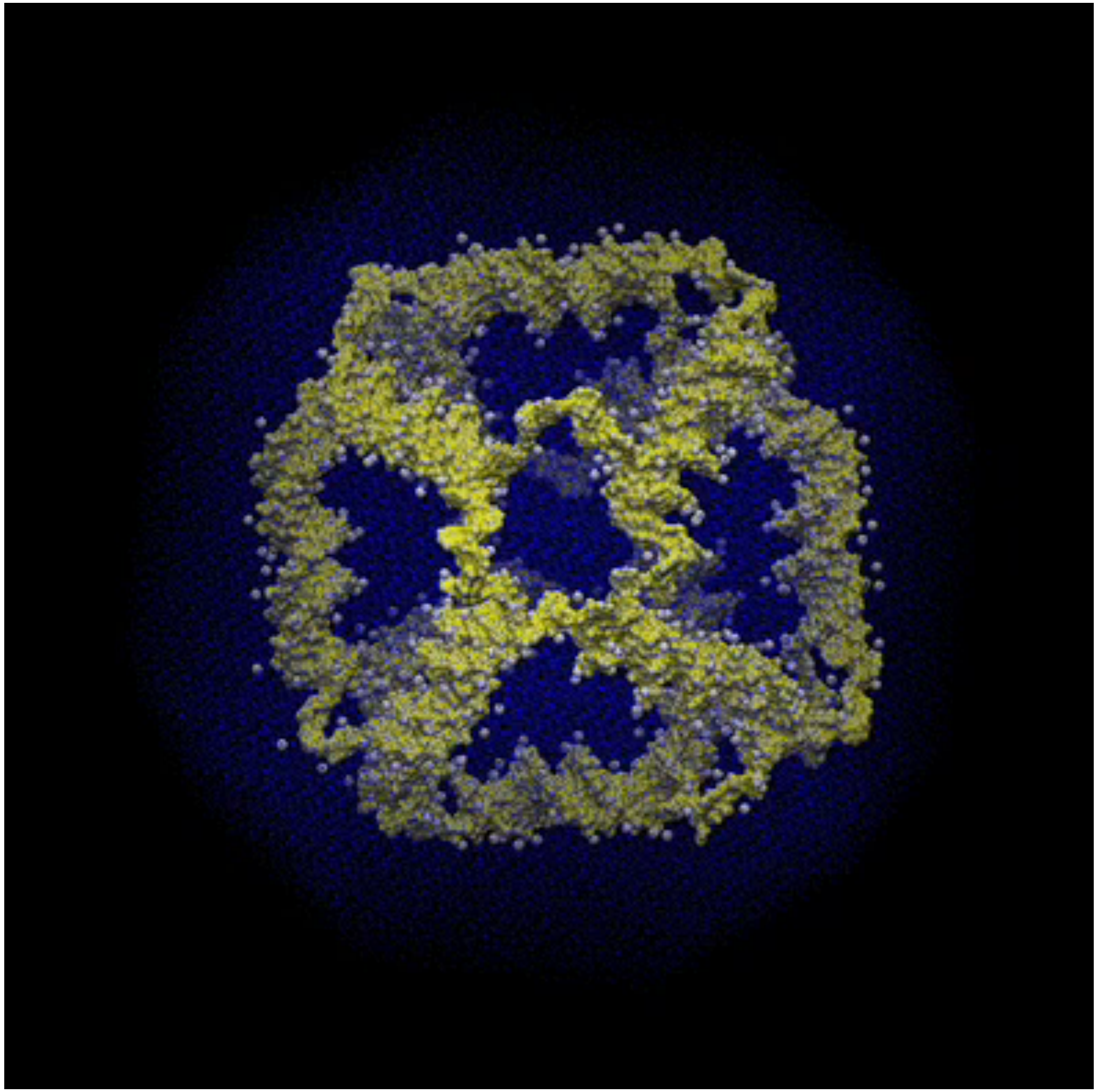
SAXS reconstruction



Andersen et al, (2007). Assembly and structural analysis of a covalently closed nano-scale DNA cage. Nucleic Acids Res. *36*, 1113-1119.

The high resolution model

Total Atoms 392955
Water molecules 124425
Na+ ions 600
Simulation box side X (Å) 171
Simulation box side Y (Å) 158
Simulation box side Z (Å) 161

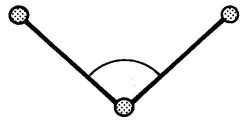


(Force Field)

TOTAL POTENTIAL ENERGY = $\mathcal{V}$

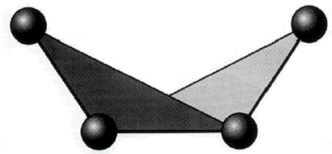
Bond stretching

+



Angle bending

+

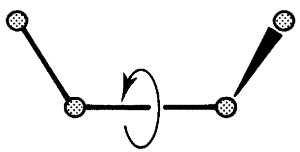


improper dihedrals

+

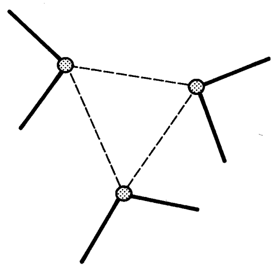
$$v(r_1,r_2,\dots,r_n)=\sum_{bonds}\frac{k_i}{2}(l_i-l_{i,0})^2+\sum_{angles}\frac{k_i}{2}(\theta_i-\theta_{i,0})^2+\sum_{\substack{improper\\dihedrals}}\frac{k_i}{2}(\xi_i-\xi_{i,0})^2$$

$$+\sum_{dihedrals}\frac{V_N}{2}(1+\cos(n\omega-\gamma))+\sum_{i=1}^{N}\sum_{j=i+1}^{N}\left(4\pi\varepsilon_{ij}\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12}-\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right]+\frac{q_iq_j}{4\pi\varepsilon_0 r_{ij}}\right)$$



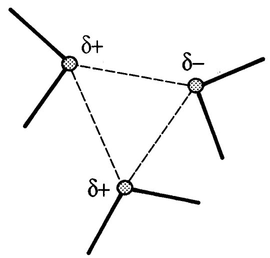
Bond rotation
(torsion)

+

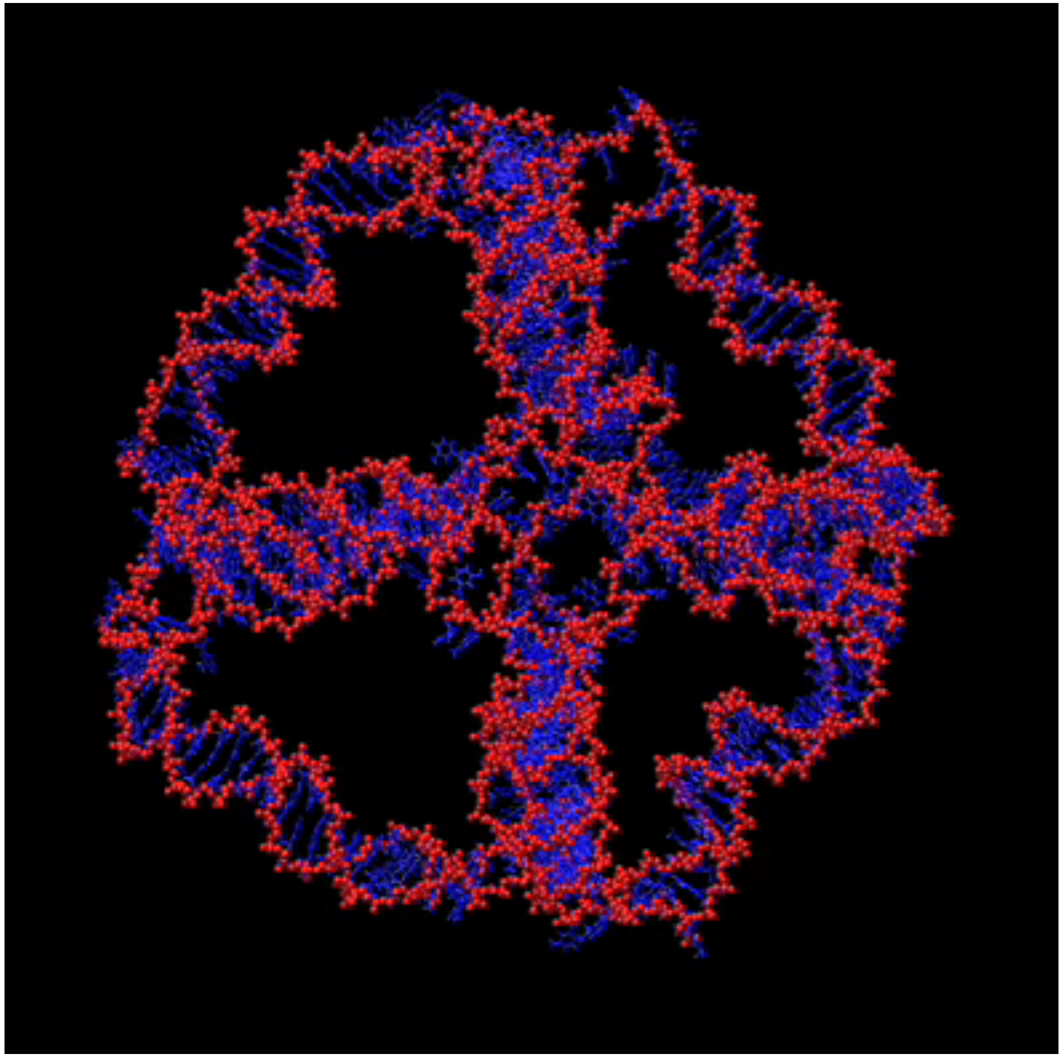


Non-bonded interactions
(van der Waals)

+



Non-bonded interactions
(electrostatic)

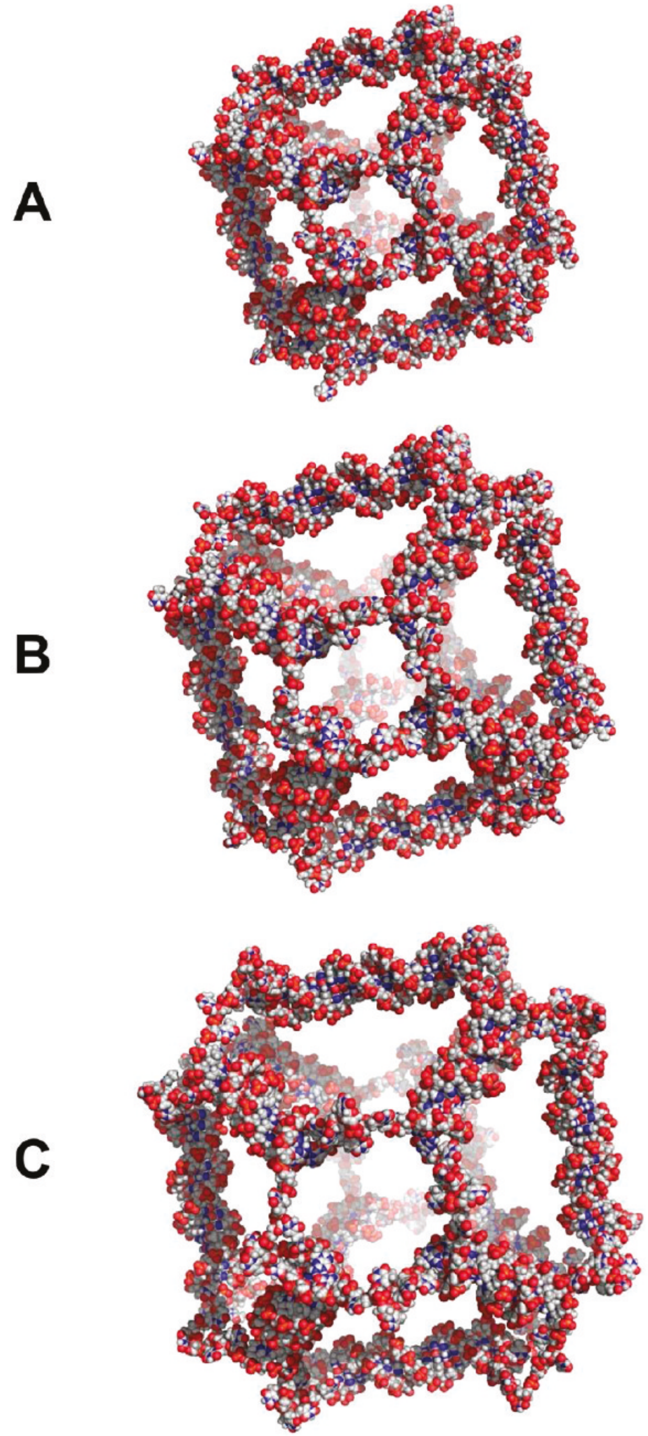


MD SIMULATION

3T

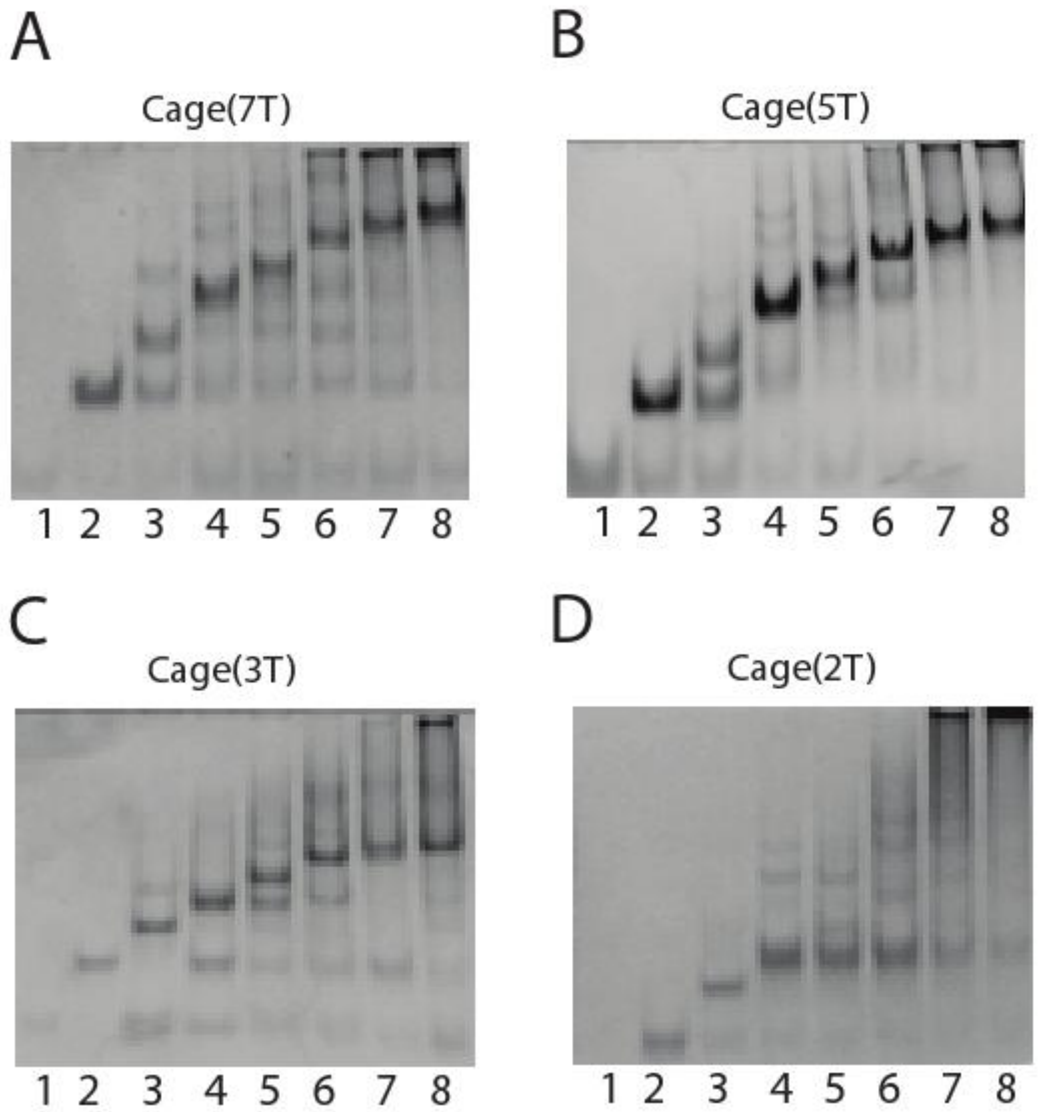
5T

7T



Self-assembly efficiency

influence of thymidine strand lenght



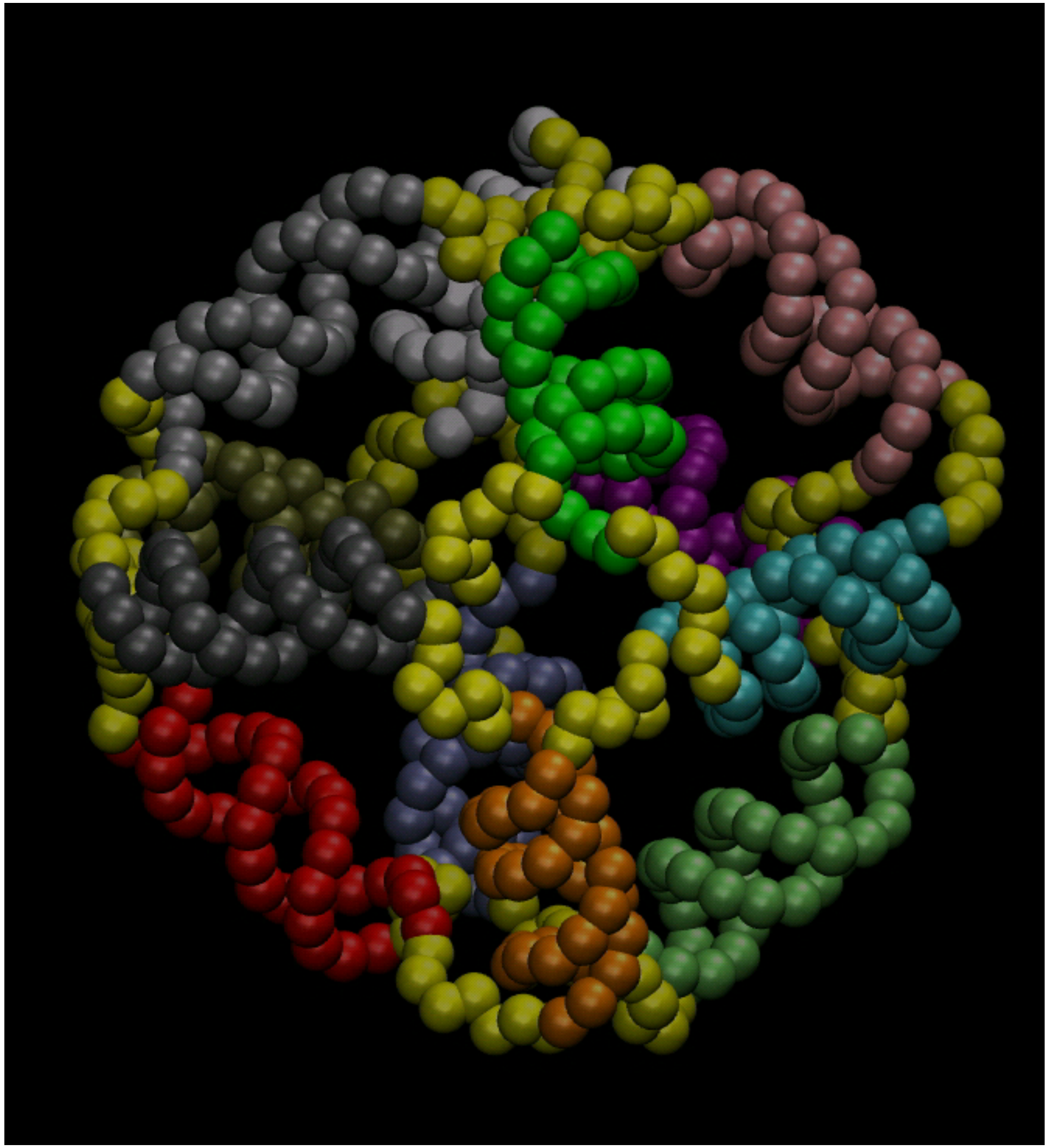
The longest are the thymidine strands,

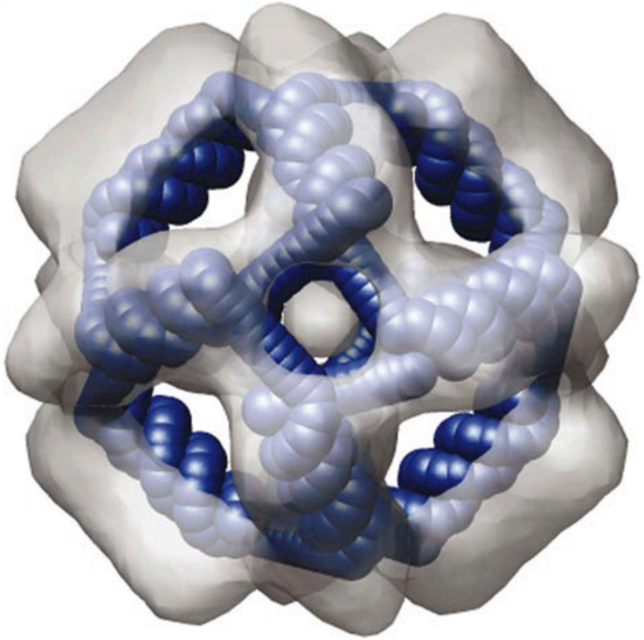
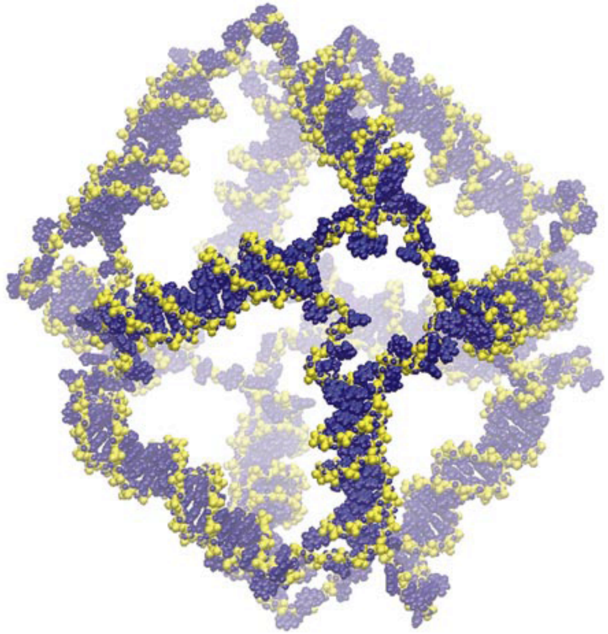
↓

The easiest is the self-assembly

MD Results

Principal Component Analysis (PCA)

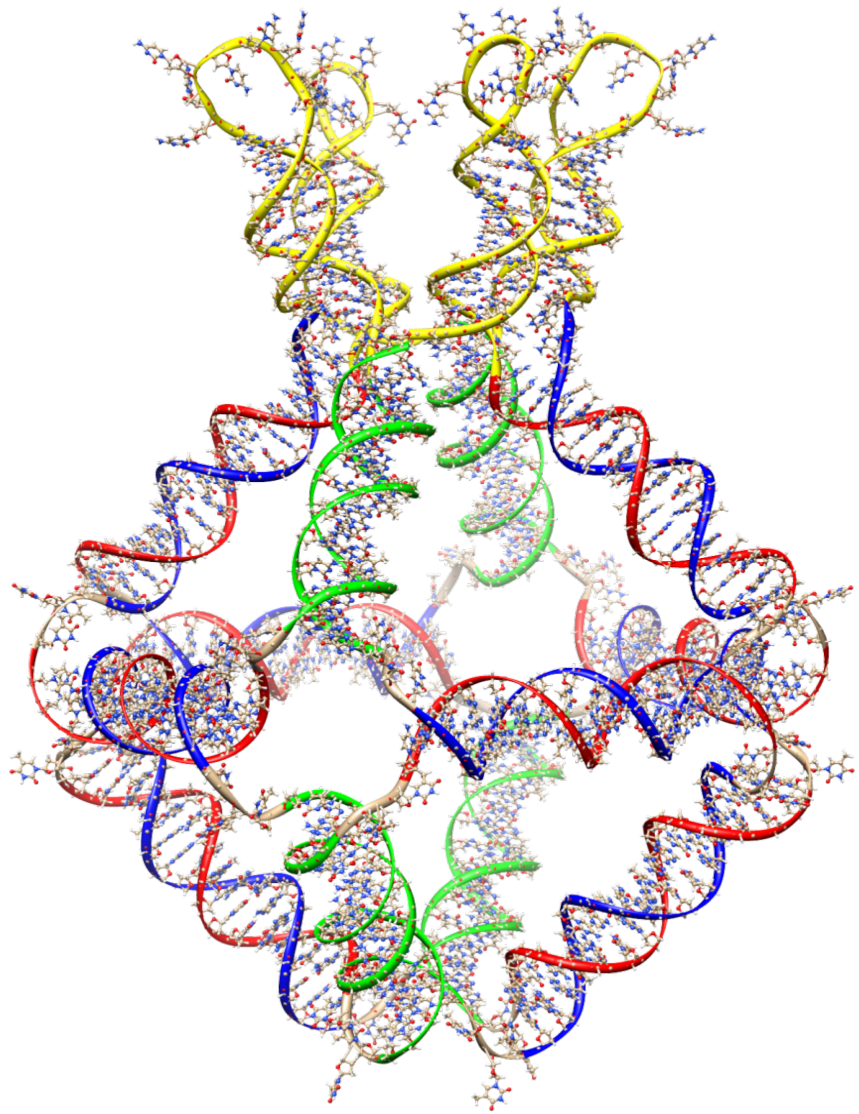


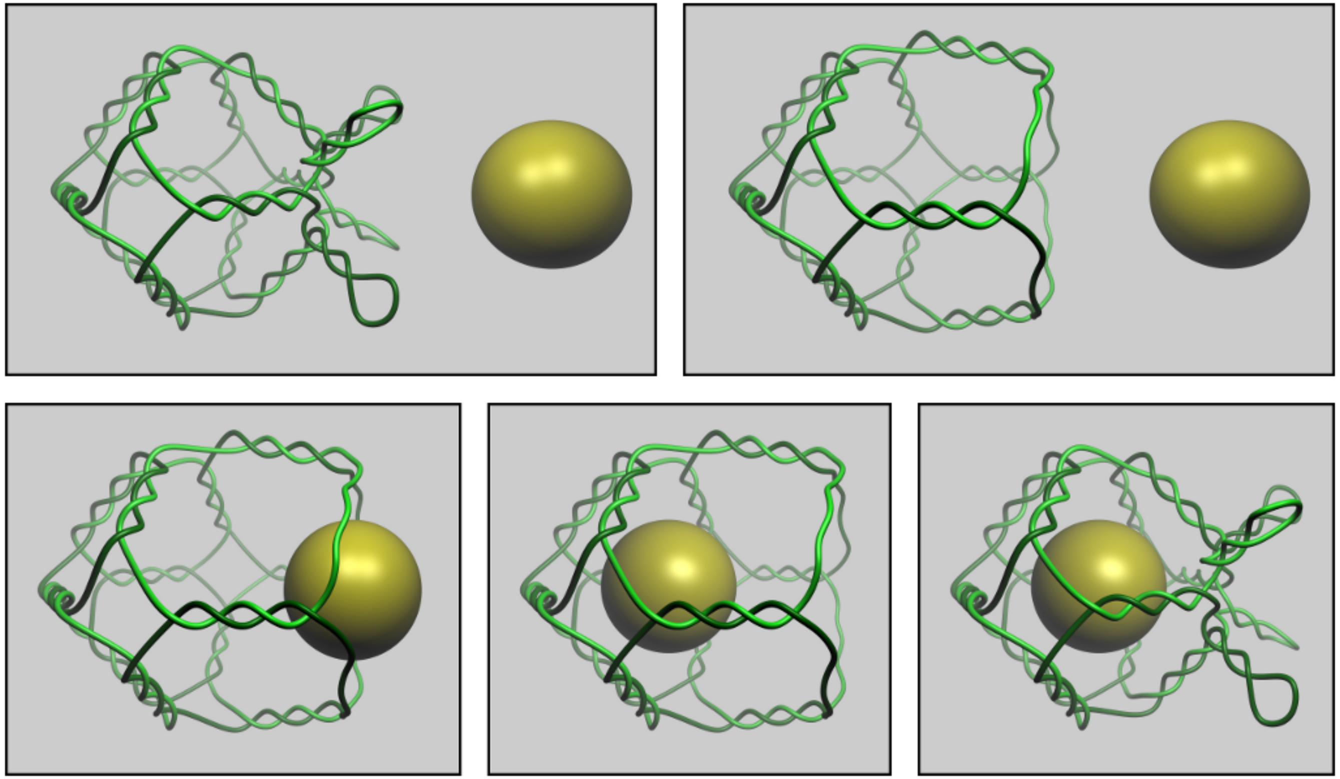
- Andersen et al NAR 2007, *3 6*, 1113-1119
- Falconi et al. ACS NANO 2009, 3, 1813-22
- Oliveira et al ACS NANO 2010, 4, 1357-1376
- Oteri et al. J. Phys. Chem C 2011, 115, 16819
- Andersen et al.2011 DNA: Microarray, Synthesis and Synthetic DNA Nova Science Publisher

IS IT POSSIBLE TO BUILD A CAGE ABLE TO TRAP AND RELEASE A PROTEIN IN A REVERSIBLE WAY ?

Atomistic Cage_{hp}(3T)

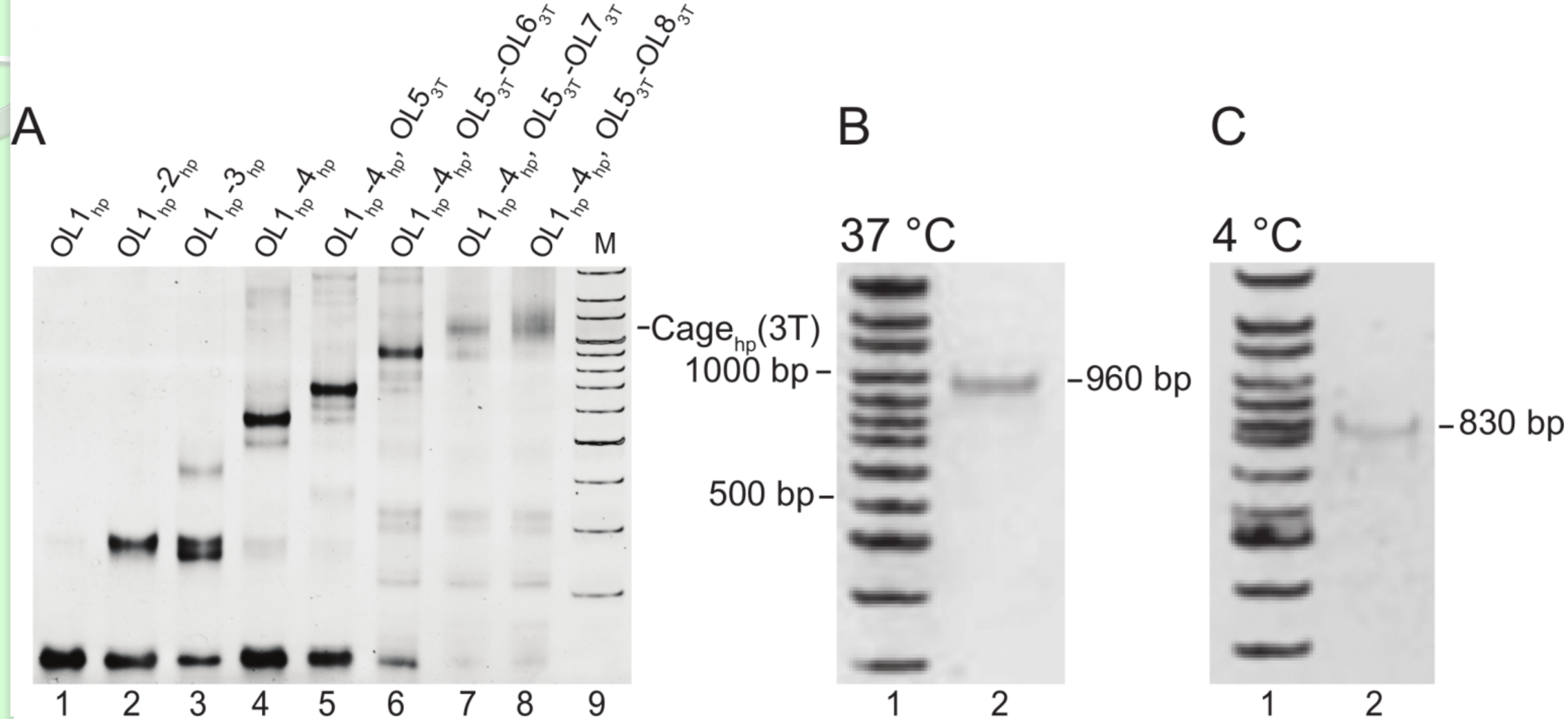


A cage designed to incorporate proteins: the $Cage_{hp}$(3T)



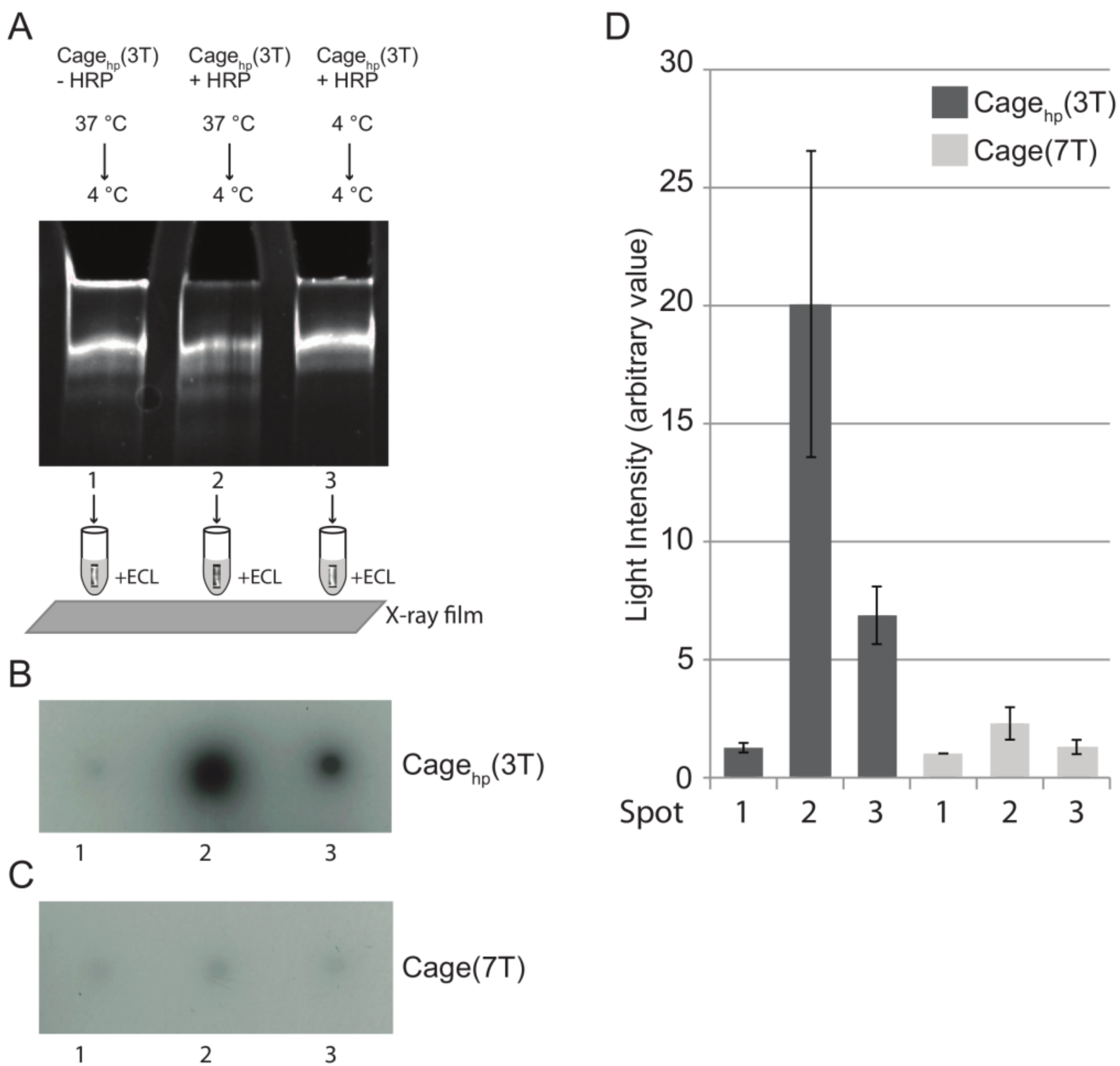
Possible mechanism of incorporation

Assembly and Gel-Electrophoretic Analyses of DNA $Cage_{hp}$(3T)



The increased mobility of $Cage_{hp}$(3T) at 4°C relative to 37°C suggests that the cage undergoes a conformational change attaining a more closed structure at low temperature and a more open structure at high temperature.

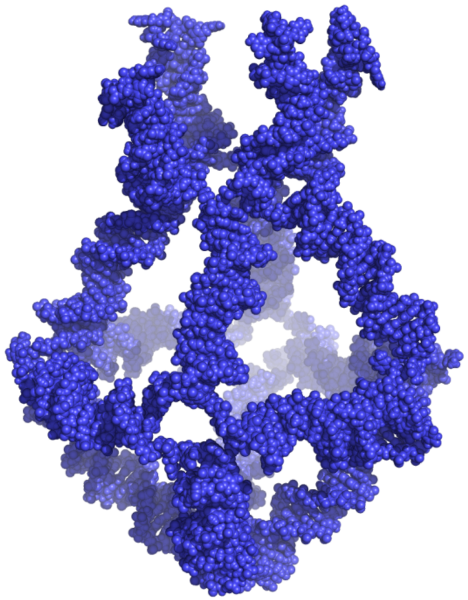
Temperature controlled encapsulation of HRP in $Cage_{hp}(3T)$



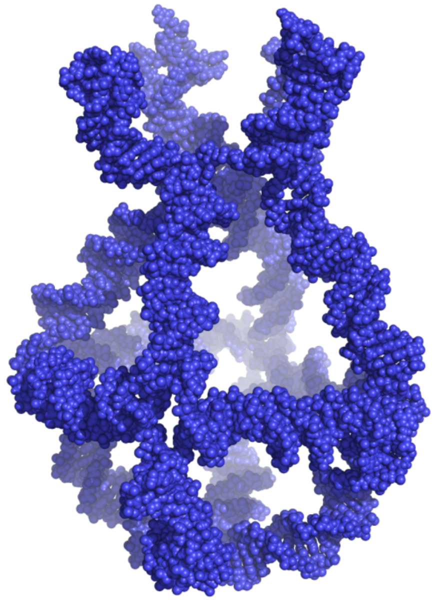
Cage$_{hp}$(3T) model building

$Cage_{hp}$(3T) MD Simulations

Two 50 ns MD simulations have been performed, starting from the same nanocage model at the experimental temperatures of 277K and 313 K:

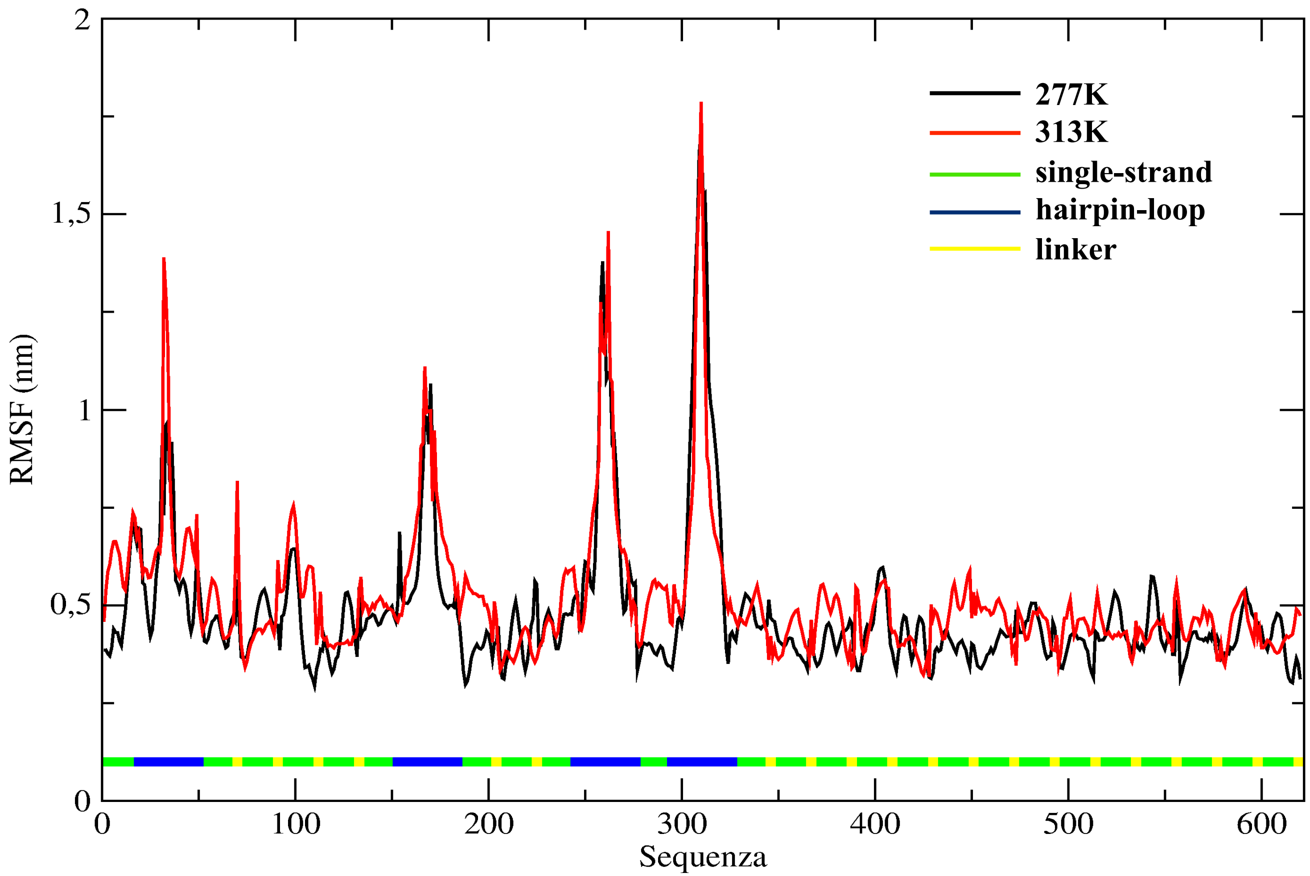


277K



313K

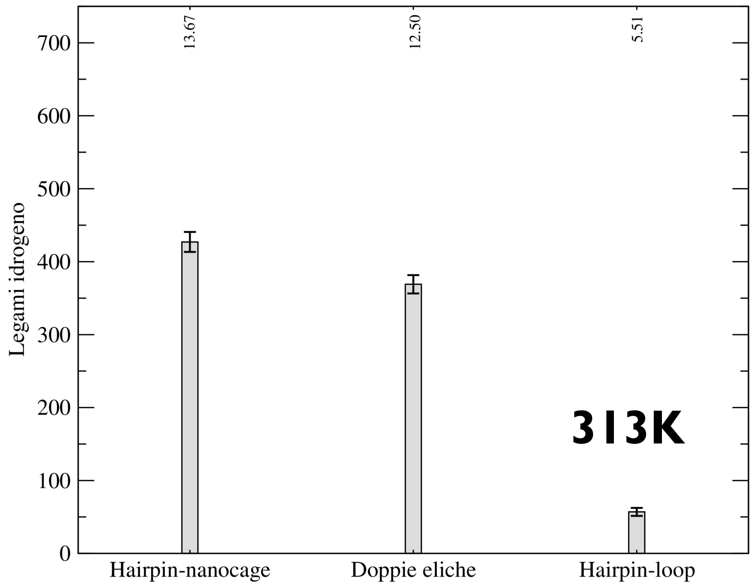
Root Mean Square Fluctuations



The hairpins-loop represent the regions with the highest level of fluctuation at both temperatures.

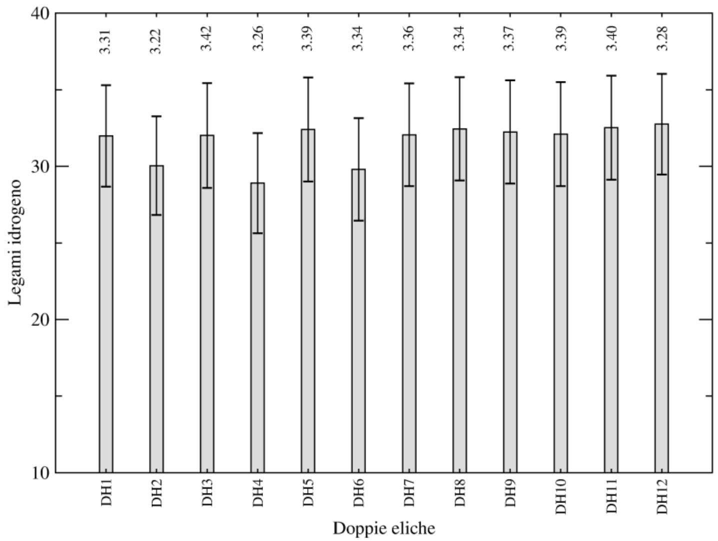
$$\text{RMSF} = \sqrt{\frac{1}{N}\sum_{t=1}^{N}(x_i - \langle x \rangle)^2}$$

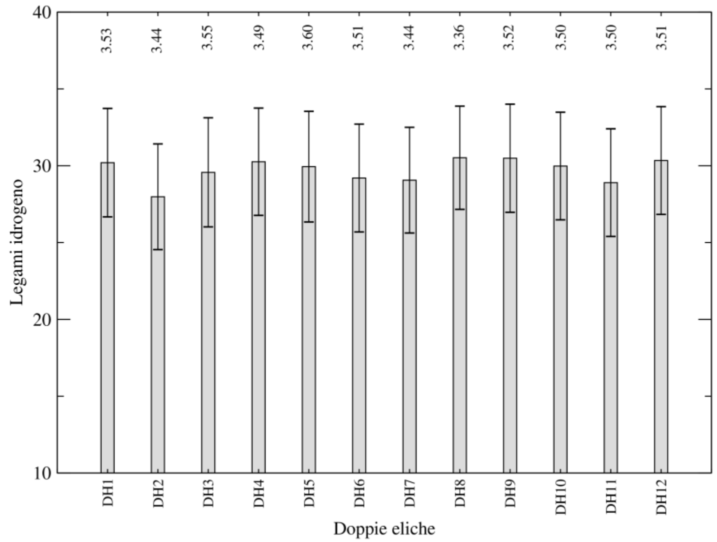
Hydrogen Bonds Analyses

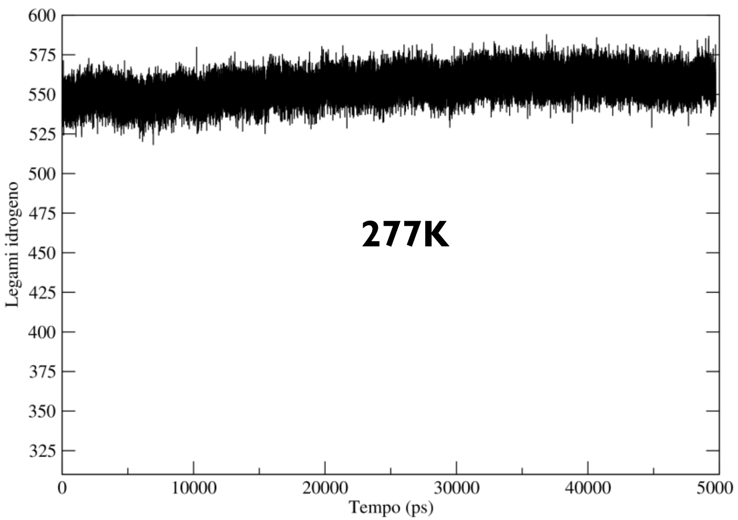


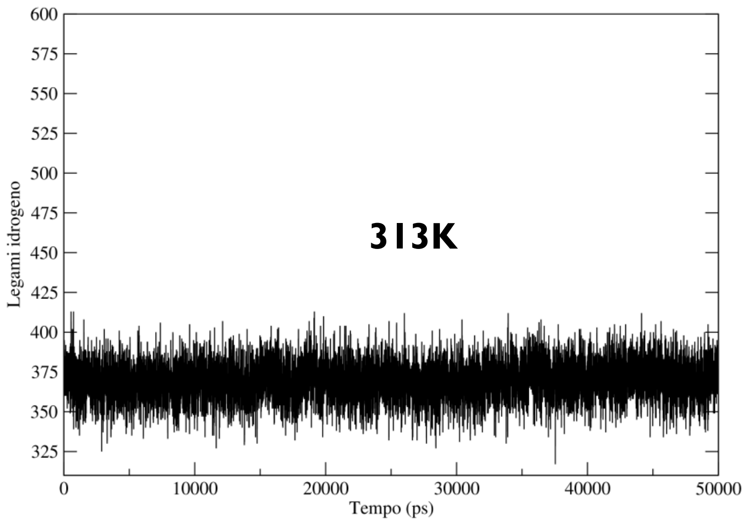
- substantial reduction in the average number of hydrogen bonds;
- double helices instability due to the kinetic energy increase .

Hydrogen bonds evaluated for each double helix

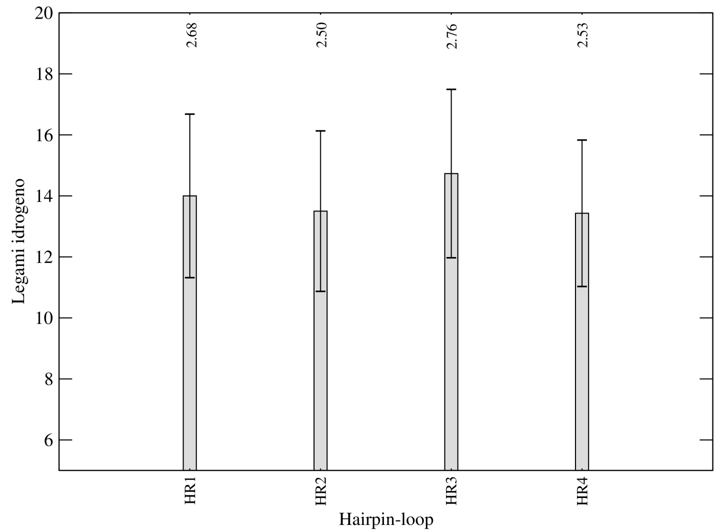


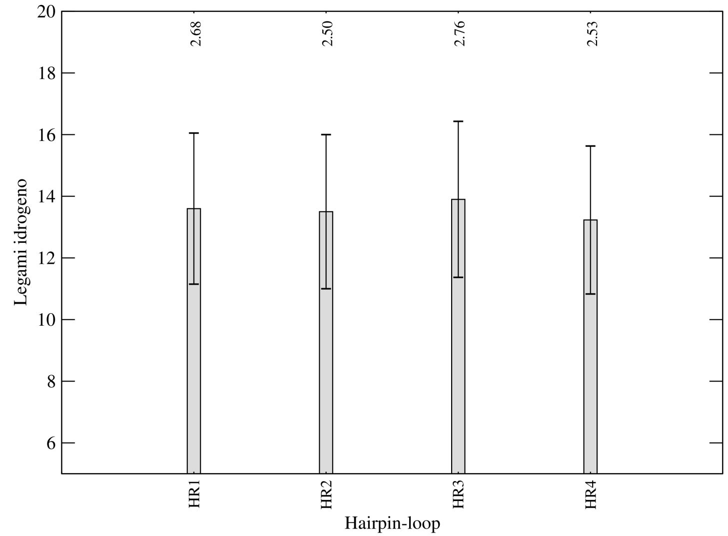


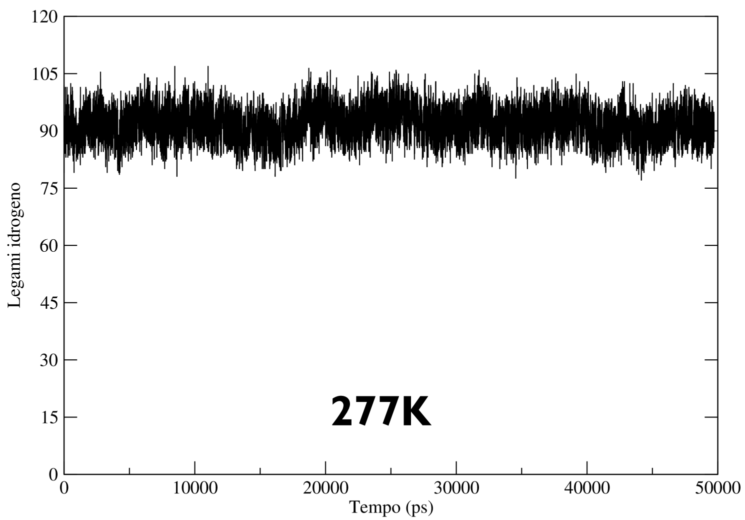


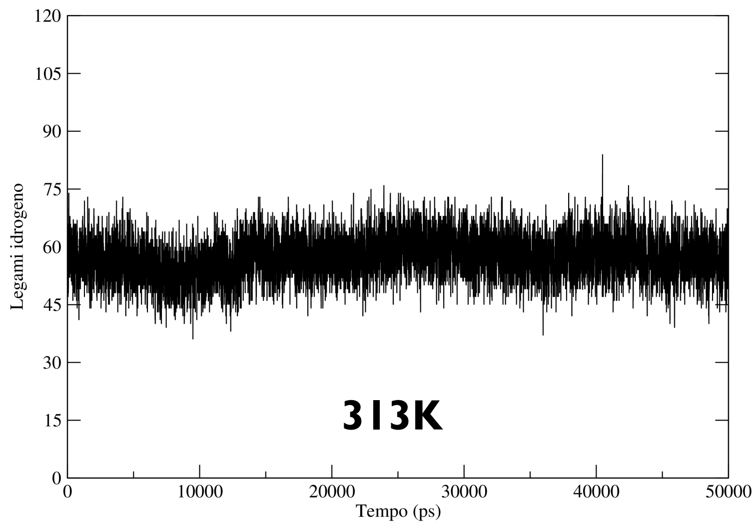


Hydrogen bonds evaluated for each hairpin-loop

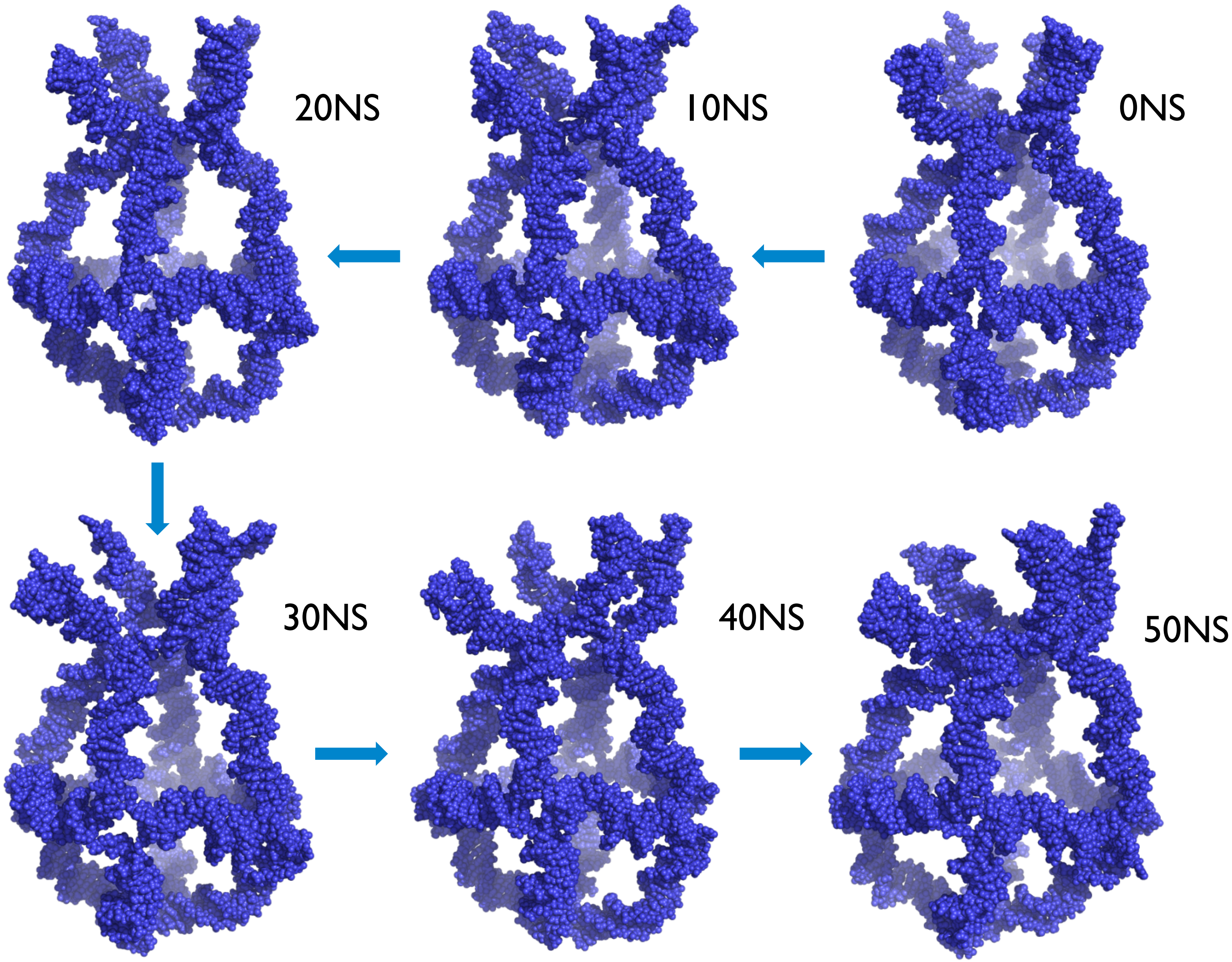




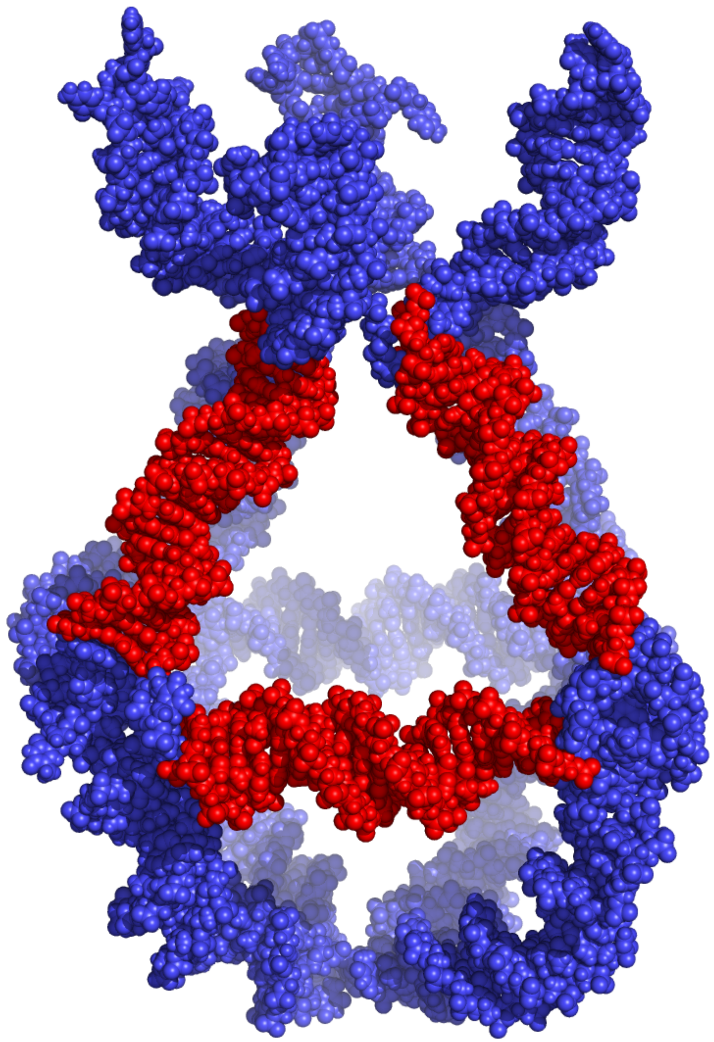




Snapshots from the 313K simulation



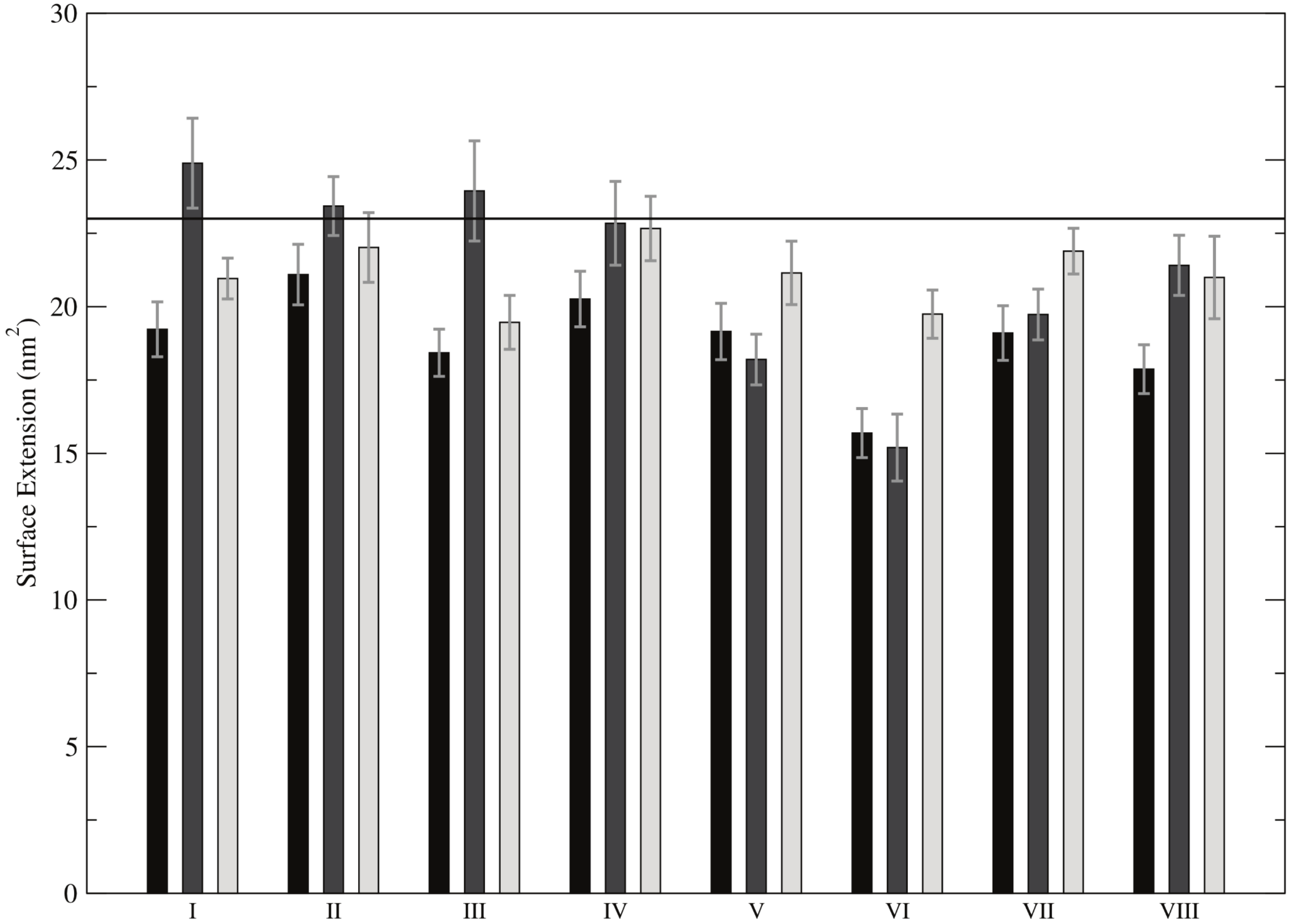
Face surface definition on the $Cage_{hp}$(3T)



Surface defined by 3 double helices, directly connected to each other through a linker region.

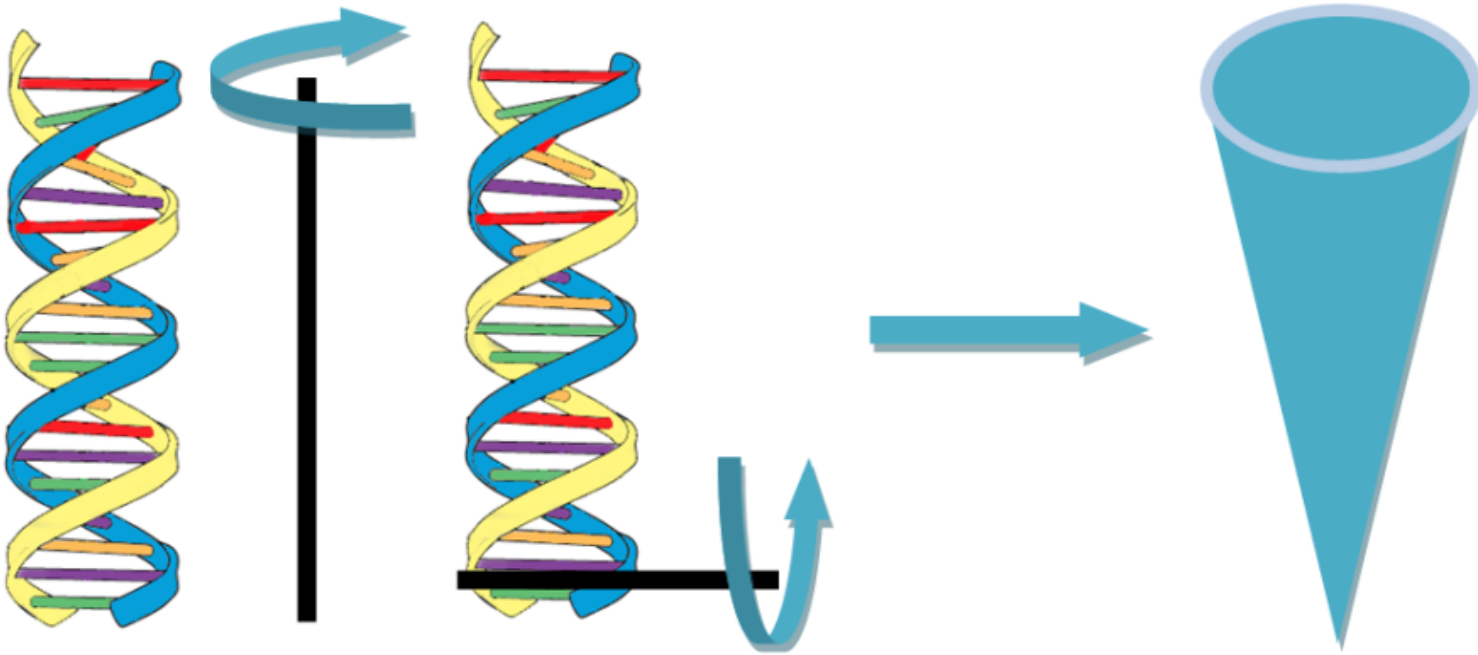
Initial Extension: 18 nm^2

Analysis of the faces extension of the $Cage_{hp}$(3T) and of the original Cage(7T)



$Cage_{hp}$(3T) at 4°C (**black bars**) at 37°C (**dark grey bars**). Cage(7T)at 37°C **light grey bars**).

Motions involved in the extension of the $Cage_{hp}$(3T) surfaces



Expansion due to two kinds of rotational motions (one parallel to the helix axis and the other perpendicular to the base-pair at the helix extremity) that lead to an increase in the faces size.

Conclusions

- HRP can be reversibly encapsulated
- Encapsulation occurs because of fluctuations/deformations occurring at high temperature
- This the first example of a reversible and controlled encapsulation and release