Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVI

Biophysical characterization of nanostructures formed by self-assembled DNA derivatives for applications in biothecnology and biomedicine

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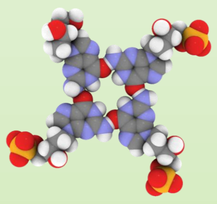
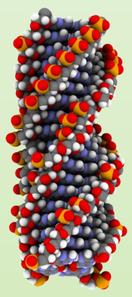
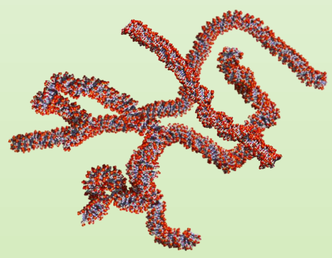
Aim of the study



G-quartet, composed by Guanosine (Gua) and guanosine 5'-monophosphate (GMP)



G-quadruplex



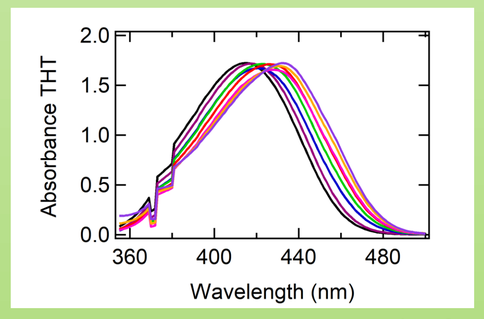
G-hydrogel

Guanosine 5'-monophosphate (**GMP**) and Guanosine (**Gua**) in water self-assemble in supramolecular, columnar helicoidal structures (**G-quadruplexes**), made by stacked planar tetramers (**G-quartets**) stabilized by non-covalent Hoogsteen bonds. Modulating G-quadruplex electrostatic repulsive and Van der Waals attractive forces by tuning the Gua/GMP molar ratio, supramolecular self-assembled **hydrogels** can be prepared. Stable G-hydrogels can be differentially hydrated modulating the amount of water from 80% and finally up to 98% v/v.

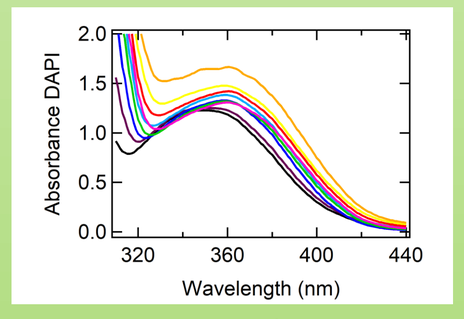
Here, a structural investigation about the interaction of some fluorescence probes (THT, DAPI and FITC-dextran) with the G-quadruplexes strands loaded on it is reported. For this purpose, a spectrophotometric UV-Vis analysis allowed to bring to light the intercalation binding for THT and the external electrostatical interaction for DAPI. Since non-binding results have been obtained for FITC-dextran, a wide range of diffusivity measurements have been analysed through the Fluorescence Recovery After Photobleaching (FRAP) technique by using Confocal Microscopy (CLSM). These results allowed to find out different diffusivity coefficients (D) as a function of different Gua/GMP molar ratios (1:1, 1:2, 1:4) and different amount of water (90-95-98 % v/v) of G-hydrogel. Thus, D values give information also on the viscosity properties and mesh-size of different hydrogel composition even in comparison with SAXS and Rheological analysis.

Results

BINDING FLUORESCENCE MOLECULES (THT and DAPI)



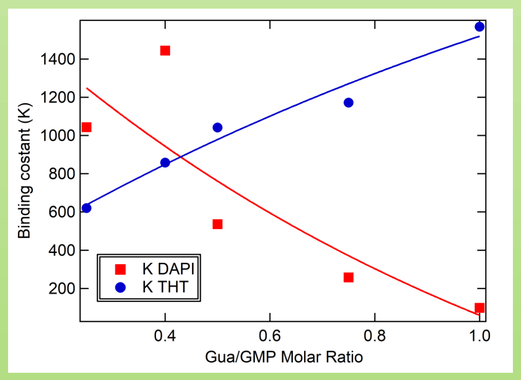
RED SHIFT = INTERCALATION mode of binding between G-quartets



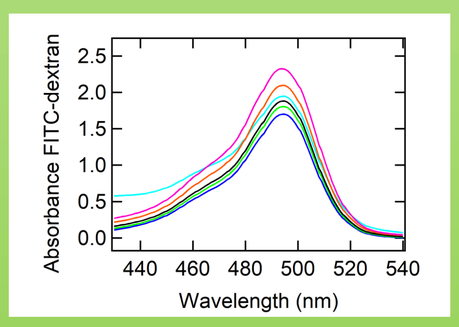
HYPERCHROMISM = electrostatic interaction on the external surface of G-quadruplexes

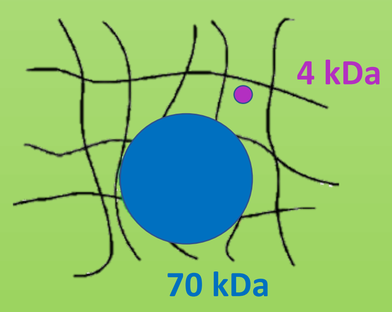
$$\frac{\frac{Gua+GMP}{4}}{\epsilon_A - \epsilon_f} = \frac{\frac{Gua+GMP}{4}}{\epsilon_B - \epsilon_f} + \frac{1}{K(\epsilon_B - \epsilon_f)},$$

Starting from the equation shown above, the abpsorbtion picks have been used to carry out the kinetic binding constant of THT and DAPI.

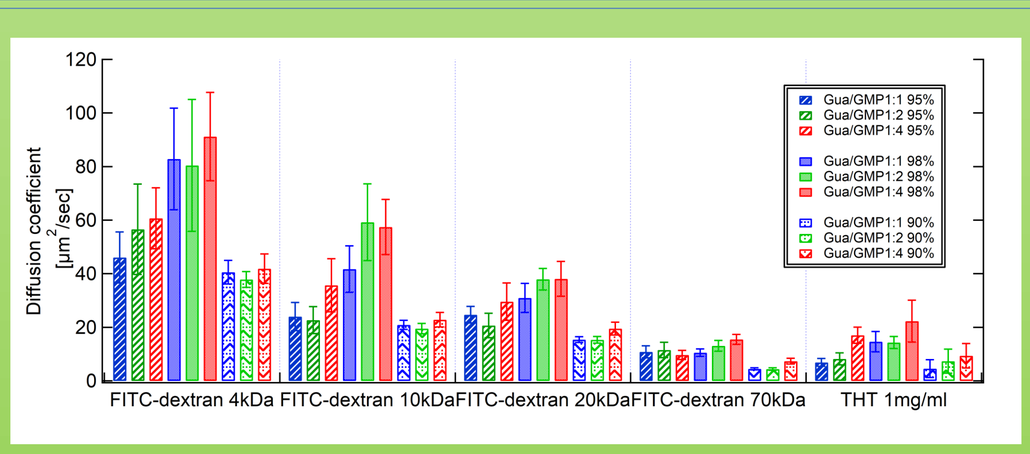


NON-BINDING FLUORESCENCE MOLECULE (FITC-dextran)





ANY SHIFT = FITC-dextran is not able to bind with the hydrogel's strand. It means that it shows diffusivities properties related to the hydrogel composition and to the MW of the FITC-dextran.

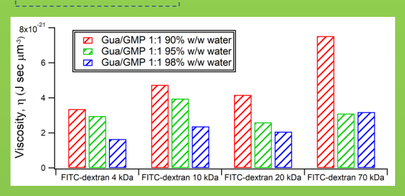


The Confocal microscopy (CLSM) has been used to investigate the FRAP techinque in order to know the diffusion coefficient of FITC-dextran (4-10-20-70 kDa) related to the different hydrogel composition in term of Gua/GMP molar ratio and % v/v of water

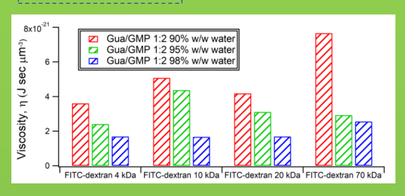
VISCOSITY ANALYSIS

$$D = \frac{k_B T}{6\pi\eta r}$$

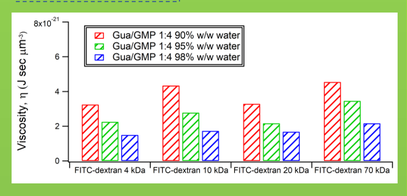
Gua/GMP 1:1



Gua/GMP 1:2



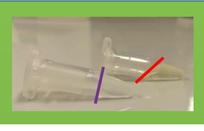
Gua/GMP 1:4



Starting from the diffusivity coefficient (D) extracted from FRAP analysis it has been calculated the viscosity by using the Stokes-Einstain Equation.
At one side, the viscosity increase as a function of FITC-dextran molecular weight (from 4kDa to 70kDa). At the other side, it increases by decreasing the mesh size of the G-hydrogel (from Gua/GMP 1:4 to 1:1).

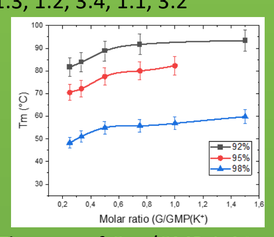
STRUCTURAL ANALYSIS

Differential Scanning Calorimetry (DSC) and SAXS data

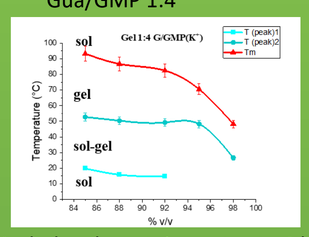


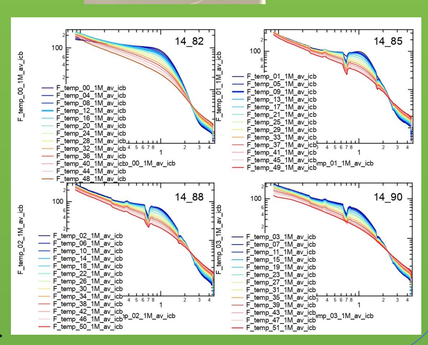
1:4 95% v/v
1:4 88% v/v

Phase diagram from DSC for Gua/GMP 1:4, 1:3, 1:2, 3:4, 1:1, 3:2



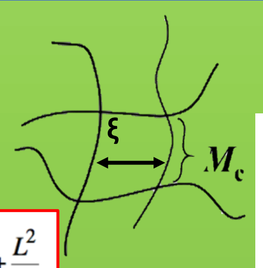
Phase diagram from DSC for Gua/GMP 1:4





The case of Gua/GMP 1:4 is particolarly relevant. In contrast with the others cases analysed this hydrogel shows two transition phase. In fact, at lower hydration level it appears liquid, while it is a gel just in a certain range of T (°C).

MESH-SIZE ANALYSIS
Comparison between CLSM and SAXS data



1

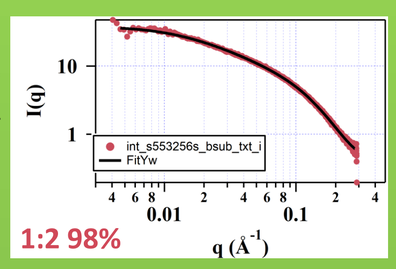
$$R_g^2 = \frac{R^2}{2} + \frac{L^2}{12}$$

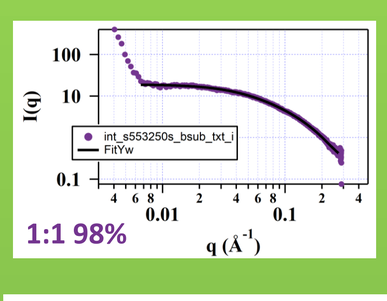
2

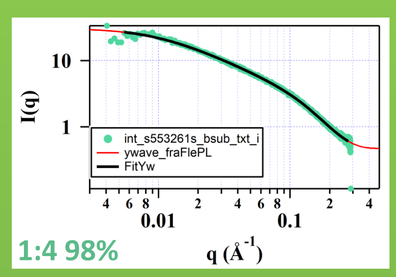
$$\frac{D_S}{D_0} = \exp\left(-\frac{\pi(r_h + r_f)^2}{(\xi + 2r_f)^2}\right)$$

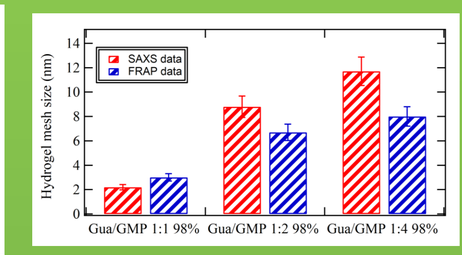
3

$$\xi = \varphi^{-\frac{1}{3}} C_n^{\frac{1}{2}} l \left(\frac{2M_c}{M_r}\right)^{\frac{1}{2}}$$









Gua/GMP	Corr. Length SAXS (nm)	Mc CLSM (nm)
1:1 98%	2,2	3,0
1:2 98%	8,8	6,7
1:4 98%	11,7	8,0

SAXS → Correlation length (L in Eq. 1) derived by fitting from Fractal Flexible Cylinder model.
FRAP → length between two crosspoint of the hydrogel network (M_c in Eq. 3) derived from diffusivity coefficient (D_s) of FRAP analysis

References

1. F. Carducci et al, *Soft Matter*, 2018, 14, 2938
2. G. Nava et al, *Soft Matter*, 2019, 15, 2315
3. M. Kang, et al, *Traffic.*, 2012, 13 (12): 1589-1600
4. Offeddu et al., *AIP Adv.*, 2018, 8(10):105006