Dynamics of G-quadruplex oligonucleotide sequences as affected by complexation with pharmaceutical molecules

2nd year sumup

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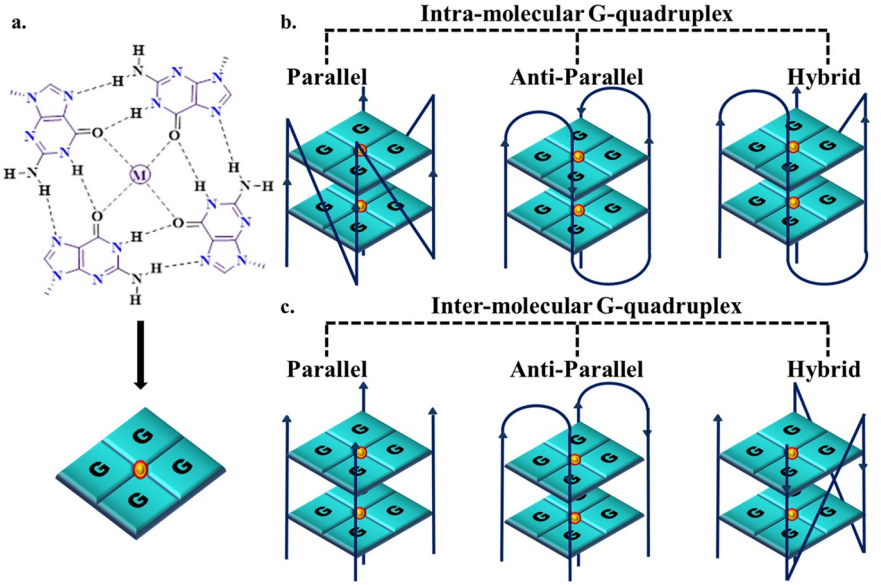
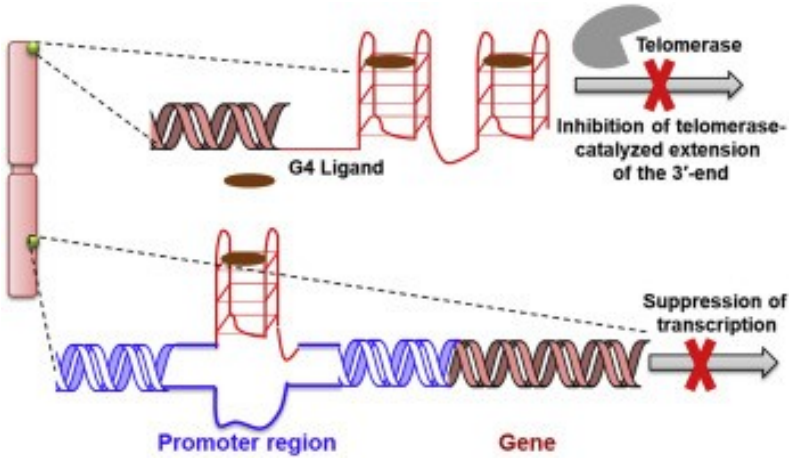
G-quadruplexes

- G-quadruplexes (G4) are nucleic acid non-canonical higher order secondary structures;
- They are found in G-rich sequences of the genome, such as telomeres and gene promoter regions;
- G4 can take different topologies depending on a large variety of conditions ;
- Structural polymorphism of G4 is linked to their transient nature;



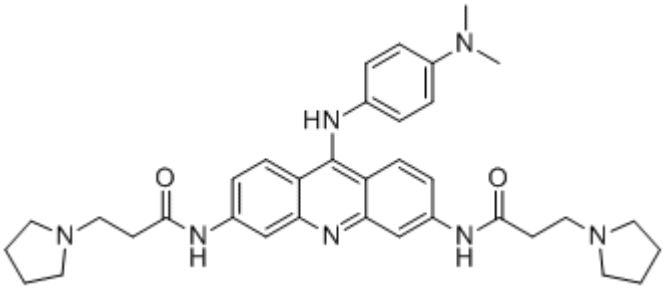


- G4 structures' stabilization in telomeric sequences leads to telomerase inhibition; in gene promoter regions it can be used to regulate gene expression;
- Understanding G4-ligand interaction mechanism is essential in the rational design of anticancer drugs having G4 as target.
- Even though investigation of G4 structure and function has been extensively performed, this is the first work ever done on G4 fast dynamics!

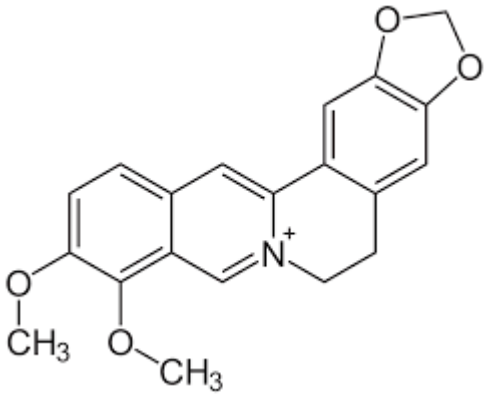
Sequence and Drugs

- **Sequence**: $AG_3(TTAG_3)_3$

- **Drugs:**
  - **BRACO19**: a trisubstituted acridine G4-interacting compound that appears to inhibit telomerase activity; higher affinity with Quadruplex DNA than duplex DNA
  - **Berberine**: a quaternary ammonium salt from the protoberberine group of benzylisoquinoline alkaloids with anticancer activity



BRACO19



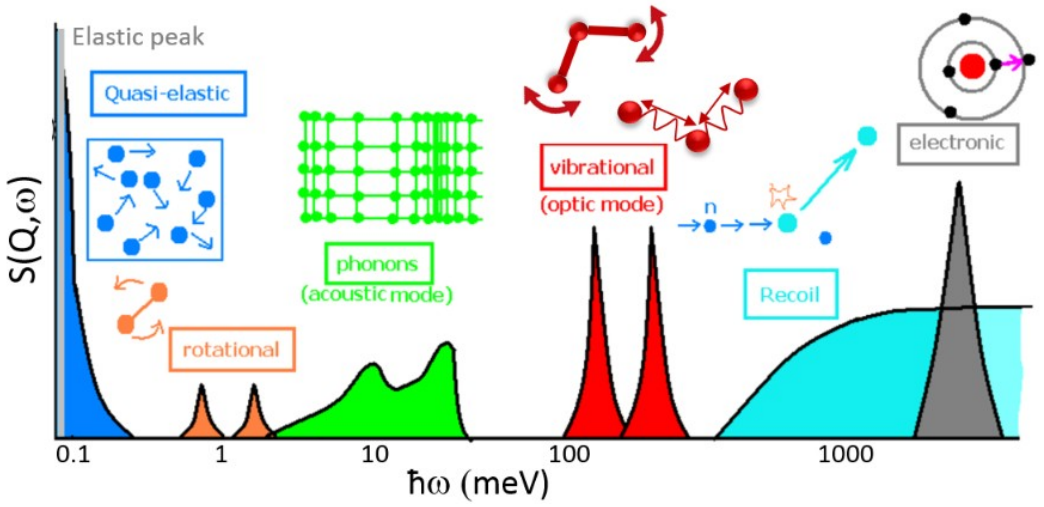
Berberine

Neutron Scattering experiments

- Incoherent neutron scattering experiments allow determination of dynamical properties of the system under study;
- Large incoherent neutron cross section for hydrogen atoms is suitable for biomolecules;
- Thermal neutrons wavelength and energy match both typical intermolecular lengths and thermal excitation energies;
- Average correlation of the same hydrogen atom at different times yields the scattering function:

$$S(Q,\omega)=\frac{1}{2\pi N}\int_{-\infty}^{+\infty} e^{-i\omega t}\sum_{i=1}^{N}\left\langle e^{-i\vec{Q}\cdot\vec{R}_i(0)}\, e^{i\vec{Q}\cdot\vec{R}_i(t)}\right\rangle$$

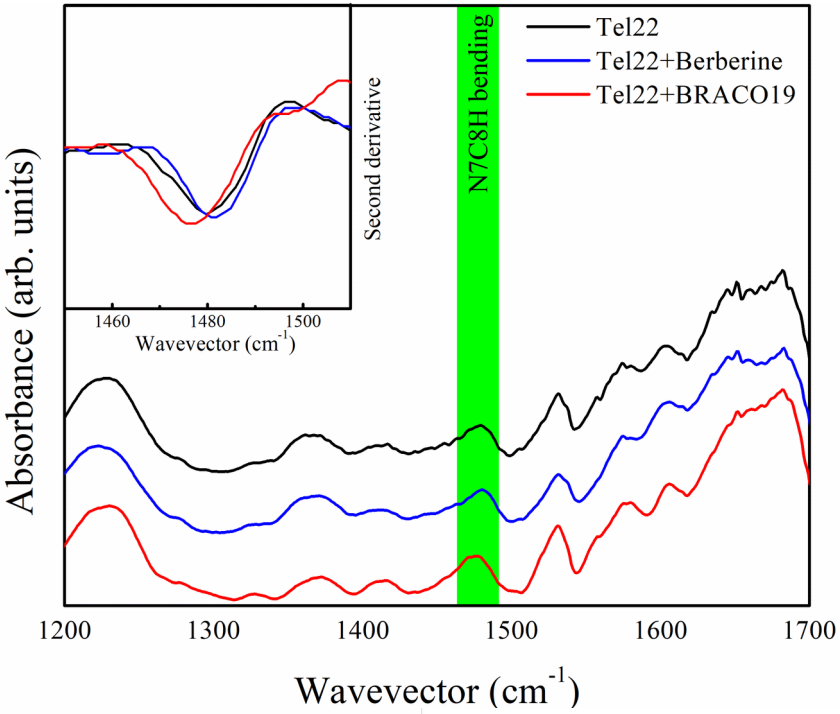
- Two techniques have been deployed:
  - Elastic Incoherent Neutron Scattering (EINS);
  - Quasi-elastic Incoherent Neutron Scattering (QENS);
- Spectrometers with different resolution allow to “tune” onto motions taking place at different timescales.



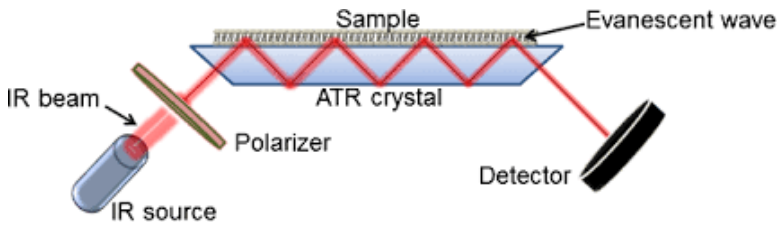
Are we measuring G-quadruplexes?

- Spectroscopic methods (e.g. CD) are available to probe **formation and structure** of biomolecules, **<u>in solution</u>**;
- **Neutron measurements** are made on **<u>hydrated powders</u>**!
- How can we be sure that we are not just measuring a “structureless” nucleic acid sequence?

ATR-FTIR!!!

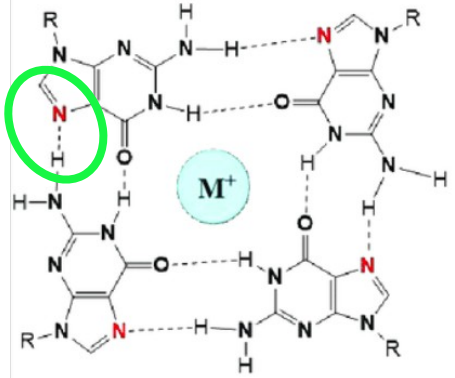


- ATR-FTIR is a spectroscopic technique useful to investigate molecular vibrations;
- It can be used to analyze powders!

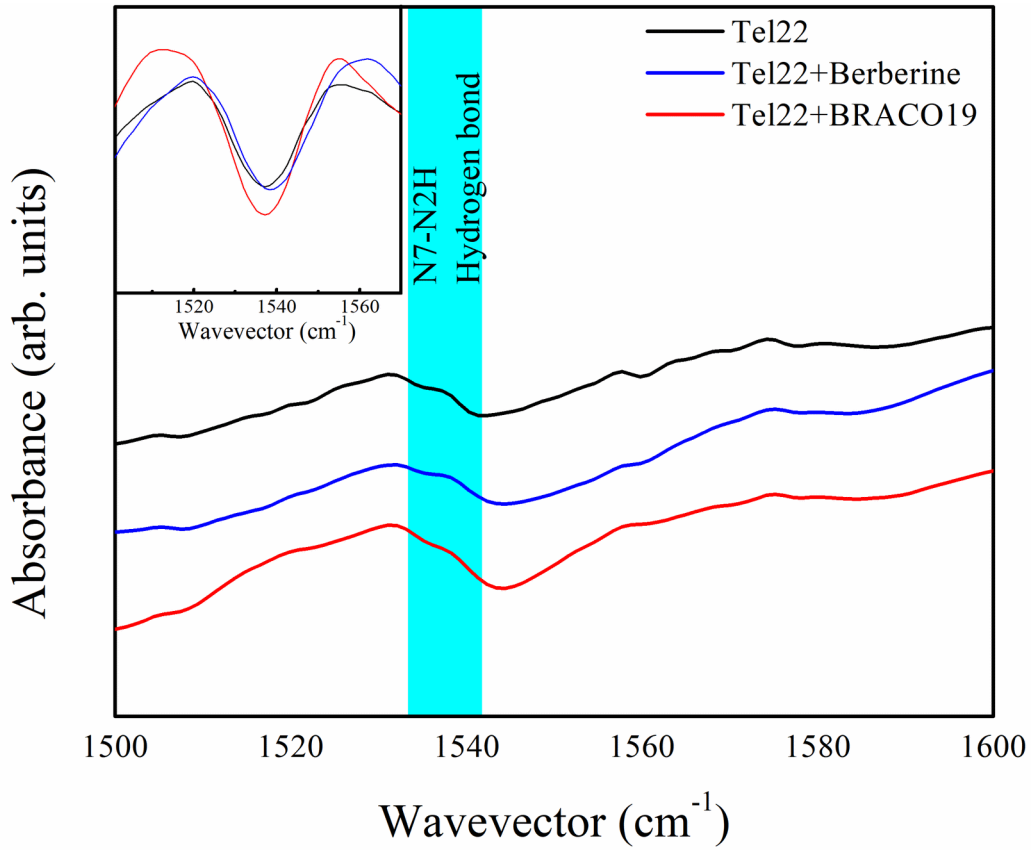


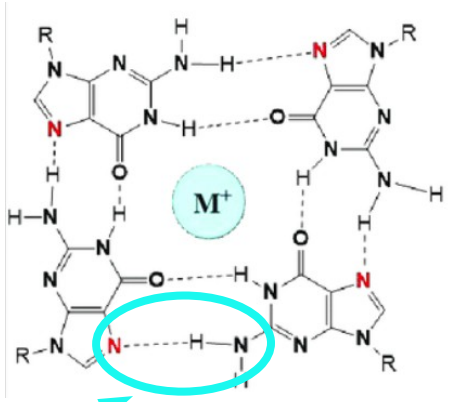
N7C8H
1480cm^{-1}

<u>Quadruplex formation[1]!!!</u>



Are we measuring G-quadruplexes?





N7-N2H

Quadruplex formation[1]!!!

- Another evidence of G4 formation is the presence of a peak at 1537cm^{-1} consistent with the presence of the N7-N2H hydrogen bond (Hoogsteen base pairing)

[1]: Guzmán, M. Romero, et al. "Characterization of parallel and antiparallel G-tetraplex structures by vibrational spectroscopy." Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 64.2 (2006): 495-503.

EINS model

- The elastic intensity can be written as:

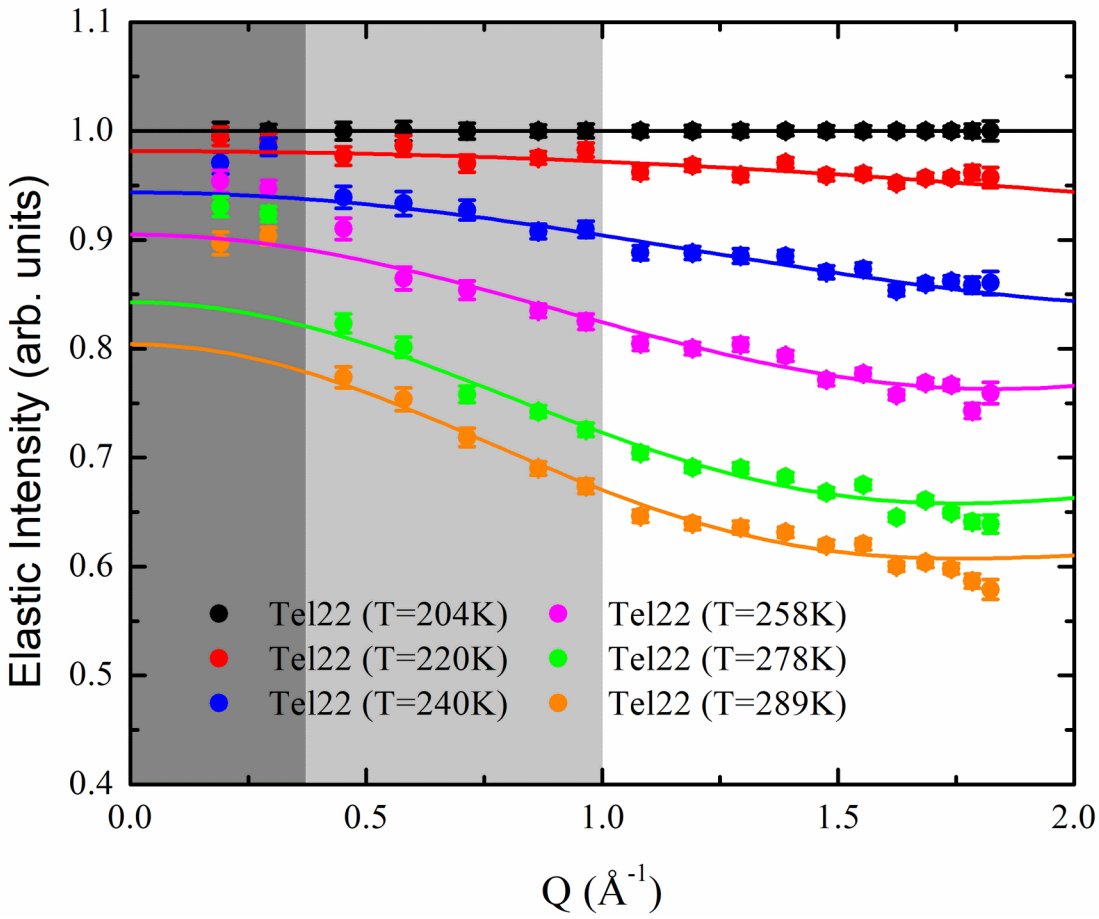
$$S(Q,\omega=0)=\frac{1}{N}\sum_{i=1}^{N}\exp\left(\frac{-Q^2\langle u_i^2\rangle}{3}\right)$$

- In the limit $Q\rightarrow 0$ (Gaussian approx.):

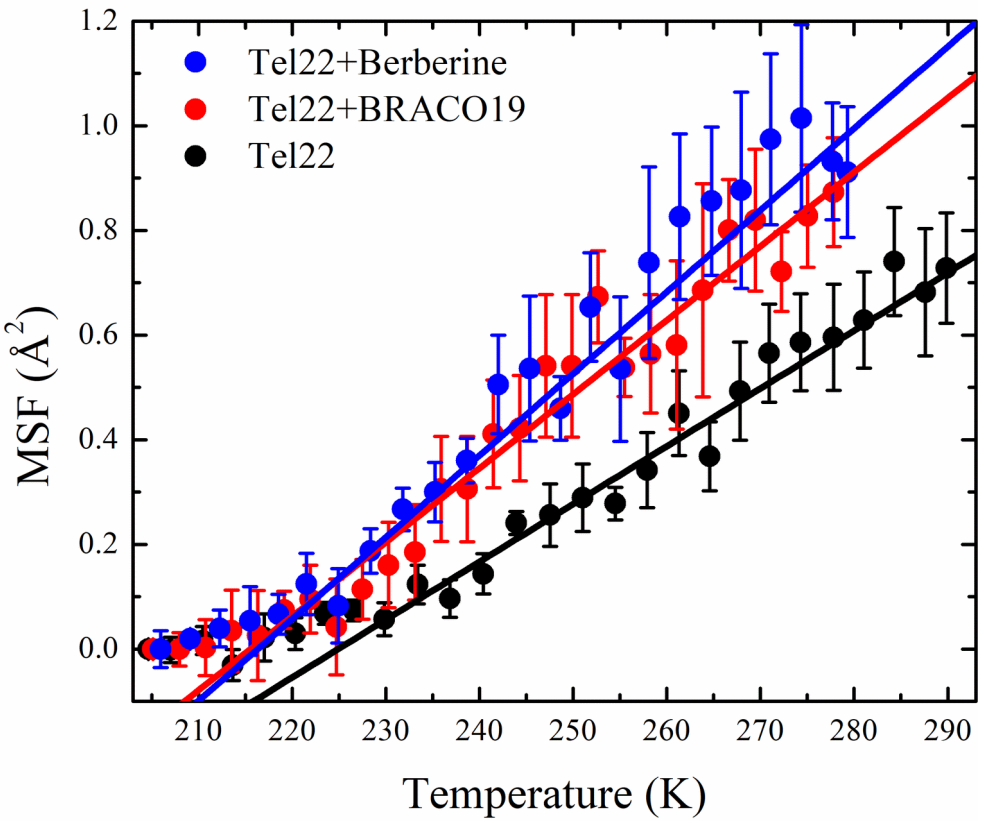
$$S(Q,\omega=0)=\exp\left(\frac{-Q^2\langle u^2\rangle}{3}\right)$$

- But to fit in the high Q-range we need a cumulant expansion introducing a quartic term to account for dynamical heterogeneity:

$$S(Q,\omega=0)=\exp\left(\frac{-Q^2\langle u^2\rangle}{3}\right)\left(1+\frac{Q^4}{18}\sigma^2\right)$$



EINS results (IN16b)



	Tel22	Tel22-Berberine	Tel22-BRACO19
k_f (N/m)	0.37 ± 0.02	0.26 ± 0.03	0.29 ± 0.02
F_u (pN)	41 ± 3	29 ± 2	32 ± 2

- The MSF are greater in the presence of the drugs: complexation leads to enhanced overall mobility;
- Dynamical transition at ~ 235K in Tel22, at ~ 220K in the presence of drugs;
- The slope of the MSF as a function of T quantifies thermal softness of the protein (random walk in confining harmonic potential):

$$\langle u^2 \rangle = \frac{3 k_B T}{k_f}$$

- Lindeman Criterion can be use to estimate unfolding forces:

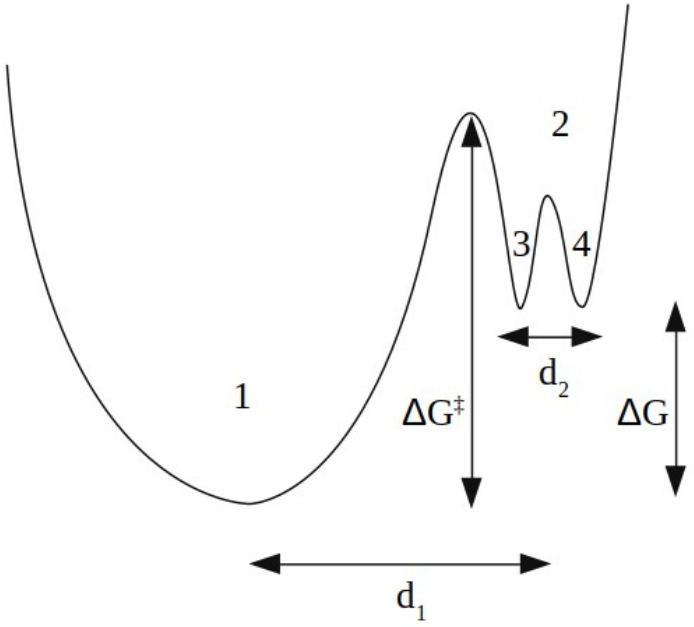
$$\sqrt{\langle u^2 \rangle_{unf}} = 0.17 L_t \qquad F_u = k_f \sqrt{\langle u^2 \rangle_{unf}}$$

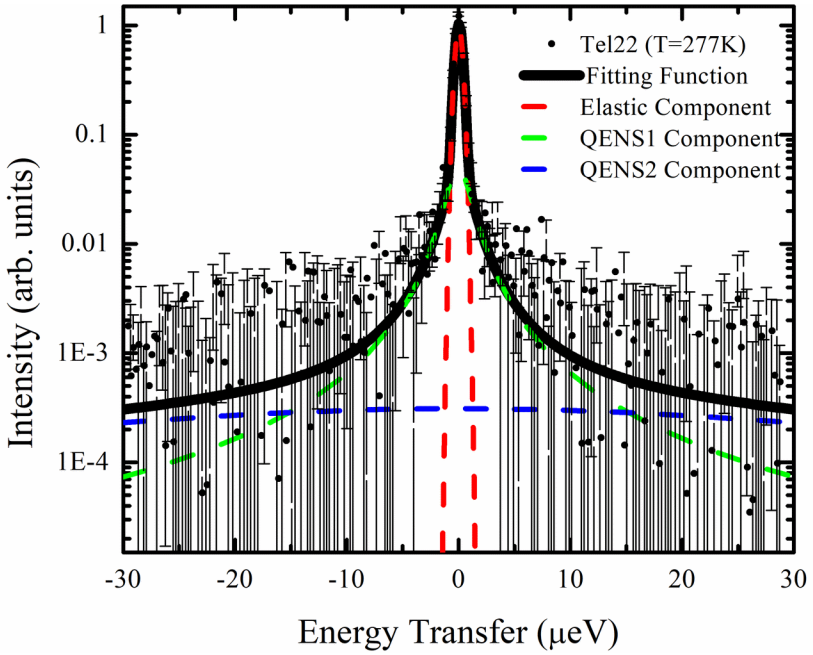
- Results are of the same order of magnitude of those from SM experiments.

IN16b Energy resolution: ~ 0.75 μeV

QENS model

- Resolution function as fitted from Vanadium standard;
- Two dynamical components, a slow (S) and a fast (F) one;
- Theoretical model: energy surface consisting of two double wells, one embedded in the other;
- Jumps from well 1 to well 2 represents S dynamics, jumps within well 2 (between sub-wells 3 and 4) represents F dynamics.





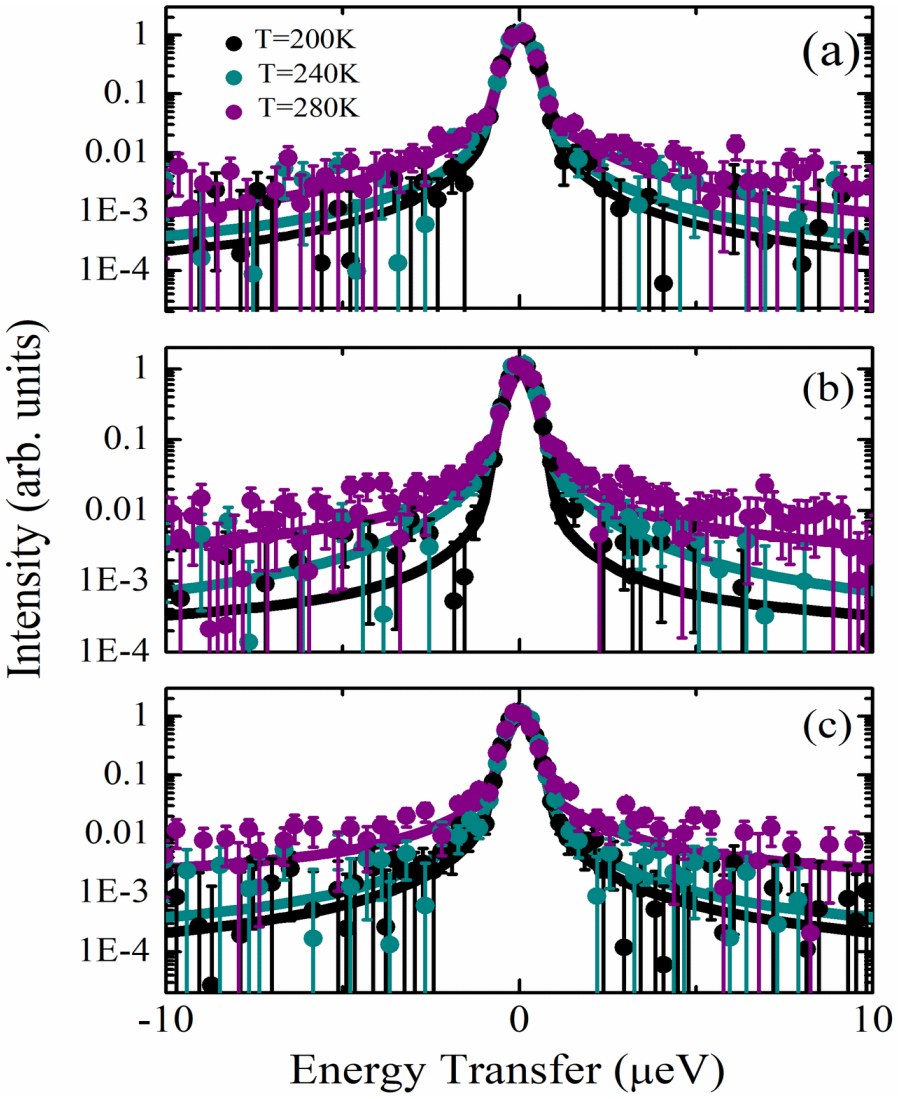
- The fitting function is:

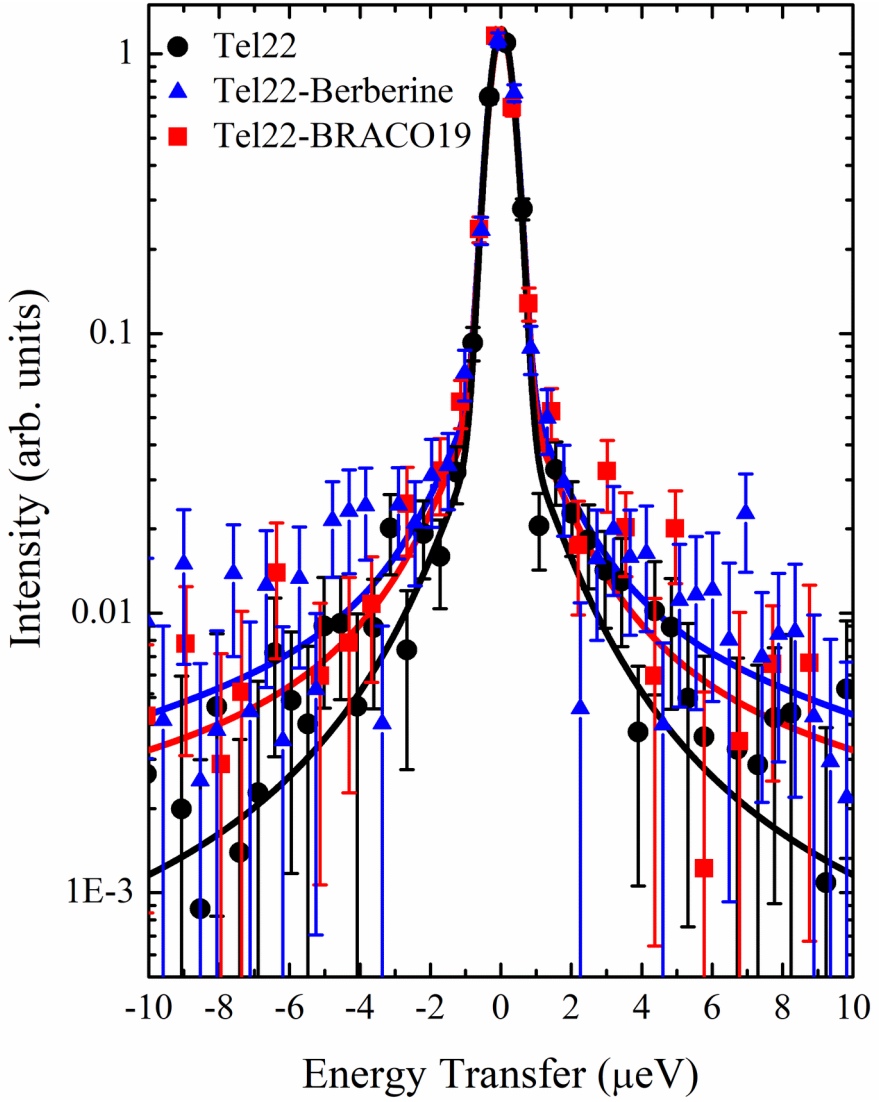
$$S(Q,\omega)=A(Q)g(\omega)+B(Q)V_1(\omega,\Gamma_1)+C(Q)V_2(\omega,\Gamma_2)$$

$$A(Q)=1-B(Q)-C(Q) \qquad B(Q)=2(1-f)p'_1p'_2\frac{\sin(Qd_1)}{Qd_1} \qquad C(Q)=\frac{f}{2}\frac{\sin(Qd_2)}{Qd_2}$$

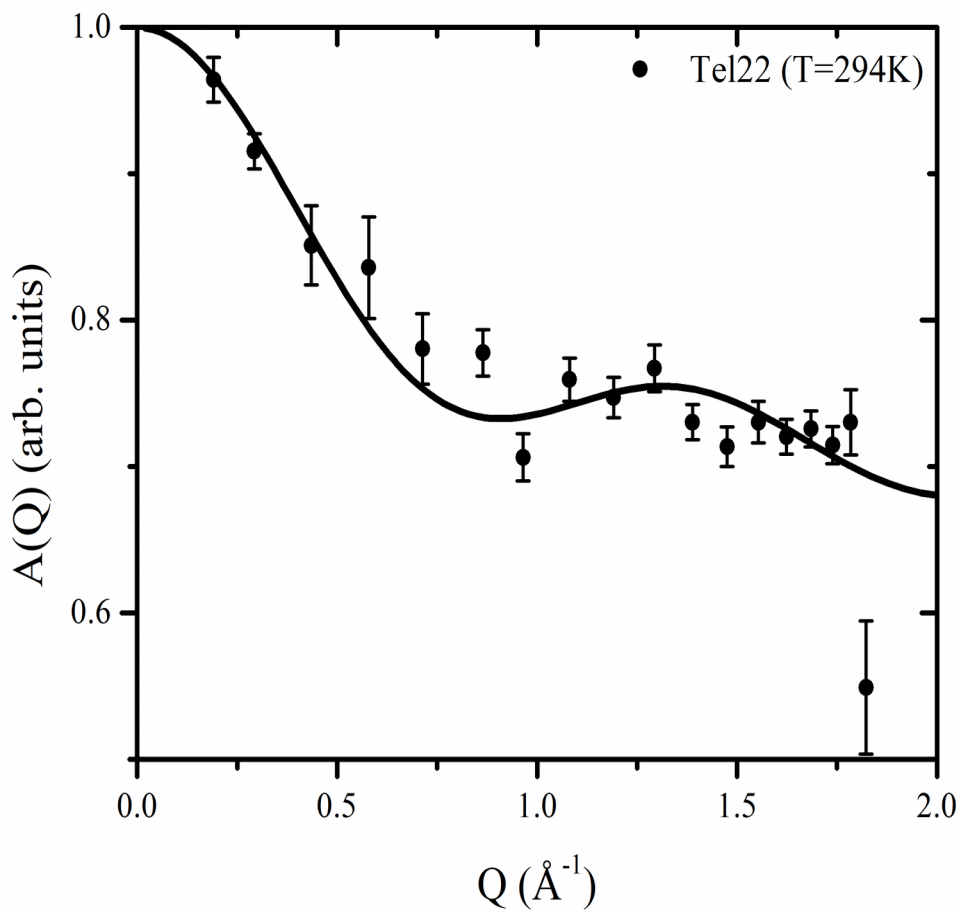
$$V(\omega;\sigma,\Gamma)=\int_{-\infty}^{+\infty}d\omega' G(\omega',\sigma)L(\omega-\omega',\Gamma) \qquad L(\omega,\Gamma)=\frac{\Gamma}{\pi(\omega^2+\Gamma^2)}$$

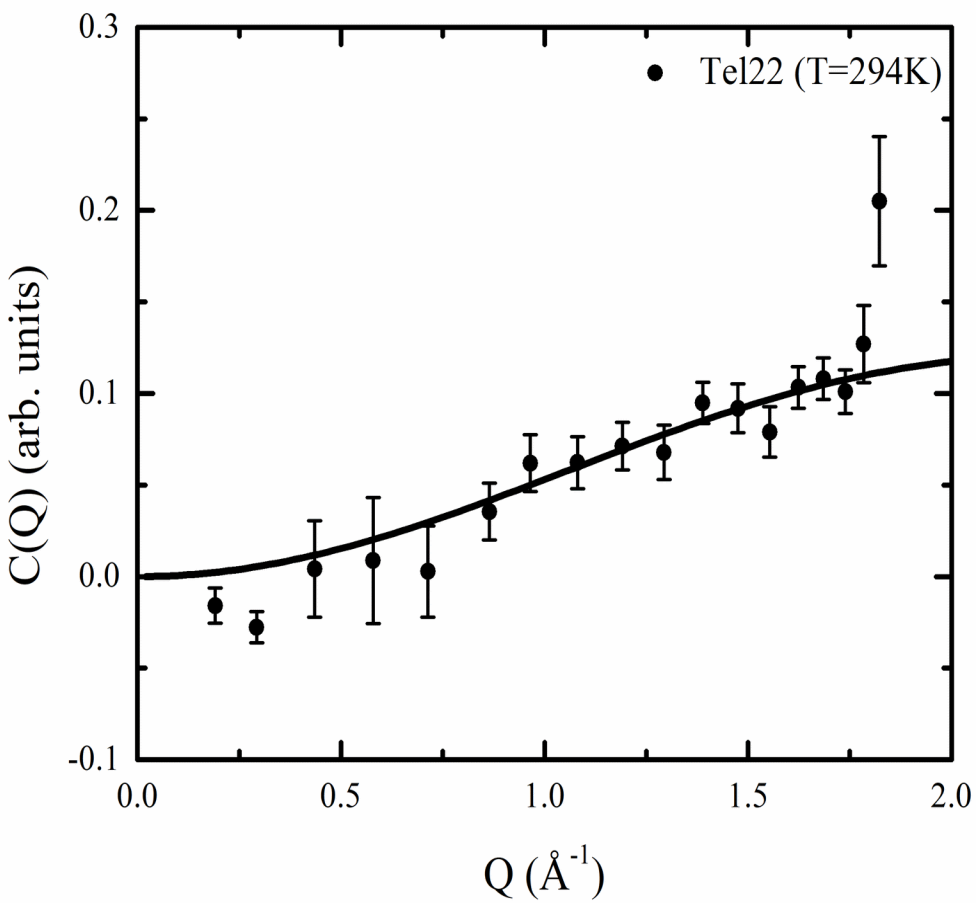
QENS results (IN16b)



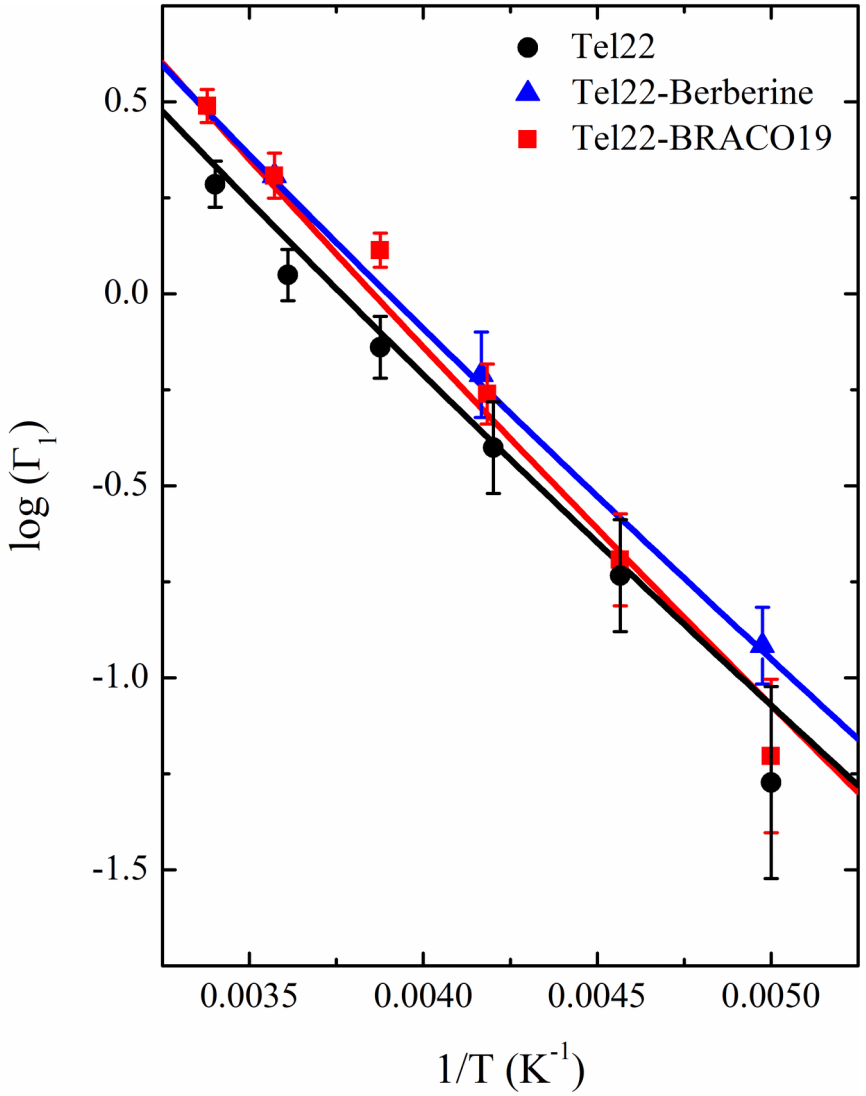


QENS results (IN16b)





QENS results (IN16b)



- Γ_2 has been found to be constant as a function of both Q and T, with the same value of 20μeV for all the samples;
- d_2 also appears to be almost unaffected by complexation;
- d_1 is increased upon complexation;
- Γ_1 (as a function of temperature) show that complexation prompts faster motions in the system; it can be written as:

$$\Gamma_1 = \frac{k_B T}{h} e^{\frac{-\Delta G_0}{k_B T}}$$

	Tel22	Tel22-Berberine	Tel22-BRACO19
d_1 (Å)	5.2 ± 0.2	6.5 ± 0.3	6.3 ± 0.2
d_2 (Å)	2.1 ± 0.3	1.6 ± 0.4	2.4 ± 0.2

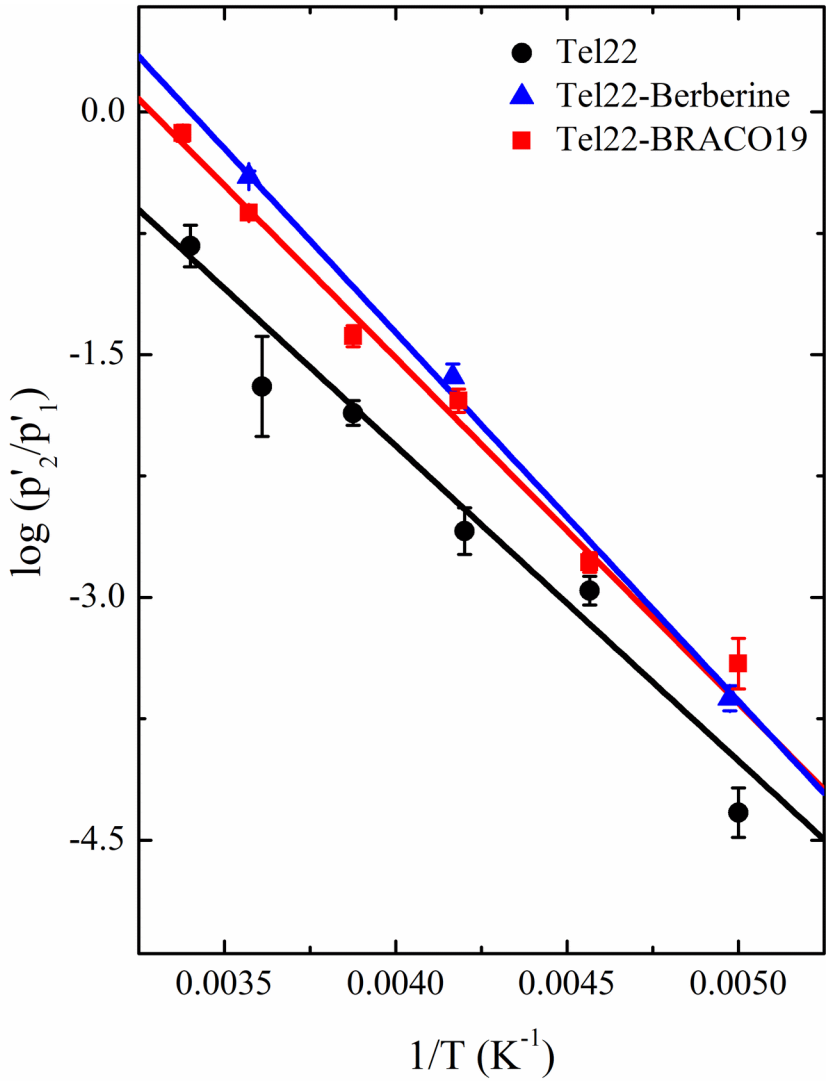
QENS results (IN16b)

- The population of the second well is increased upon complexation;
- We have:

$$\frac{p'_2}{p'_1} = \exp\left(\frac{\Delta G}{k_B T}\right) = \exp\left(\frac{\Delta S}{k_B} - \frac{\Delta H}{k_B T}\right)$$

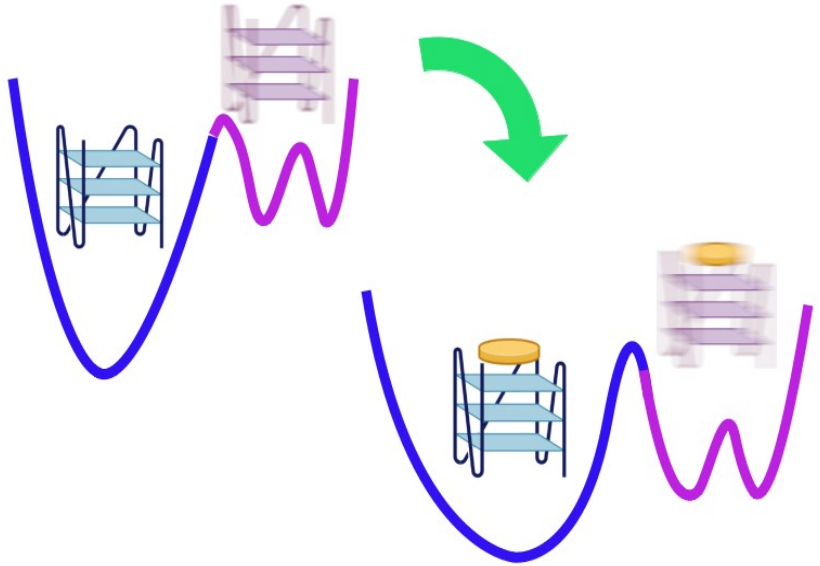
- The decrease in free-energy difference upon complexation is entropy-driven. The extracted values are reported below:

	Tel22	Tel22-Berberine	Tel22-BRACO19
$\Delta H^{\ddagger}$ (kJ mol^{-1})	5.3 ± 0.4	5.3 ± 0.1	5.9 ± 0.6
$\Delta S^{\ddagger}$ (J mol^{-1}K^{-1})	-224 ± 1	-223.0 ± 0.3	-221 ± 2
ΔH (kJ mol^{-1})	16 ± 1	18.9 ± 0.7	17.7 ± 0.7
ΔS (J mol^{-1}K^{-1})	48 ± 6	64 ± 3	58 ± 3

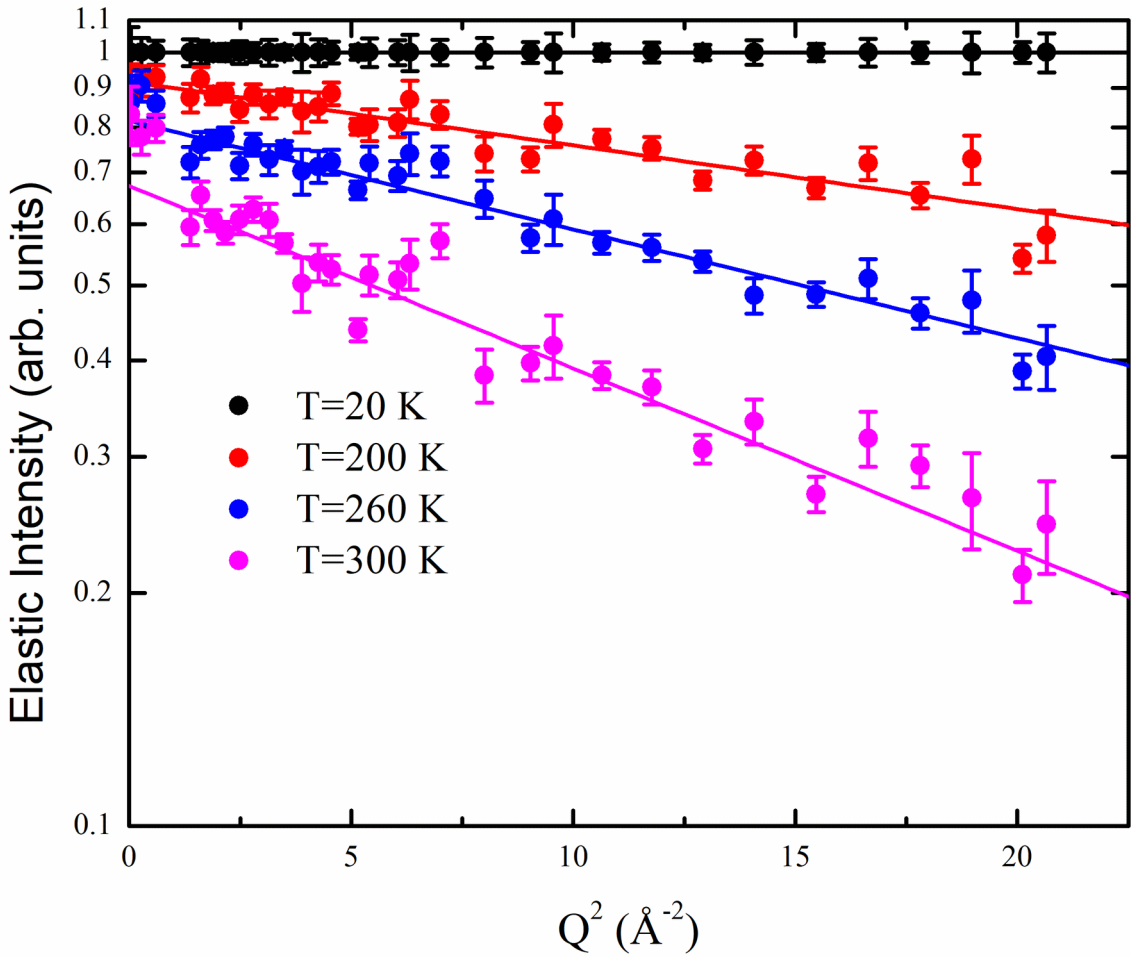


IN16b results in brief

- **Complexation** with selected drugs results in the **enhancement of the dynamics** of Tel22;
- Stabilization was expected to result from complexation, but:
  - hydrated powder samples were measured (only internal dynamics);
  - Specific timescale (ns and sub-ns at IN16b) was investigated;
  - D_2O hydrate powders (hydration water is not visible).
- The system is **dynamically heterogeneous**;
- Two dynamical regimes have been found of **slow high amplitude motions** and **fast low amplitude motions**;
- Enhanced dynamics is prompted by:
  - **Increased number of scatterers** participating **to quasielastic motions** originating from decreased free energy differences between the wells bottom in the potential energy;
  - **Faster transition between the wells** in the potential energy due to slightly slower energy barriers.
- The **enhancement of the dynamics** of Tel22 upon complexation results to be **entropy-driven**.
- The hydration of the three samples was the same: reasonable to suppose that complexation leads to a **different hydration water coordination**;
- **Changes in hydration water network** may reflect into different mobility observed in neutron scattering experiments.
- The role of hydration water needs to be further clarified.



Influence of ions and complexation in G4 fast dynamics (IN13)



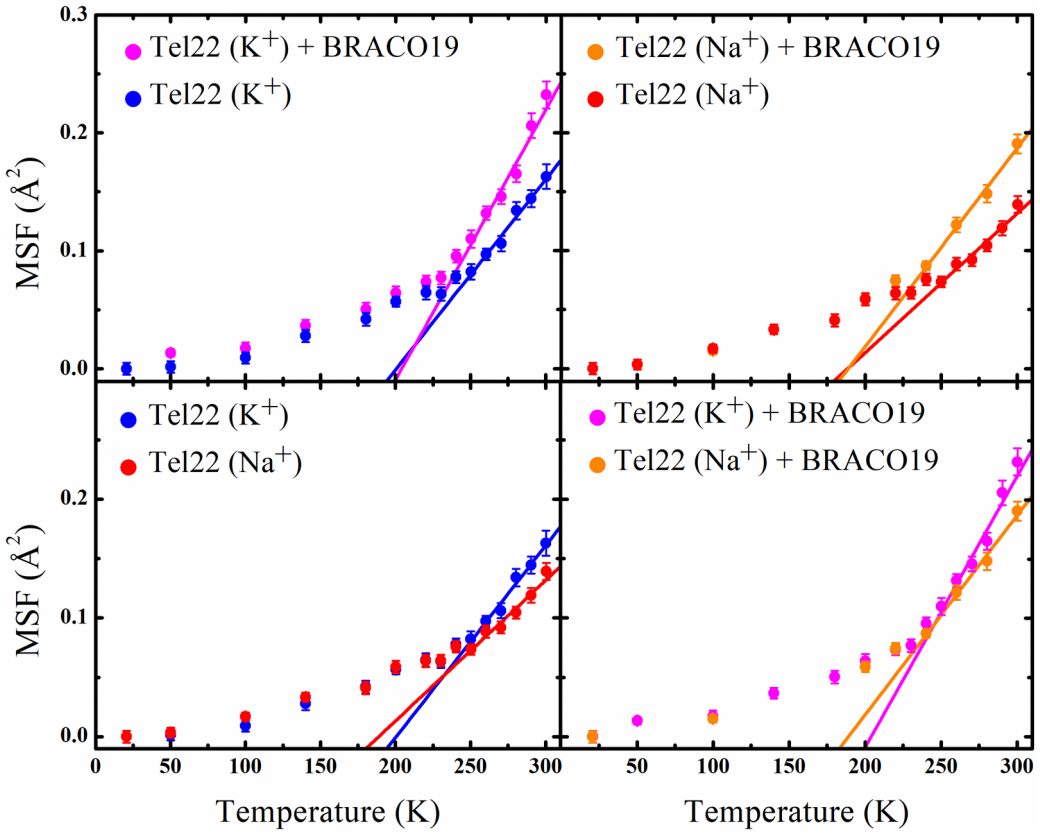
IN13 Energy resolution: 8 μeV

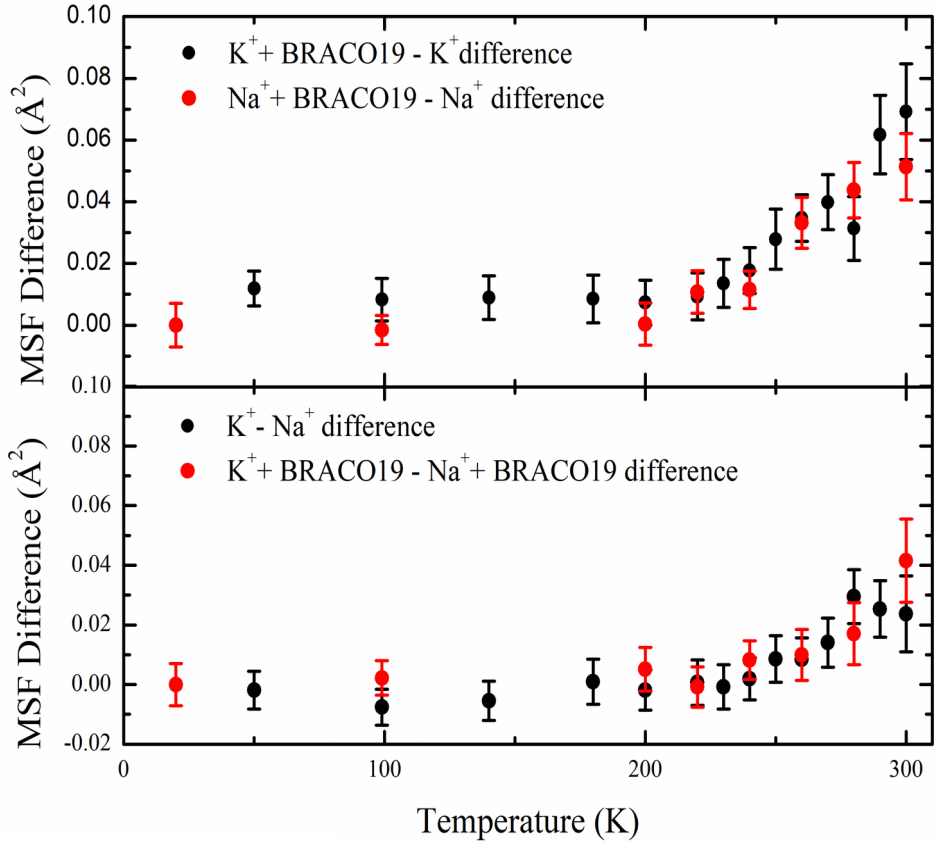
- Measurements of Tel22 stabilized with K^+ and with Na^+, both alone and in the presence of BRACO19;
- Only EINS temperature scans were carried out, from 20K to 300K;
- Interestingly enough, the sample shows dynamical homogeneity;
- This allows us to obtain MSF using the Gaussian approximation:

$$S(Q,\omega=0)=\exp\left(\frac{-Q^2\langle u^2\rangle}{3}\right)$$

- We observe greater MSF in Tel22-K^+ than Tel22-Na^+;
- Again, enhanced mobility in the presence of the ligand is visible;
- The two effects (ion difference and complexation) seem to act independently on the MSF.

IN13 results

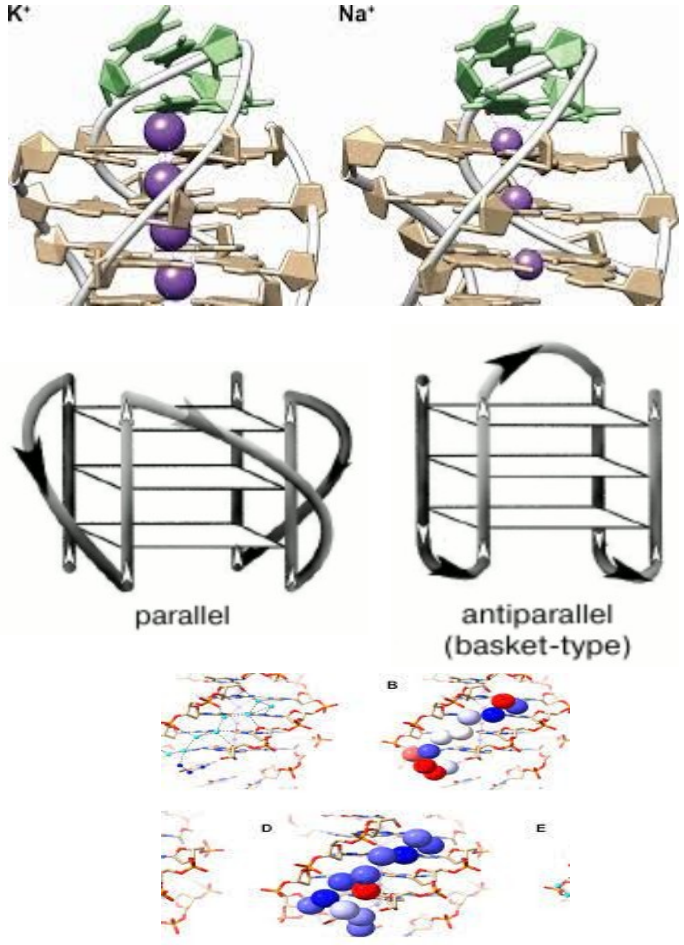




	Tel22 (K^+)	Tel22 (Na^+)	Tel22 (K^+) + BR19	Tel22 (Na^+) + BR19
k_C (N/m)	86 ± 6	116 ± 11	60 ± 6	82 ± 11
F_u (pN)	39 ± 3	53 ± 5	27 ± 3	37 ± 5

Dynamical differences: just hydration water or different secondary structure?

- Different ions exert different coordination degree of water molecules, with sodium possibly facilitating formation of water networks;
- Ion position among stacked tetrads is different; more ions exposed to water may change the number of water molecules coordinated by the biomolecule;
- But...different **ions** can induce **different G-quadruplex conformations**, which may also contribute to their dynamics…
- Furthermore, different conformations can involve different water structures (e.g. water spines), with different mobility.
- Complexation may also induce a change in G-quadruplex structure…
- G4 **structure is known** to be antiparallel in Na^+ and mostly parallel in K^+[3] **in solution**...what about **powders**?

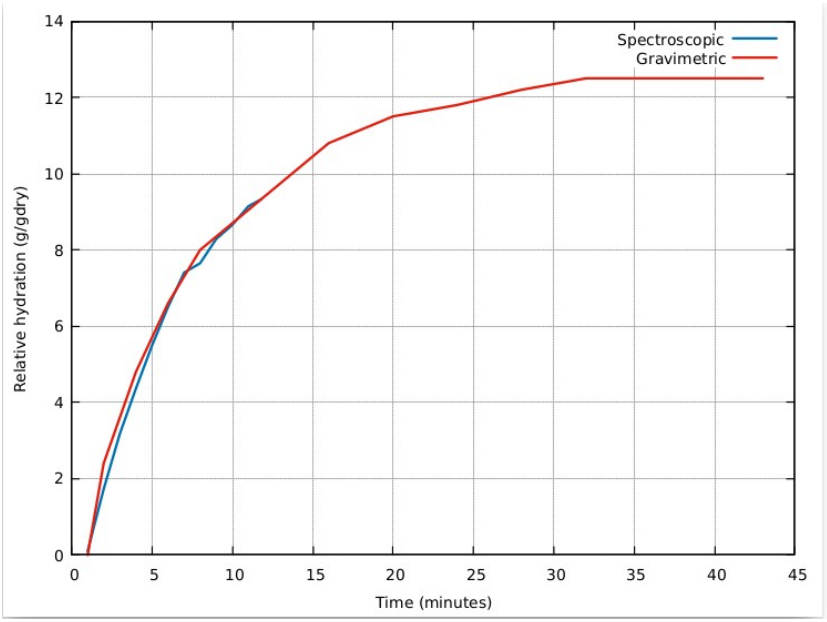


[2] Lim, Kah Wai, et al. "Structure of the human telomere in Na+ solution: an antiparallel (2+ 2) G-quadruplex scaffold reveals additional diversity." Nucleic acids research 41.22 (2013): 10556-10562.

[3] Ambrus, Attila, et al. "Human telomeric sequence forms a hybrid-type intramolecular G-quadruplex structure with mixed parallel/antiparallel strands in potassium solution." Nucleic acids research 34.9 (2006): 2723-2735.

FTIR for structure determination

- We now understand that, in order to **understand** dynamics we need to understand **hydration water interaction and structure**;
- Again, FTIR can be the answer, but the task is more complicated than formation detection…
- Two major problems:
  - Hydration level control;
  - WATER!
- If we expect the ligand to induce differences in water network, we must be sure that hydration is the same.
- We can try to estimate the hydration level by monitoring the OH stretching band (in the powder state);
- Gravimetric measurements might be useful for calibration!



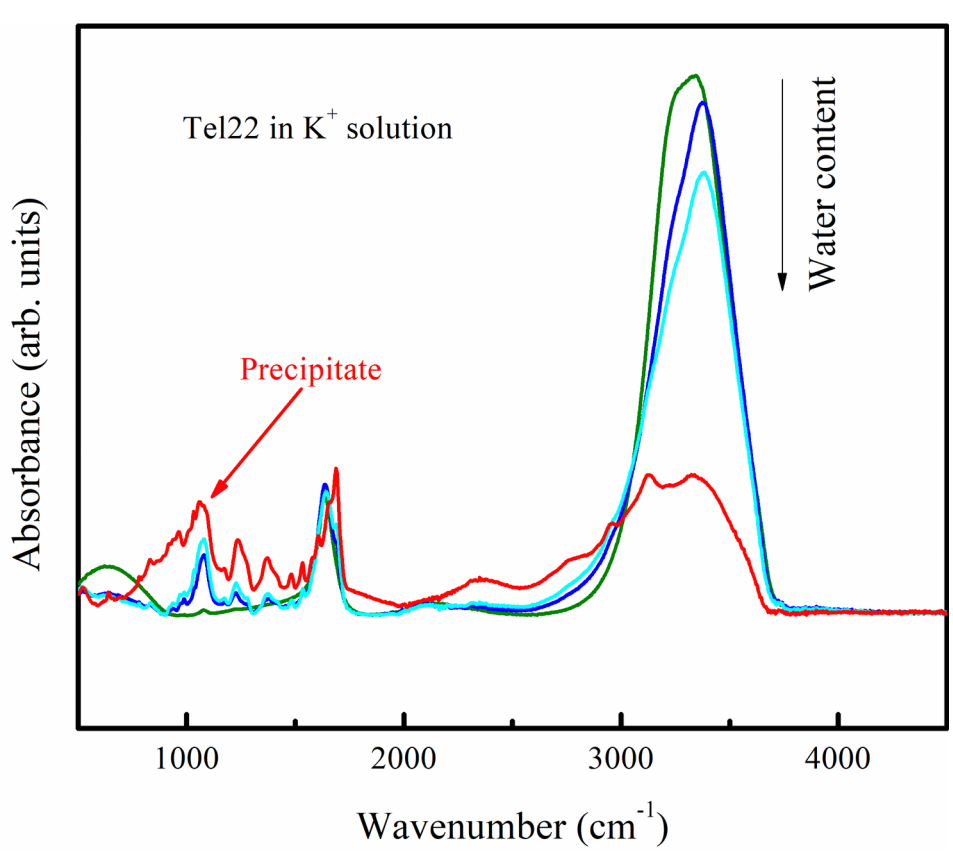
$$h_g(t) = m_0\left(1 - e^{\frac{-t}{\tau_g}}\right)$$

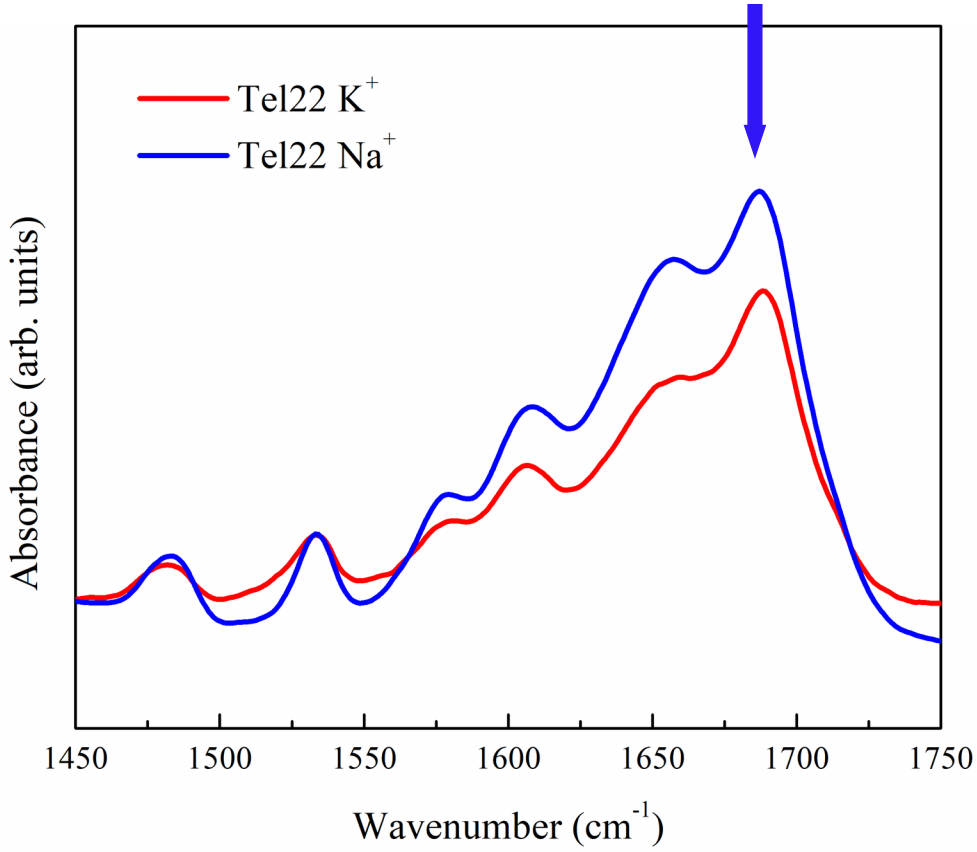
$$h_s(t) = I_0\left(1 - e^{\frac{-t}{\tau_s}}\right)$$

FTIR spectra: solution

[1]: Guzmán, M. Romero, et al. "Characterization of parallel and antiparallel G-tetraplex structures by vibrational spectroscopy." Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 64.2 (2006): 495-503.

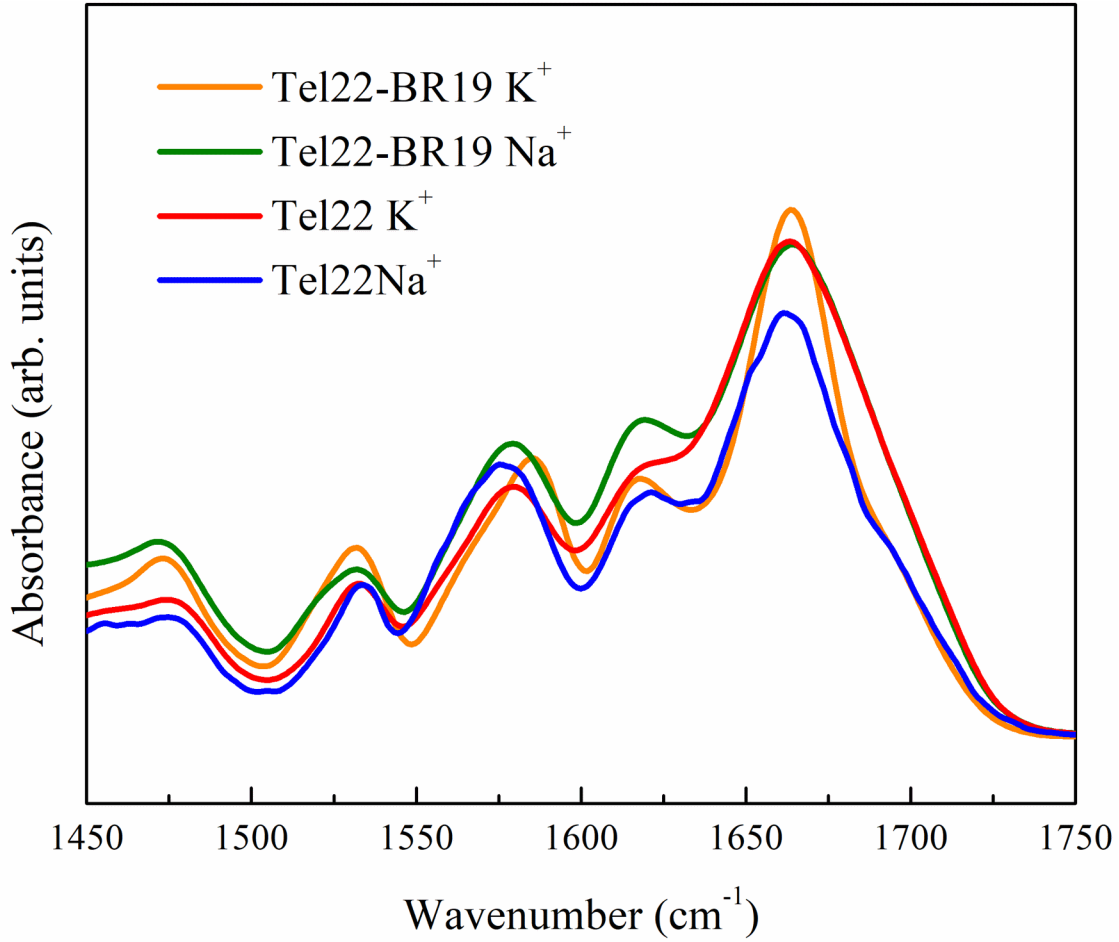
1682cm^{-1}: AP strand[1]

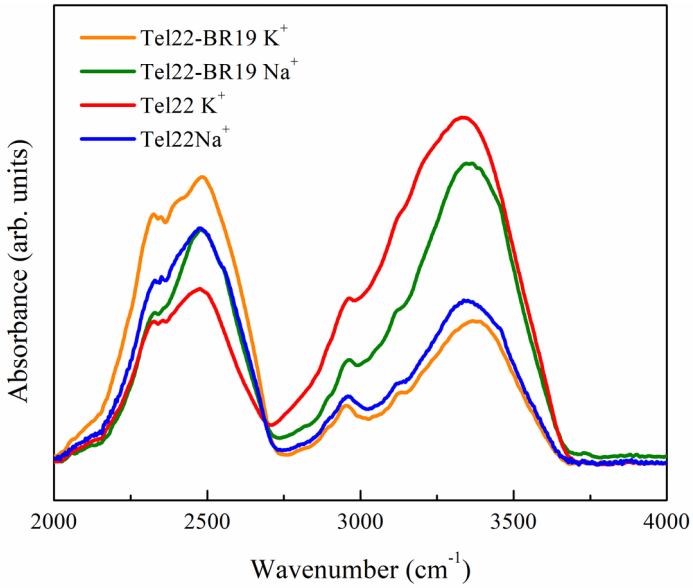




BUT, even though we expect Na+ to favour AP conformation in solution, K+ should induce a mixture of populations. Besides, OH bending is present in the region!

FTIR spectra: powder state (D_2O)

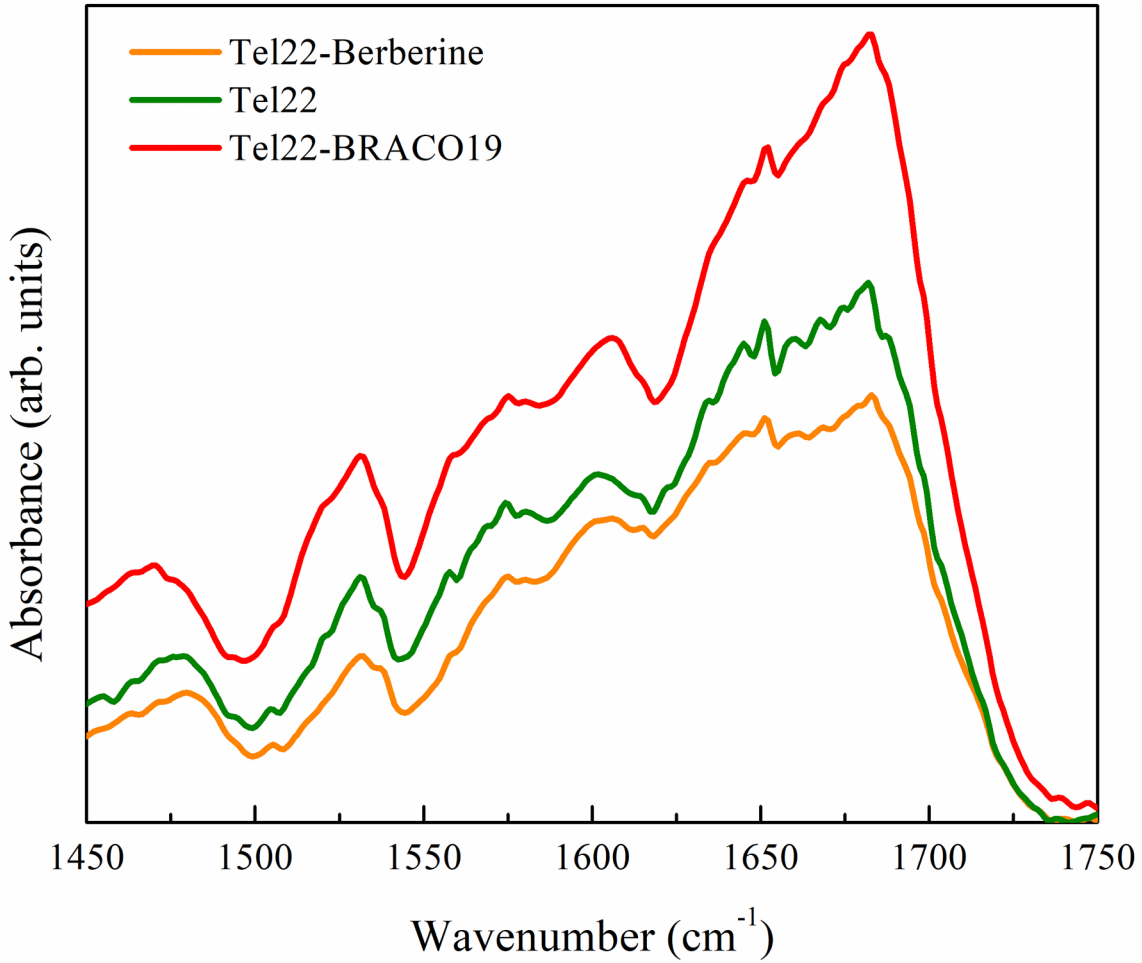


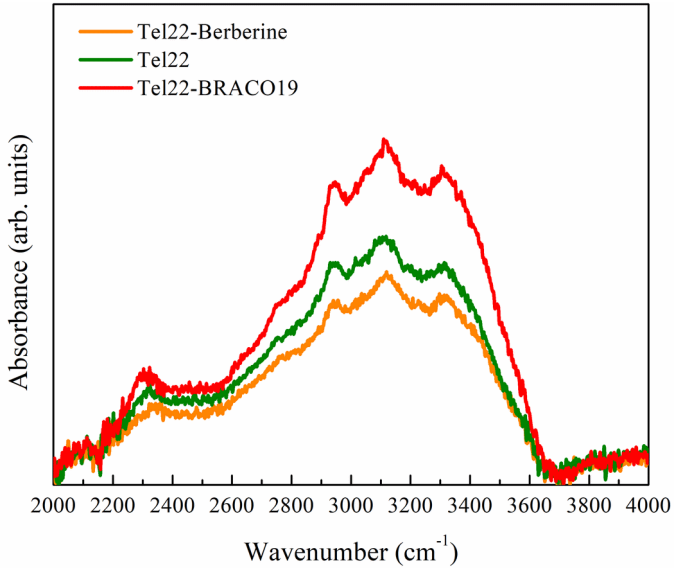


Hydration control (hopefully!)

Using D_2O moves water bands at lower frequencies. However, we see the same peaks at 1682cm^{-1}. But during the measurements there is D_2O-H_2O exchange.

FTIR spectra: powder state (H_2O)





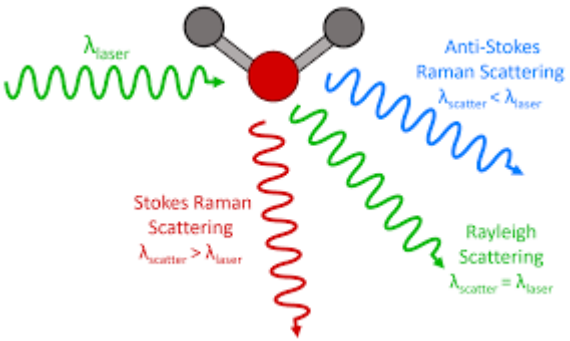
Hydration control (hopefully!)

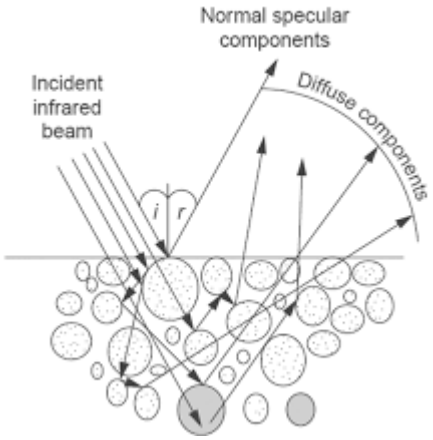
Measurements at lowest hydration (H_2O). The peak at 1682cm^{-1} is still present. Is it possible that at extremely high concentration only AP conformation is favoured?

Next steps

- Clarify the issues with structure determination;
- It is possible to **DRIFT** to measure completely dehydrated samples **OR**…
- Use **Raman spectroscopy**[4]!

- Investigate aggregation, either via IR (a marker needs to be found) or SAXS;
- Perform simulations in order to interpret SAXS results about aggregation.
- Use a different sequence with available PDB structure and more stable conformation to investigate dynamics.





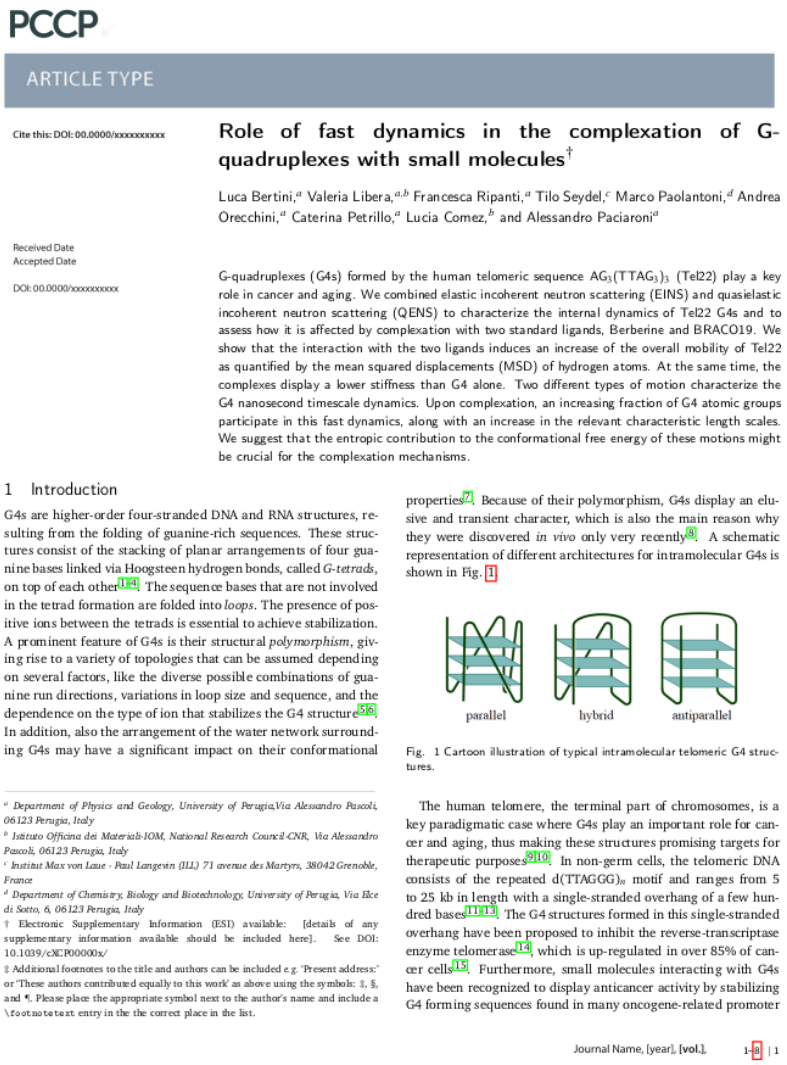
[4] Palacký, Jan, et al. "Does Raman spectroscopy recognize different G-quadruplex arrangements?." Journal of Raman Spectroscopy 51.2 (2020): 301-312.

II year activities and upcoming

- Corso avanzato di Python per uso scientifico (27-29 october 2021, INFN Perugia);
- Serie di Webinar sulla progettazione in Horizon Europe- Gestione in Horizon 2020:
  - Lump Sum Funding in Horizon Europe: How does it work? How to write a proposal? (19 May 2022);
  - Info session on Horizon Results Booster – steering research towards a strong societal impact (25 May 2022);
  - Horizon Europe Coordinators' Day on Grant Agreement Preparation (15 June 2022).
- G4thering conference (27 june- 1 july 2022) in Mariánské Lázně (CZ):
  - Poster Session (Role of fast dynamics in the complexation of G-quadruplexes with small molecules);

II year activities and upcoming

- Seminar “Brillouin Scattering as a probe for mechanical and morphological characterization of materials (19 May 2022, IOM-CNR, Perugia)
- “Biofotonica” course (Oct-Dec 2022, Dipartimento di Fisica e Geologia, Perugia);
- Paper: “Role of fast dynamics in the complexation of G-quadruplexes with small molecules” (Revison submitted);
- Paper: “The effect of ions and complexation with small molecules on G-quadruplex fast dynamics”; (in preparation, estimated submission on 15 December);
- UPCOMING: European Conference on Neutron Scattering (20-23 Marzo 2023) with (hopefully) a talk about the PCCP article results.



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Role of fast dynamics in the complexation of G-quadruplexes with small molecules†

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G-quadruplexes (G4s) formed by the human telomeric sequence $AG_3(TTAG_3)_3$ (Tel22) play a key role in cancer and aging. We combined elastic incoherent neutron scattering (EINS) and quasielastic incoherent neutron scattering (QENS) to characterize the internal dynamics of Tel22 G4s and to assess how it is affected by complexation with two standard ligands, Berberine and BRACO19. We show that the interaction with the two ligands induces an increase of the overall mobility of Tel22 as quantified by the mean squared displacements (MSD) of hydrogen atoms. At the same time, the complexes display a lower stiffness than G4 alone. Two different types of motion characterize the G4 nanosecond timescale dynamics. Upon complexation, an increasing fraction of G4 atomic groups participate in this fast dynamics, along with an increase in the relevant characteristic length scales. We suggest that the entropic contribution to the conformational free energy of these motions might be crucial for the complexation mechanisms.

1 Introduction

G4s are higher-order four-stranded DNA and RNA structures, resulting from the folding of guanine-rich sequences. These structures consist of the stacking of planar arrangements of four guanine bases linked via Hoogsteen hydrogen bonds, called *G-tetrads*, on top of each other[1,4]. The sequence bases that are not involved in the tetrad formation are folded into *loops*. The presence of positive ions between the tetrads is essential to achieve stabilization. A prominent feature of G4s is their structural *polymorphism*, giving rise to a variety of topologies that can be assumed depending on several factors, like the diverse possible combinations of guanine run directions, variations in loop size and sequence, and the dependence on the type of ion that stabilizes the G4 structure[5,6]. In addition, also the arrangement of the water network surrounding G4s may have a significant impact on their conformational properties[7]. Because of their polymorphism, G4s display an elusive and transient character, which is also the main reason why they were discovered *in vivo* only very recently[8]. A schematic representation of different architectures for intramolecular G4s is shown in Fig. 1.

parallel hybrid antiparallel

Fig. 1 Cartoon illustration of typical intramolecular telomeric G4 structures.

The human telomere, the terminal part of chromosomes, is a key paradigmatic case where G4s play an important role for cancer and aging, thus making these structures promising targets for therapeutic purposes[9,10]. In non-germ cells, the telomeric DNA consists of the repeated $d(TTAGGG)_n$ motif and ranges from 5 to 25 kb in length with a single-stranded overhang of a few hundred bases[11,13]. The G4 structures formed in this single-stranded overhang have been proposed to inhibit the reverse-transcriptase enzyme telomerase[14], which is up-regulated in over 85% of cancer cells[15]. Furthermore, small molecules interacting with G4s have been recognized to display anticancer activity by stabilizing G4 forming sequences found in many oncogene-related promoter

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‡ Additional footnotes to the title and authors can be included e.g. 'Present address:' or 'These authors contributed equally to this work' as above using the symbols: ‡, §, and ¶. Please place the appropriate symbol next to the author's name and include a \footnotetext entry in the the correct place in the list.

Thank you for the attention!