PHD COURSE IN LIFE AND ENVIRONMENTAL SCIENCES

Report Form for PhD student annual evaluation (XXXVI and XXXVII cycles)

Name of PhD student: Alessia Pepe
Title of PhD research: Biophysical characterization of nanostructures formed by self-assembled DNA

Name of PhD supervisor: Prof. Paolo Mariani
Research lab name: Molecular Biophysics Lab

Cycle:
[✔] XXXVI
[ ] XXXVII

PhD Curriculum::
[ ] Marine biology and ecology
[✔] Biomolecular Sciences
[ ] Civil and environmental protection

DISVA instrumentation labs/infrastructure eventually involved in the project:
[ ] Actea Mobile Laboratory
[✔] Advanced Instrumentation lab
[ ] Aquarium
[ ] MassSpec lab
[ ] MaSBiC
[ ] Simulation/informatics lab
[ ] Other. Please, indicate: .....

ABSTRACT (1000 characters, including spaces):

It is well known that nucleotides self-assemble into functional nucleic acids and that the structure is organized through specific H-bonds. However, due to its chemical structure guanine adopts non-canonical Hoogsteen H-bonds with other three guanines, forming a planar tetrameric structure called G-quartets. In turn, G-quartets stack one on top of the other by π-stacking interaction, forming long 4-stranded helices called G-quadruplexes. This feature is recognised also for guanine derivatives such as guanosine (Gua) and guanosine 5'-monophosphate (GMP). Both of these two small oligomers, lead to the formation of guanosine hydrogel (G-hydrogel) in a huge amount of water (up to 99% v/v).

Several biotech applications could be imaged for G-hydrogel as a result of its captivating properties: high viscosity, high stability, biocompatibility, biodegradability, reversibility and adaptability to environmental changes (e.g. pH, temperature, enzymatic activity). Here, it is described one of the possible biophysics applications regarding the delivery of protein microcrystals to get their structure in an X-ray source (synchrotron and free electron laser facility).

Part 1. Scientific case of the PhD Research (2 to 3 pages, including figures)

- BACKGROUND

Hydrogels can be defined as three-dimensional hydrophilic polymeric networks capable of absorbing a large amount of water. In this context, the present research activity examines the biophysical characterization of the supramolecular guanosine hydrogel (G-hydrogel), bringing up an alternative to the traditional polymeric hydrogel. The self-assembly process involves guanosine (Gua) and guanosine 5'-monophosphate (GMP) leading to the formation of a physical 3D network. This *fishnet*-like structure is composed of highly flexible G-quadruplexes structure, characterized by negative phosphate groups of GMP that cover their external region. The singular properties that could be controlled in the whole system concerning the different molar ratios of Gua/GMP (1:4, 1:2, 1:1) and the percentage of water (from 82% to 98% v/v). The first point allows to regulate of the negative phosphate charges in order to control the repulsion/electrostatic forces among the G-quadruplexes strands. The second feature gives rise to a swollen structure that can be compared to the native extracellular matrix.

- SCIENTIFIC AIMS

This report describes a structural characterization of the G-hydrogel as an extension of the one started last year (A.Y. 2021/2022). For this aim, two have been the main techniques stressed:

- X-ray diffraction experiments (SAXS/WAXS) were performed in a synchrotron facility in Europe (Elettra in Italy, and Diamond Light Source in the UK). We studied the orientational properties of different types of hydrogel (1:1, 1:2, 1:4 ratio of Gua:GMP and different % of water from 82% to 98%).
- Rheological analyses were performed at Grenoble's Partnership for Structural Biology (PSB). These were some preliminary measurements, in order to find out the different viscosity properties of different hydrogel compositions.

On the other side, we have found two applications the G-hydrogel may deal with:

- A biophysical application was studied in collaboration with the group of Dr. Martin Weik at the Institute de Biologie Structural (IBS) in Grenoble. This work's purpose was to optimise the G-hydrogel as a new viscous carrier to embed different types of protein microcrystals inside it. This was done in order to perform a crystallography analysis at x-rays free electron laser (XFEL) source in Japan.
- We proved that G-hydrogel could be used as a polymeric material to generate nano and microparticles. In the future, this could be really challenging to load and deliver pharmaceutical molecules for instance, in the particles.

- WORKPLAN AND RESEARCH ACTIVITIES

WP 1. Objective. Study of the structural properties of G-hydrogel

The performed experiment concerns the structural characterization of G-hydrogels prepared in different conditions (composition, temperature, presence of salts, water content and of G-hydrogels containing silica nanoparticles of different dimensions and surface chemistry (25 and 50 nm diameter; no coating and carboxylic acid and amino groups coatings). They were carried out at the Elettra Synchrotron in Trieste (Italy) in January 2022. The two techniques used were SAXS and WAXS in order to have more information about the orientational properties of the G-hydrogel and even to study any conformational changes by adding salts and silica NPs.

Methods. **SAXS/WAXS analysis**

The SAXS/WAXS setup was used, covering the 0.1-4.5 nm-1 and 15-25 nm-1 Q-ranges. Samples were prepared in capillaries of 1 mm diameter and mounted on a capillary rack. Sample temperature was controlled from 20 to 80°C. G-hydrogels were prepared at different Gua/GMP molar ratios (1:4, 1:2, 1:1)

and at 5 different hydrations (85, 88, 90, 95 and 98% water, w/w). KCl and MgCl2 were added to obtain concentrations of 0.04 and 0.4 M. Silica nanoparticles concentrations were of 1.0 and 0.1 mg/ml.

Expected/Obtained Results.

The main interesting results that we considered here, concerning samples prepared at Gua:GMP ratio of 1:4. We investigated 5 different hydration levels mentioned above, each one at the range temperature between 20°C and 80°C. The case of 1:4 molar ratio is crucial: this hydrogel has a crucial composition. Since the high concentration of GMP/K^+, at a lower amount of water (from 82 to 90% v/v tested) the texture is liquid, while from 95% v/v of water it becomes a hydrogel. Moreover, the liquid samples are sensitive to high temperatures (~50°C) when it looks like a gel. SAXS results reveal that there is a change in the structure of the hydrogel depending on the temperatures tested, but it is not completely clear the switching composition. The results are illustrated in Fig. 1.

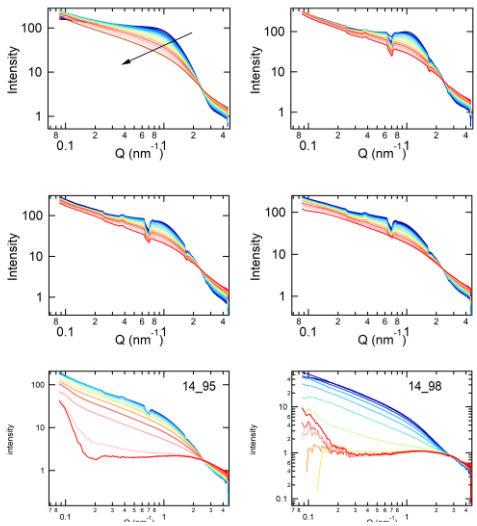

Figure 1. *SAXS results from hydrogels 1:4 82-85-90-95-98% v/v*

WP 2. Objective. Study of the viscoelastic properties of G-hydrogel

G-hydrogel is characterized by two main properties. On one side, the gel-sol transitions occur by changing the temperature. This is demonstrated not only by the differential scanning calorimetry (DSC), but also by the rheological analysis. On the other side, self-heling and swelling properties that allow G-hydrogel to be injectable. For this purpose, the study of rheological behaviour can allow an understanding of the ability of this polymeric material to be localized in the desired site.

Methods. **Rheological and DSC measurements**

The visco-elastic behaviour of the G-hydrogel was studied by using the HAAKE MARS III Rheometer (Thermo Fischer) installed at Partnership for Soft Condensed Matter (PSCM) at the Institute Laue-Langevin (ILL) in Grenoble (France). The preliminary tests were done for the following samples: 1:1 98%, 1:2 98%, 1:2 95%. The analysis were done by using the Amplitude sweep and the Frequency sweep. For both of them, the % of the Controlled Deformation (CD, % γ) has been used to get the results. Since the gel-sol transitions, these measurements were performed between 20°C and 60°C.

Expected/Obtained Results.

By taking into account the case of hydrogel 1:2 98% v/v, Fig. 2 illustrates the results obtained by using the amplitude sweep test (a,b) and the frequency sweep test (c), as a function of temperature. In the first case, we can claim that by increasing the temperature there is a shift of the inflexion point to the left of the graph. It means that there is a decrease of the elasticity (G') and viscosity (G'') of the G-hydrogel. Moreover, Fig. 2c) shows the viscosity as a function of the oscillation frequency. The decreasing of the viscosity depends on the temperature that it is strictly connected to the hydrogel phase transition.

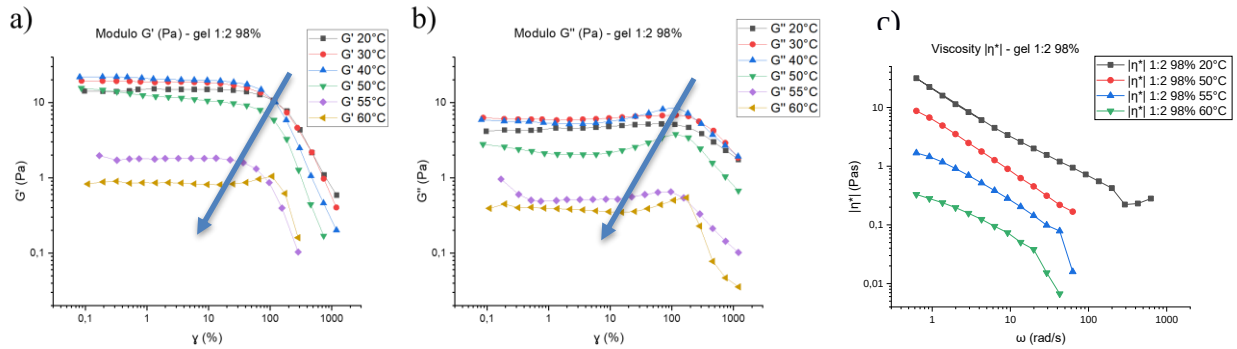

Figure 2. *a) Amplitude sweep test considering G' modulus, b) Amplitude sweep test considering G'' modulus, c) Frequency sweep test considering the viscosity, η^**

WP 3. Objective. G-hydrogel as a new viscous carrier to embed protein microcrystals

This year I was a visiting PhD student for several months in the group of Martin Weik at the Institute de Biologie Structurale (IBS) in Grenoble (France). This led to a collaboration to study a new biophysical application of the G-hydrogel for transporting protein microcrystals into the X-ray beam when X-rays at synchrotron and at free electron laser (XFEL) sources are used.

Methods. Microscopy analysis, injection experiments, UV-Vis absorption, XFEL

The crystallographic experiments used for this work were time-resolved, in order to study the conformational changes of considered proteins (Lys, CarH, rsEGFP2) embedded inside the G-hydrogel (1:1 90%, 1:2 95%) Since the quality of the G-hydrogel with embedded microcrystals inside it should be transparent and stable, we made several analyses before running the experiment in an X-rays facility. Optical microscopic tests were done to check if crystals can survive in a new viscous medium, UV-Vis absorption was done to check the photo-switching of crystals CarH and rsEGFP2, off-line injection experiments allowed us to understand the quality of the jet generated by samples and also to extrapolate the velocity of it. Finally, diffraction was checked at XFEL in Japan (June 2022) to obtain the structure of the CarH crystal.

Expected/Obtained Results.

All the results allow claiming that G-hydrogel may be a potential candidate as a new viscous carrier for the delivery of protein microcrystals to perform X-ray diffraction. Firstly, it is necessary to optimize the G-hydrogel with the buffer condition of each protein. G-hydrogel shows wide adaptability considering the different crystallization conditions of proteins. In fact, all three considered do not change their shape in the hydrogel and then, CarH and rsEGFP2 have the same photo-sensitivity both in the hydrogel and in their mother liquor. Since the injection experiment gave us some good results about the quality of the injection, the Serial Femtosecond Crystallography (SFX) was made in the XFEL facility in Japan. As a consequence, the structure of the chromophore of CarH crystal was the first one extrapolated in an XFEL facility. It is possible to claim that the resolution (3Å) remains unchanged also when this protein is resolved in a different kind of viscous medium.

WP 4. Objective. Production and characterization of G-hydrogel particles

Among all of the biotech applications in which G-hydrogel could be involved, it could be used not only as an injection polymer but also to produce some hydrogel beads. For this aim, a $CaCl_2$ solution of 1.2 % was used to dissolve a hydrogel of 1:1 90%.

Methods. SEM and polarized microscopy, SAXS/WAXS

The microscopy techniques were useful to characterize the size of the hydrogel particles obtained which is around 25 μm. From SAXS and WAXS we got information about the presence of the G-quadruplexes in

these particles treated by using salt: the SAXS profile reveals the presence of a cylindrical structure, while WAXS data detects the presence of the peak at 3.4 Å about the staking of G-quartets.

Expected/Obtained Results.

This study was a preliminary test conducted just using a single condition both of hydrogel composition and salt concentration. The results obtained from all of the previous methods used are really challenging: Although we managed to get for the first time microparticles starting from G-hydrogel, it is not possible yet to control the size of the beads and to coat them in order to have more stability.

- REFERENCES

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G. Nava et al, *Soft Matter*, 2019, 15, 2315
Kovacsova G. et al., *IUCrJ*, 2017, 4, 400-410
Sugahara, et al., *Nat Methods*, 2015, 12, 61- 63
Engler AJ. Et al., *Cell*, 2006 ; 126(4) : 677-89

Part 2. PhD student information on the overall year activity (courses/seminars/schools, mobility periods, participation to conferences)

List of attended courses/seminars/schools

1. "Phyton for Data Analysis" online course of 3 days organized by Università di Pavia

List of periods spent abroad

1. From March to June 2022 and From September to October 2022 I made an exchange in Martin Weik's group at the Institute de Biologie Structural (IBS) in Grenoble (France).

List of conferences/workshops attended and of contributions eventually presented

1. Talk presentation in the 2nd MaSbiC – DiSVA Annual Symposium "Protein structure and function in Biology, Medicine and Nanotechnology" (Ancona, Italy). Talk title: "G-hydrogel for the delivery of protein microcrystals: a new adjustable-viscous carrier matrix for serial protein crystallography experiments at XFELs and synchrotrons".
2. Talk presentation in "G-quadruplex Meeting" (Napoli, Italy). Talk Title: "Self-orienting anisotropic structure and diffusivity properties of guanosine hydrogels".

Part 3. PhD student information on publications

If not yet published, please indicate the publication status (submitted, accepted, in preparation...)

List of publications on international journals

J1. Mariaconcetta Sicurella, Maddalena Sguizzato, Paolo Mariani, Alessia Pepe, Anna Baldisserotto, Raissa Buzzi, Nicolas Huang, Fanny Simelière, Sam Burholt, Peggy Marconi and Elisabetta Esposito, "Natural Polyphenol-Containing Gels against HSV-1 Infection: A Comparative Study", *Nanomaterials*, 2022, 12, 227.

List of publications on conference proceedings

C1. ...

C2. ...

List of other publications (books, book chapters, patents)

B1. ...

B2. ...

14/10/2022

Student signature

Supervisor signature

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