QUADRUPLEX KNOTS AS NETWORK NODES: NANO-PARTITIONING OF GUANOSINE DERIVATES IN SUPRAMOLECULAR HYDROGELS

Giovanni Nava[1], Federica Carducci[2], Rosangela Itri[3], Juliana Sakamoto Yoneda[3], Tommaso Bellini[1], Paolo Mariani[2]

1- Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Università di Milano, via Vanvitelli 32, 20129 Milano, Italy

2- Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy

3- Departamento Física Aplicada, Instituto de Física, Universidade de São Paulo, Sao Paulo, Brazil

ABSTRACT: Aqueous solutions of guanosine-5'-monophosphates (GMP) – known to form columns of G-quadruplex and liquid crystal phases – can be induced to turn into high water content gels by the addition of guanosine (Gua). By a combination of Light Scattering (LS) and AFM we show that Gua/GMP hydrogels are microscopically heterogeneous, formed by Gua-rich disordered microcoils of intertwined filaments ("knots") connected by GMP-rich long linear threads. The different thermal stability of knots and threads controls the gel transition.

Sample preparation

Guanosine 5'-monophosphate, acid free form (Santa Cruz Biotechnology, 99% purity), was converted into potassium salt by chemical titration using KOH 1M (Carlo Erba Reagents) until pH 9, followed by 2 times washing using absolute ethanol and lyophilisation of the recovered solution. The pellet was resuspended in distilled water, at the concentration of 10% wt/v. Guanosine (Sigma, St. Louis, USA; 99% purity) was suspended in water, at the concentration of 6% wt/v. Hydrogels were prepared by mixing into a glass vial different volumes of Gua and GMP solutions and then adding distilled water to reach the required water concentration. In particular 2 samples were prepared, as indicated in the table.

G/GMP	GMP (mg/ml)	Gua (mg/ml)	GMP (M)	Gua (M)	Ionic force
1:2	36.0	14.0	.0990	.0496	.297
3:4	31.5	18.5	.0869	.0652	.261

Table 1: Sample preparation for 1:2 and 3:4 samples used in this work

Mixtures were then homogenised by heating the vials up to 90°C (i.e. till the formation of a liquid fluid state) and then left to cool down at room temperature. After 30 min equilibration at 20°C, the pre-heated mixtures resulted transparent and clear. The pH of the gel was 7.9. As Gua is poorly soluble in water, errors in adding Gua solution to the mixture were minimised by pipetting the stock solution several times before delivery. Errors on the Gua/GMP molar ratio not larger than 5% were estimated by gravimetric assay on delivery testing.

LS setup and measurement procedures

Light scattering measurement were performed in a custom modified setup (Scitech 100) at wavelength $\lambda = 532\ nm$ at constant angle $\alpha = 90°$. Both the excitation light and the scattered light were introduced and fetched by means polarization-maintaining fibers. The sample was put in a cylindrical glass capillary (O.D.=3 mm I.D. 2.4 mm) and flame sealed. Sample and fibers are immersed in circulating silicon oil providing thermalization.

The intensity time autocorrelation $g_2(\tau)$ was obtained using a digital correlator (flex-03d Correlator.com). Each $g_2(\tau)$ was obtained after averaging 900s.

At each investigated T, measurements were performed after 2 hours of thermalization. At each T, we performed various measurements of $g_2(\tau)$, alternating measurements on still cells and measurements on samples that were continuously rotating by means of a motor embedded in the setup ($\omega = 6\ mrad/s - T = 100\ s$). Measurements on rotating cells were performed to detect kinetic arrest, as explained in the main text.

AFM observations of Gua/GMP hydrogels

AFM micrographs were performed at the Chemistry Department of FFCLRP-USP (BR) by using a Shimadzu SPM-9600 Scanning Probe Microscope (Shimadzu Corporation, Japan) operating in dynamic mode. Scanning was performed in air at 23±1 °C by using silicon probes with a resonance frequency ranging from 324 to 369 kHz (Nanosensors, Switzerland). Scan rates of 0.2–0.5 Hz were used to prevent tip-induced sample deformations and/or damages. The cantilevers spring constant was approximately 38.8 N m^{-1} and the value of the resonance frequency was approximately 336±67 kHz. The roughness values were determined by SPM Offline software, from Shimadzu.

Gua/GMP hydrogel samples for AFM analysis were prepared at c_G = 50 mg/ml and further diluted 3 and 11-fold, to reach concentrations of 12.5 and 4.2 mg/ml, respectively. 5 µL of the final sample was dropped onto freshly cleaved mica substrates, left to dry at room temperature and imaged by AFM.

AFM image analysis for the 1:2 sample

The analysis of AFM images obtained from 1:2 sample and reported in the text is here resumed:

- Line scan analysis was performed on the regions containing individual filaments, bundles and knots. Figure S1 shows that single wires (as along the A-B and G-H lines) have homogenous heights in the range of 2–2.5 nm, which are consistent with the G-quadruplex diameter (around 2.4 nm). Moreover, the height scans also indicate that bundles and knots are due to the wrapping of quadruplexes around one another (see the C-D and E-F lines).

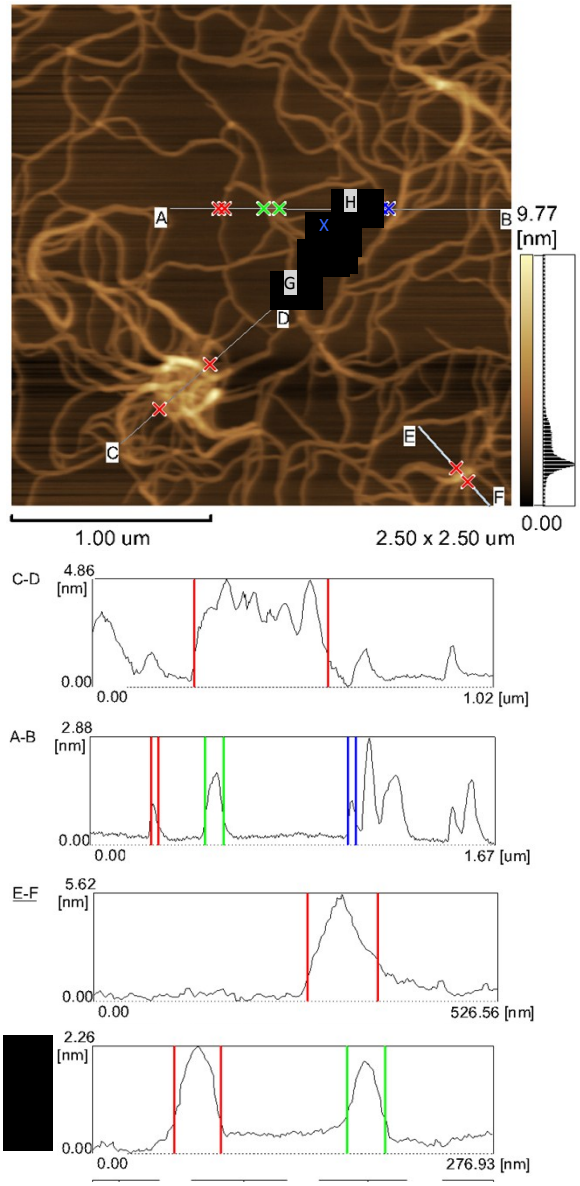

Figure S1: line analysis of AFM image from 1:2 sample extra diluted 11-fold.

Knots probably show higher height, as evidenced by the 3D image reported in Figure S2: height as larger as 10 nm can be easily observed.

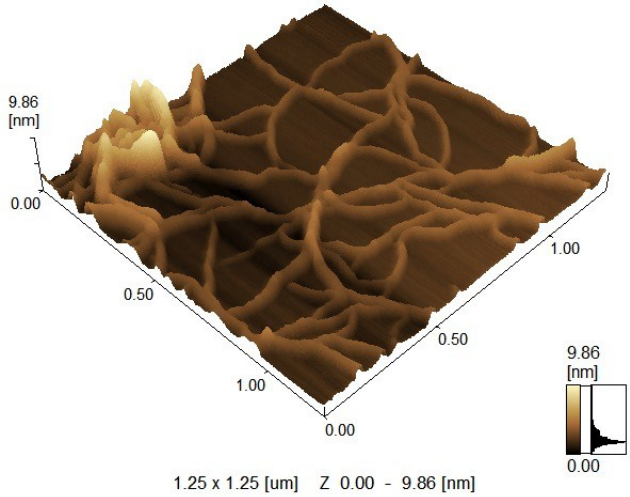

Figura S2: 3D AFM image from 1:2 sample extra diluted 11-fold

- Knot dimensions have been also derived from the AFM images. Images have been analyzed using ImageJ software using the thresholding MaxEntropy method to define the color limit and then to delineate the knots. Results are reported in Figure S3.

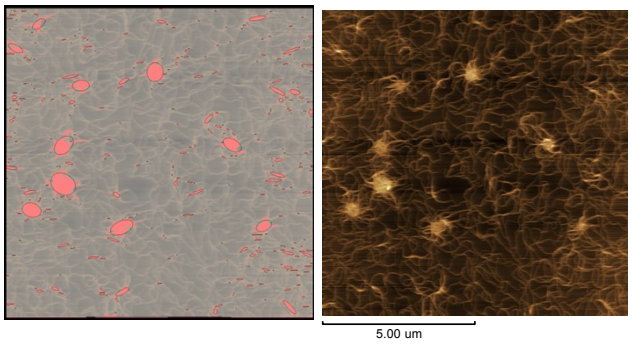

Figura S3: contour-image on thresholded knots compared to the original AFM image from 1:2 sample extra diluted 11-fold.

Analysis results are reported in Figure S4, in term of knot radii distribution. It can be observed that larger knots range from 150 to 350 nm radii.

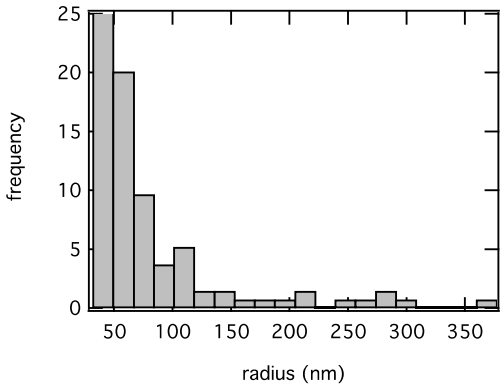

Figura S4: knot radius distribution in AFM image from 1:2 sample extra diluted 11-fold.