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UNIVERSITÀ DEGLI STUDI  
DI PERUGIA

# Interazione di G-quadruplex DNA e molecole fotosensibili

**LUCIA COMEZ**

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c/o Dipartimento di Fisica e Geologia, Università di Perugia

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Consiglio Nazionale delle Ricerche



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## Settori PTSR

- Fisica delle biomolecole e dei materiali avanzati per terapia

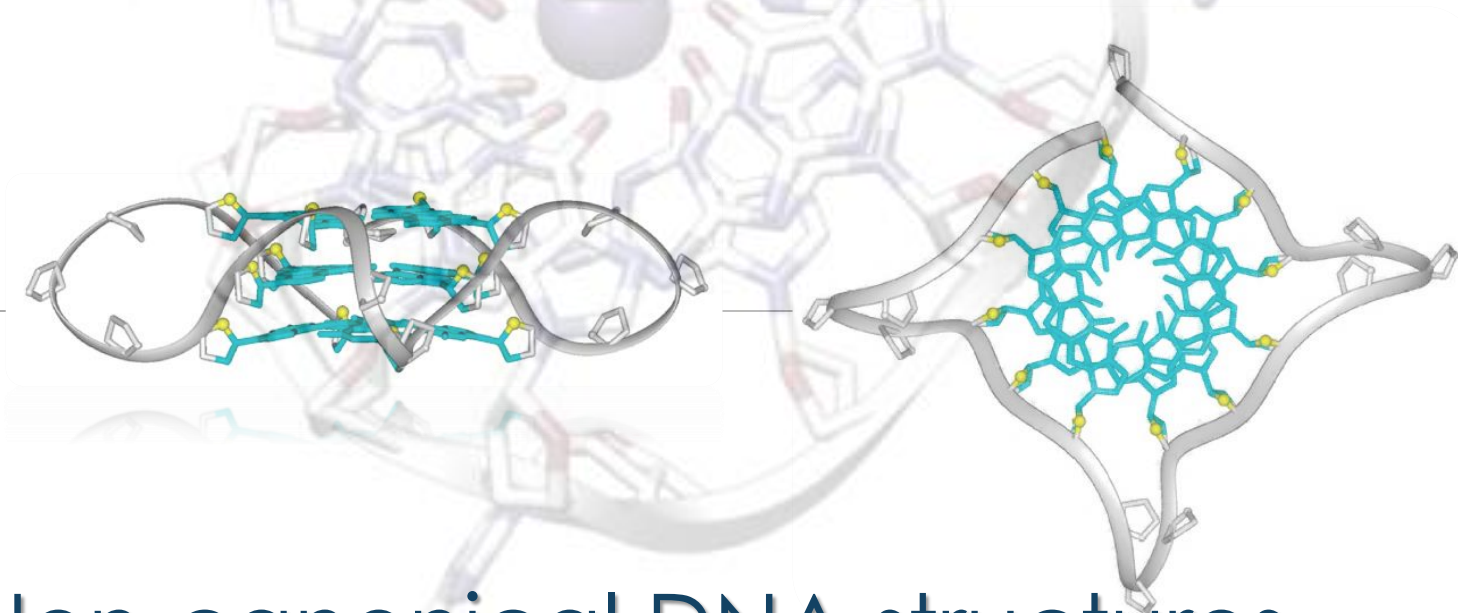
**Azioni collaborative di Ateneo coinvolte:** Azione1 (Salute), Azione1-1 (WP: Ciclo della vita: processi naturali e patologici), Azione1-3 (WP: Sviluppo di prodotti e tecniche innovative diagnostiche e terapeutiche)

- Nanoscienze, Materia Soffice

(AMBITI DI RICERCA: Nanoscienze, Fisica della Materia Soffice)

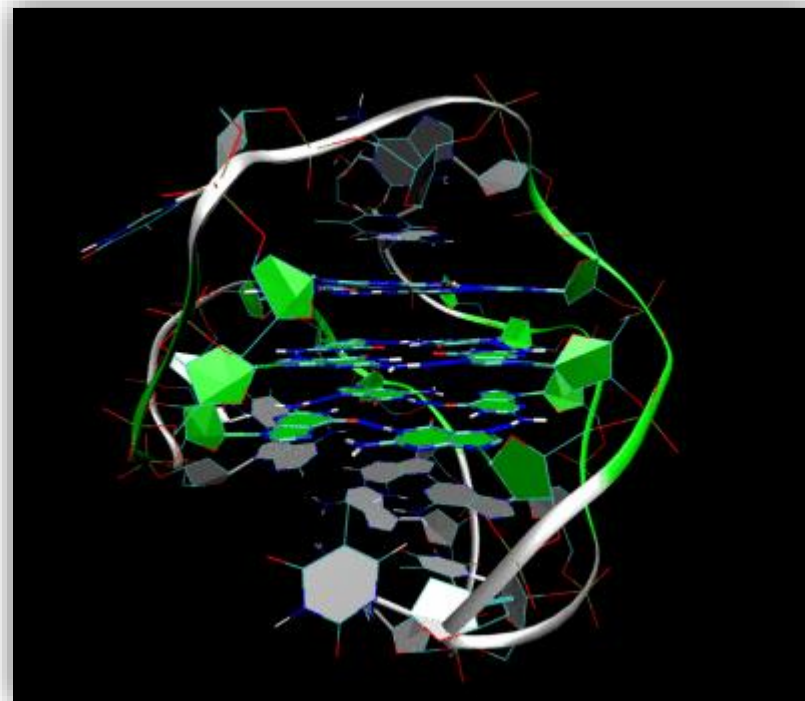
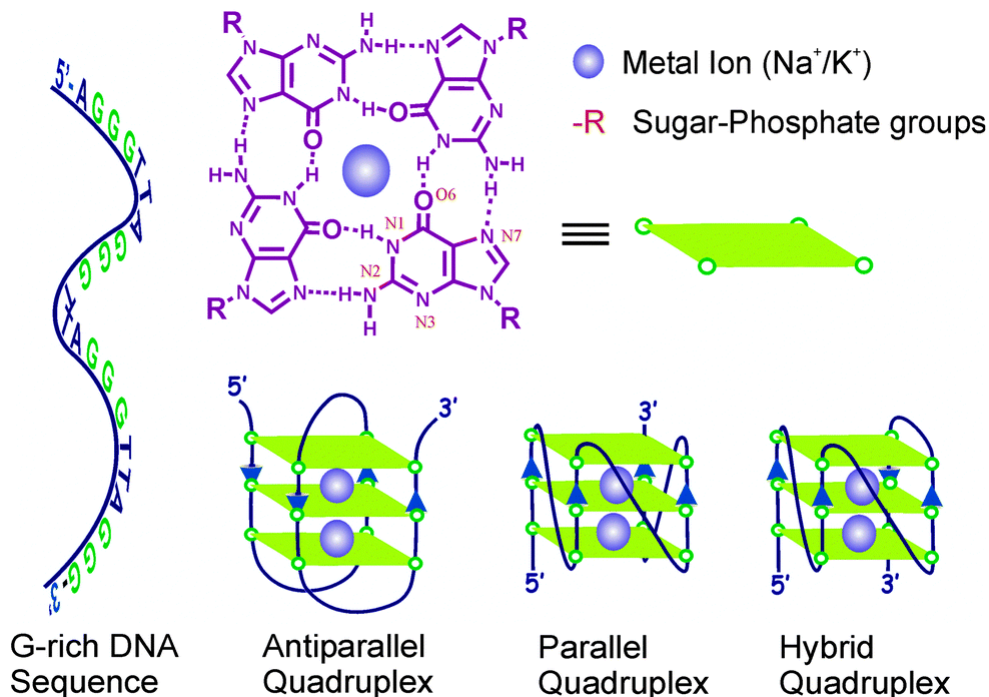
SETTORI ERC: **PE3**, PE4, **PE5**

# The nano-world of G-quadruplexes

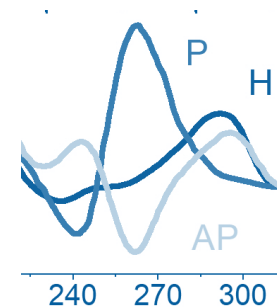


## Non-canonical DNA structures

# G-quadruplex structures: nanoDNA



- Oligonucleotides, guanine-rich sequences (tens of nucleotides).
- G-quadruplexes are polymorphic, versatile and controllable “bricks”.
- Their stability depends on temperature, pH, ligands.

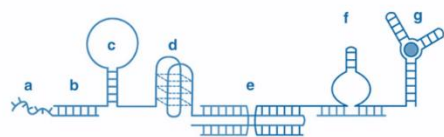


# G-quadruplex structures: nanoDNA

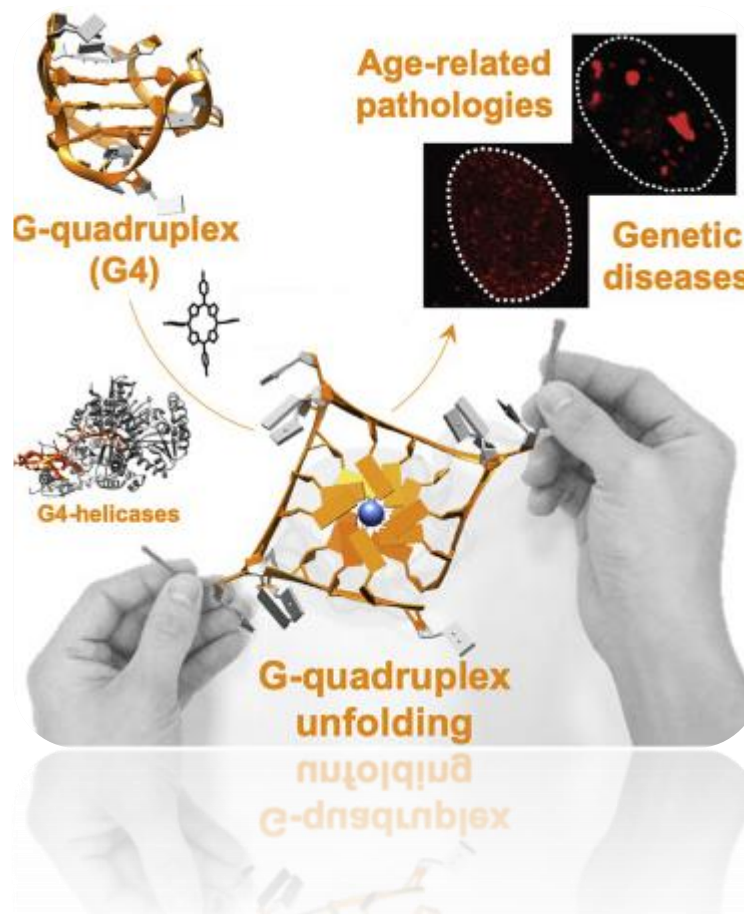
## Relevance and applications

- Nanotechnology
- Biomedicine
- Photodynamic therapy

Functional DNA nanostructures for biosensing, nanoplasmonics, nanorobotics



DNA-nanowires



# G-quadruplexes are considered as an attractive target for cancer therapy

nature  
chemistry

ARTICLES

PUBLISHED ONLINE: 20 JANUARY 2013 | DOI: 10.1038/NCHEM.1548

## Quantitative visualization of DNA G-quadruplex structures in human cells

Giulia Biffi<sup>1</sup>, David Tannahill<sup>1</sup>, John McCafferty<sup>2</sup> and Shankar Balasubramanian<sup>1,3\*</sup>

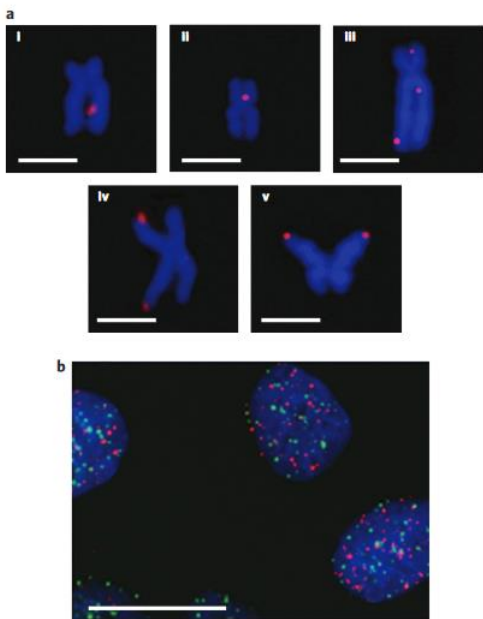
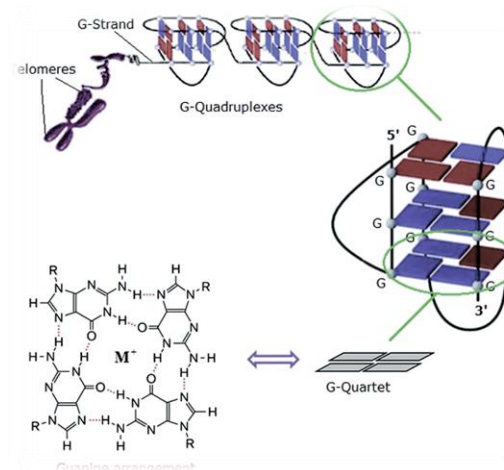


Figure 3 | Localization of G-quadruplex structures in chromosomes.



Value-added  
G4 DNA ligands



Conformational switches

Stimuli-responsive systems

Targeted modification

Binding...

...and beyond!

# G-quadruplexes are considered as an attractive target for cancer therapy

SCIENTIFIC  
REPORTS

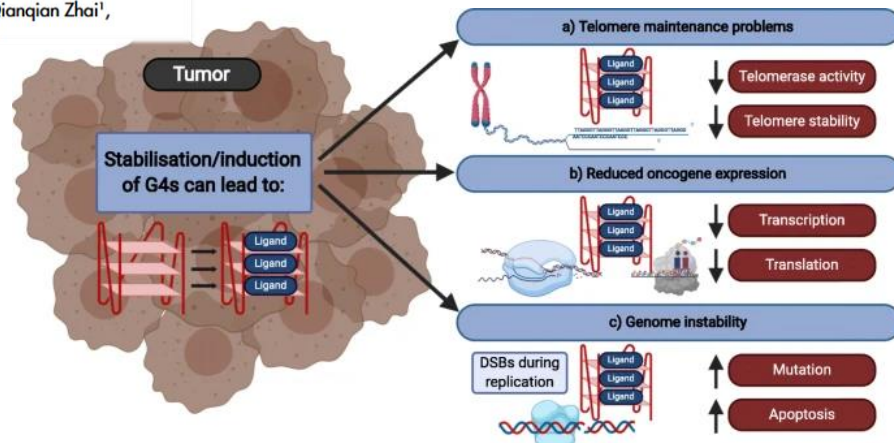
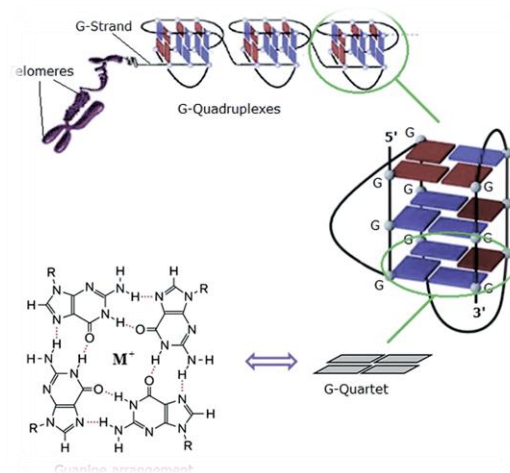


## Existence of G-quadruplex structures in promoter region of oncogenes confirmed by G-quadruplex DNA cross-linking strategy

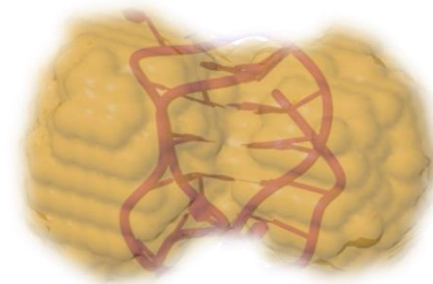
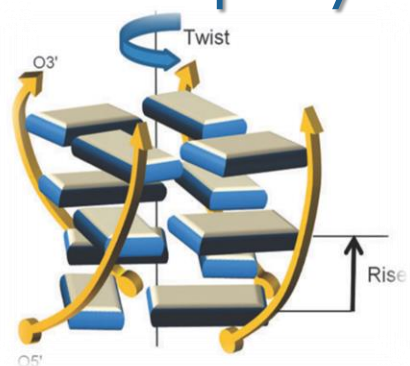
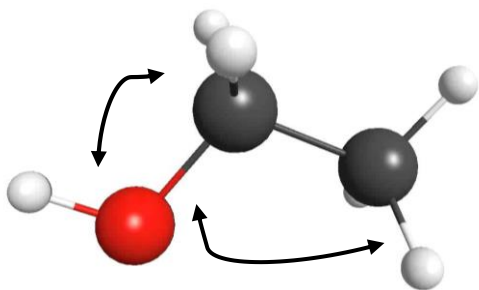
SUBJECT AREAS:  
DNA  
CHEMICAL GENETICS  
TARGETED RESEQUENCING  
NUCLEIC ACIDS

Libo Yuan<sup>1\*</sup>, Tian Tian<sup>1\*</sup>, Yuqi Chen<sup>1</sup>, Shengyong Yan<sup>1</sup>, Xiwen Xing<sup>1</sup>, Zhengan Zhang<sup>1</sup>, Qianqian Zhai<sup>1</sup>, Liang Xu<sup>1</sup>, Shaoru Wang<sup>1</sup>, Xiaocheng Weng<sup>1</sup>, Bifeng Yuan<sup>1</sup>, Yuqi Feng<sup>1</sup> & Xiang Zhou<sup>1,2</sup>

DNA G-quadruplexes have been found in the proximal location of promoters, which are mostly TATA-less, in a number of human genes involved in growth and proliferation, as potential transcriptional regulators.



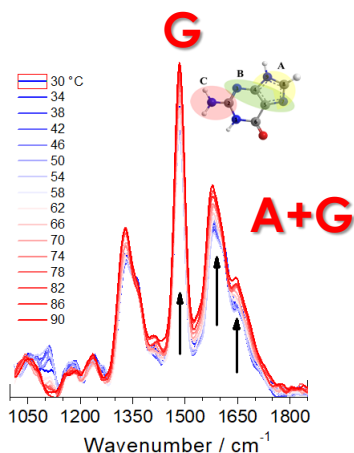
# Multi-technique biophysical method



## UV-Resonant Spectroscopy

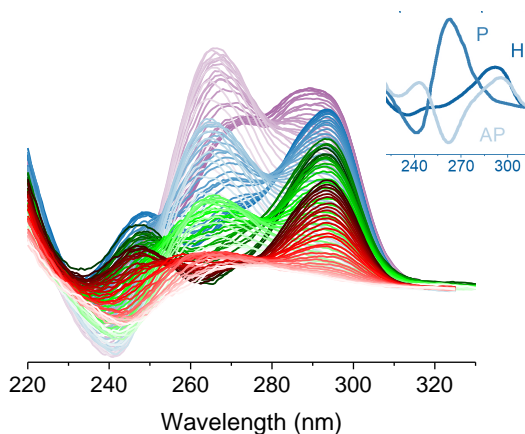
- Vibrations
- Stacking

## Raman



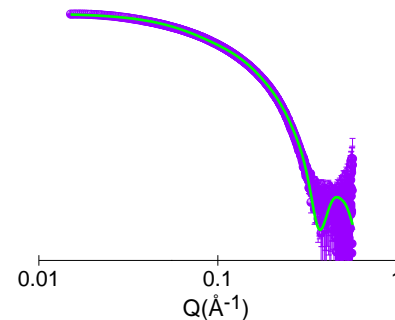
## Circular Dichroism

- Conformation
- Secondary structure



## Small angle techniques

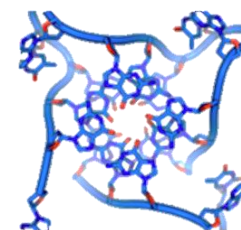
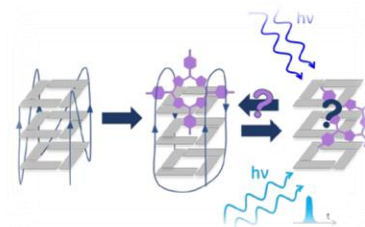
- Size
- Shape
- Low-res models





# Multi-technique biophysical method

- ❑ Ligand-induced stabilization of G4 sequences
- ❑ Photoinduced conformational changes in DNA g-quadruplex complexed with photosensitive ligands
- ❑ Self-aggregation properties of G-quadruplexes



# Ligand-Induced stabilization of G4 sequences

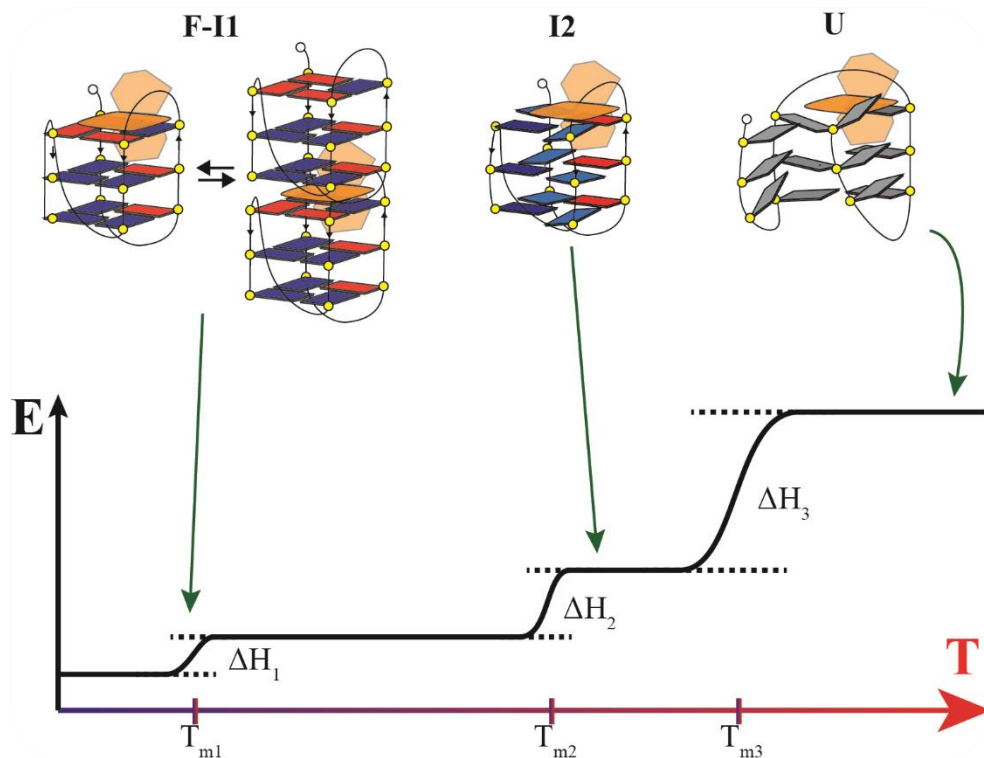
## Structure of human telomere G-quadruplex in the presence of a model drug along the thermal unfolding pathway

Federico Bianchi, Lucia Comez ✉, Ralf Biehl, Francesco D'Amico, Alessandro Gessini, Marialucía Longo, Claudio Masciovecchio, Caterina Petrillo, Aurel Radulescu, Barbara Rossi ... Show more

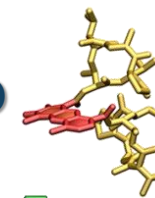
*Nucleic Acids Research*, Volume 46, Issue 22, 14 December 2018, Pages 11927–11938, <https://doi.org/10.1093/nar/gky1092>

OXFORD  
ACADEMIC

Nucleic Acids Research



ACTINOMYCIN D



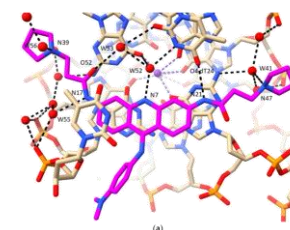
BERBERINE



PALMATINE



BRACO-19



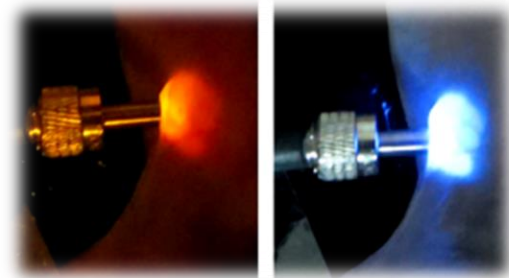
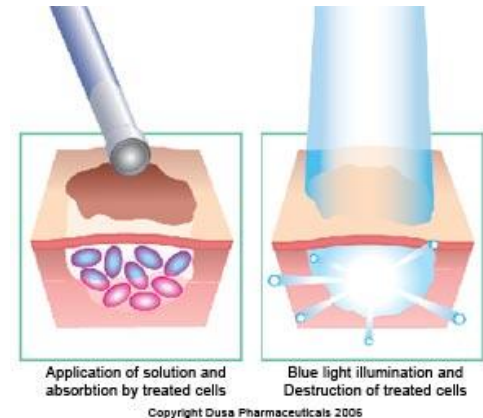
# □ G-quadruplex & Photosensitive ligands

**LIGHT** has proven to be an attractive means for the control of DNA-based systems as it is non-invasive and it can be delivered with high spatio-temporal precision.

**PHOTO-PHARMACOLOGICAL** approaches are attractive, since they allow more localised control of the therapeutic activity, reducing or eliminating off-target effects

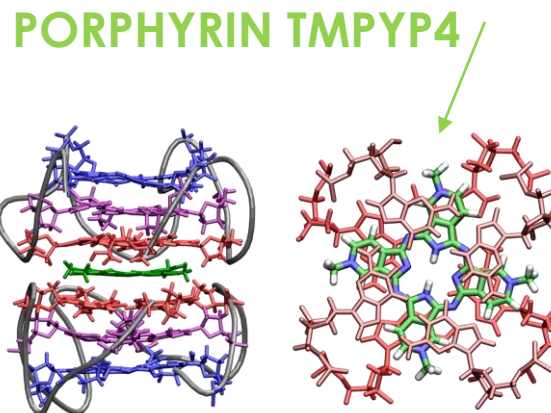
## **LIGHT-BASED MEDICAL APPLICATIONS**

have been already tested (e.g. photodynamic therapy)

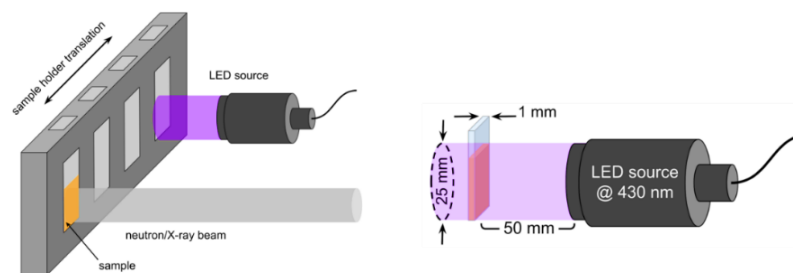


# □ G-quadruplex & Photosensitive ligands

- ▶ Porphyrins are the most widely used ligands for G4, due to both their **shape** and **size**, ~9 Å side, comparable to that of an individual G tetrad.
- ▶ They have **high affinity** for several quadruplexes.
- ▶ When irradiated, porphyrins generate singlet oxygen.
- ▶ In the interaction with G4s, singlets preferentially oxidize guanines at the exterior faces, triggering **structural rearrangements**.

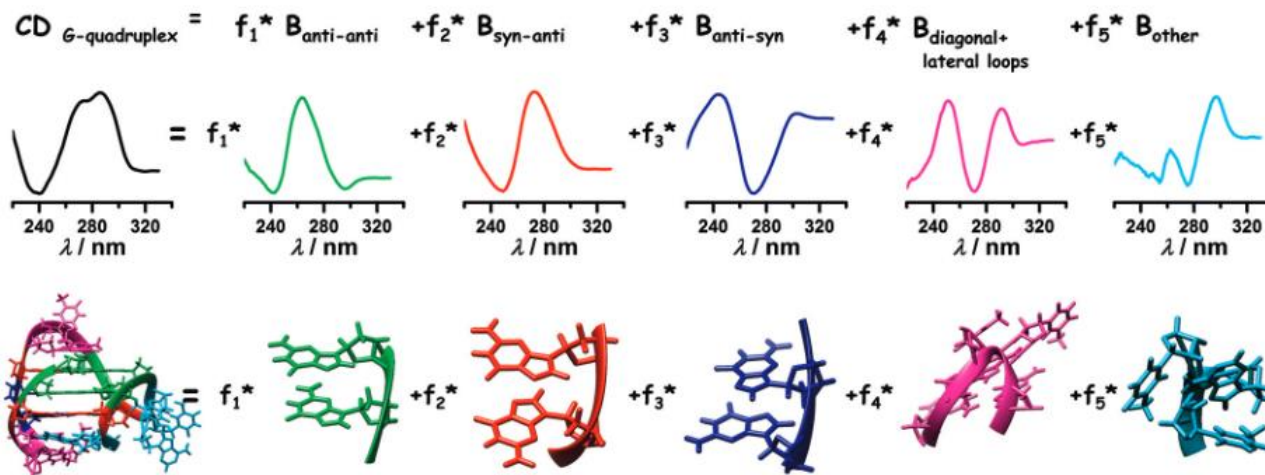


## CD & SAXS PROBES



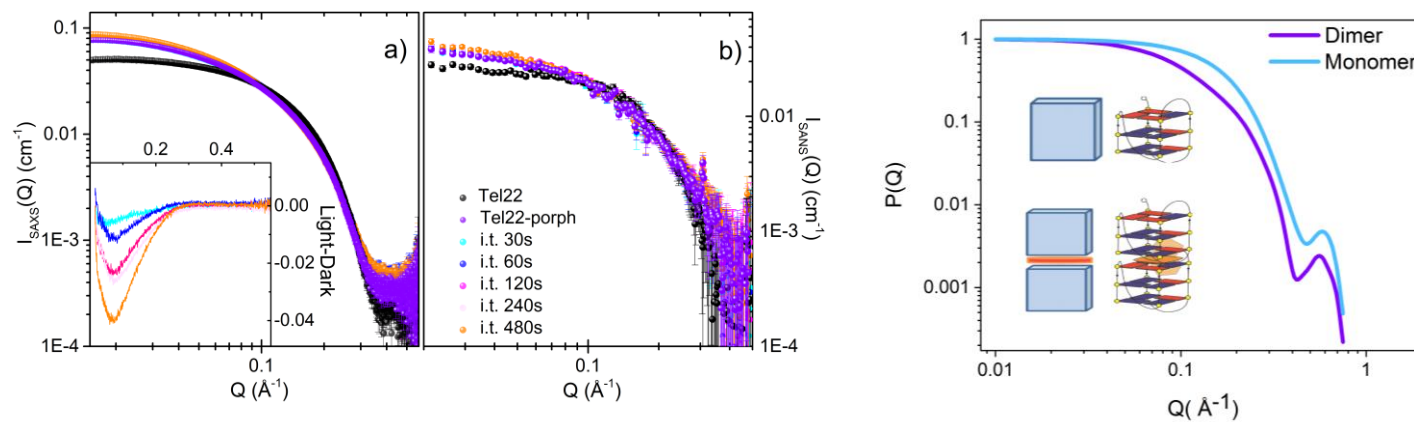
# CD: secondary structure

Specific  
SVD&PCA  
TOOLS for G4s

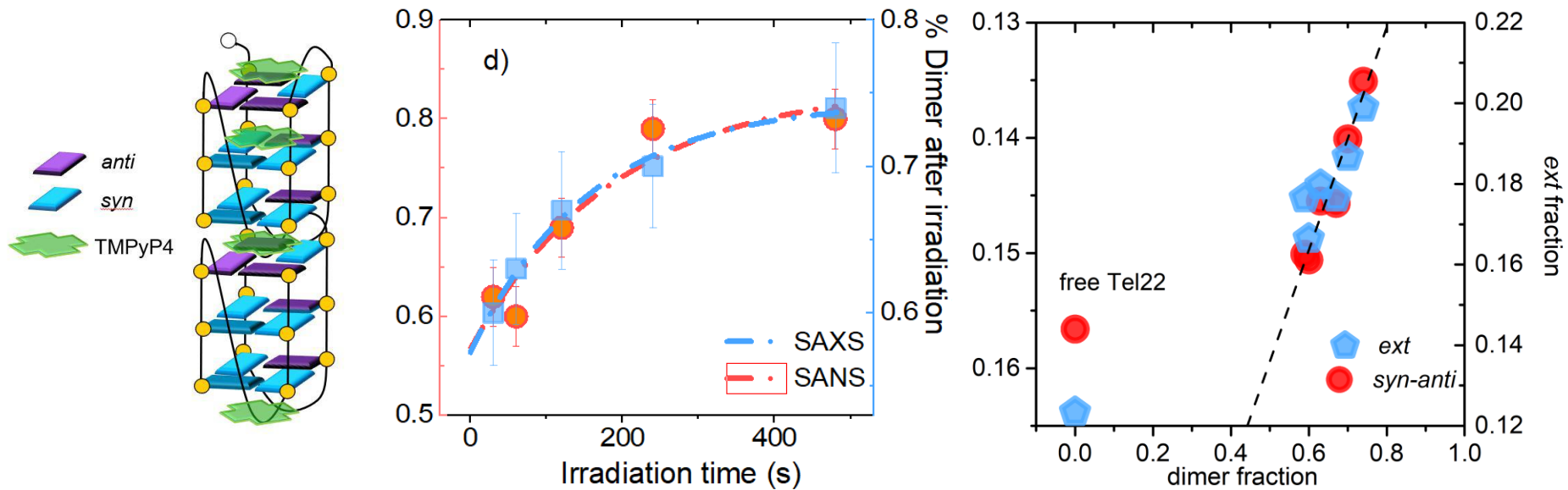


# SANS & SAXS: quaternary structure

Size &  
Oligomer  
distributions



# G4 modelling & Secondary vs Quaternary structure correlation



We found a correlation between the **secondary** and the **quaternary** structure, which opens the possibility of **controlling** and **predicting** the fraction of dimers produced during the whole process.

The combined use of information at different structure levels will represent a diagnostic strategy to implement the **photocontrol of G4 dimerization and stabilization**, enabling us to tune the telomerase activity in cancer cells.

*Porphyrin Binding and Irradiation Promote G-Quadruplex DNA Dimeric Structure*; V. Libera et al, J. Phys. Chem. Lett. 2021

# Photoresponsive Control of G-quadruplex DNA Systems

**Ligand: dithienylethene derivative (DTE),** which is a **photosensitive and photoswitchable** molecule

- two stable isomeric conformations (1o & 1c)
- from 1o, after irradiation with LED source @450 nm, 1c is obtained
- from 1c, after irradiation with LED source @635 nm, 1o is perfectly restored
- The process is completely reversible (reversibility tested to be valid until 7 times)
- the two configuration show a different absorbance profile → we have **spectral markers!**



## COMMUNICATION

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View Journal | View Issue



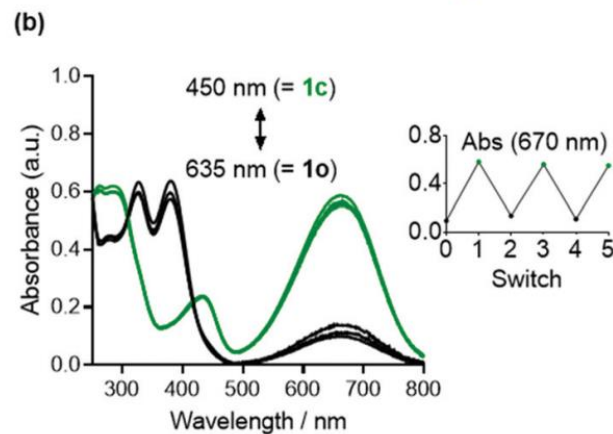
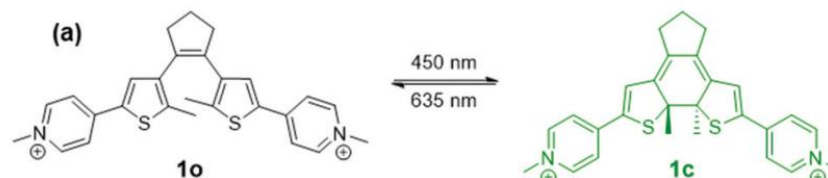
Cite this: *Chem. Commun.*, 2020, 56, 5186

Received 28th February 2020,  
Accepted 18th March 2020

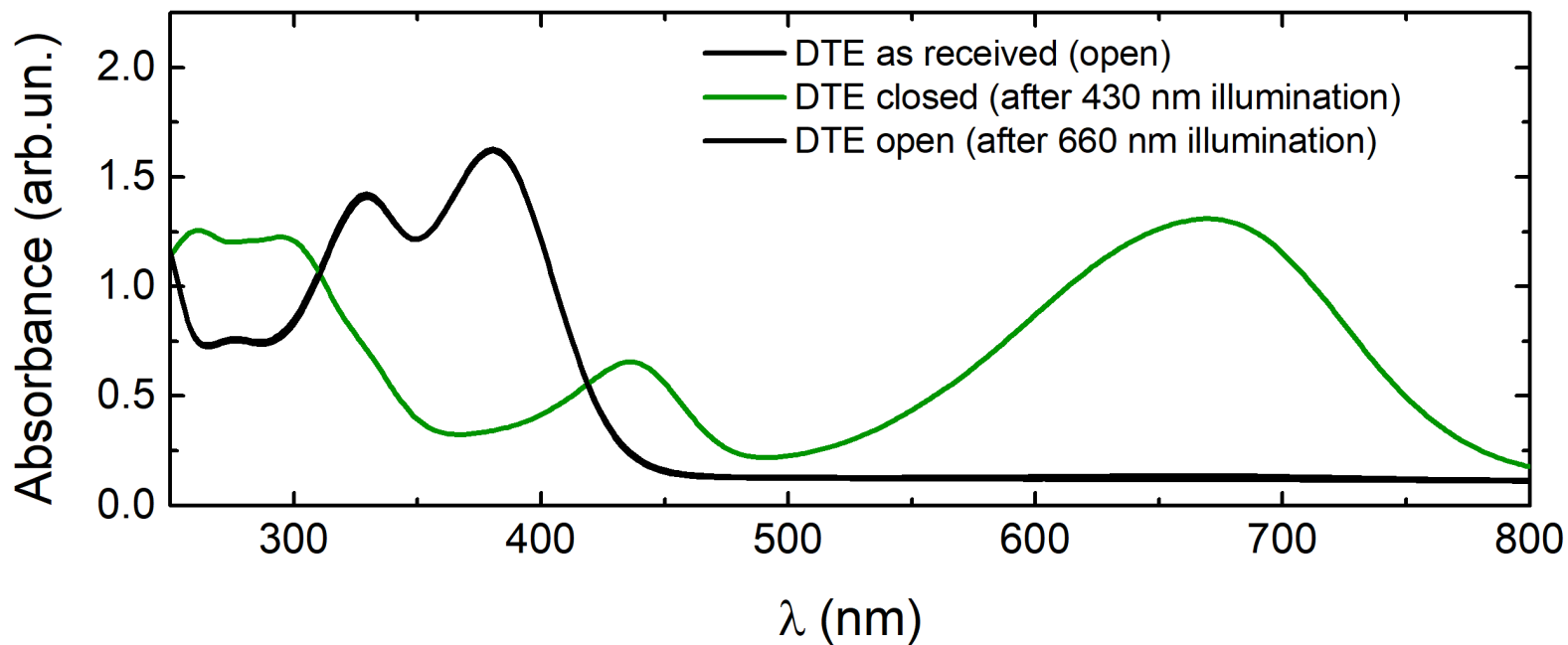
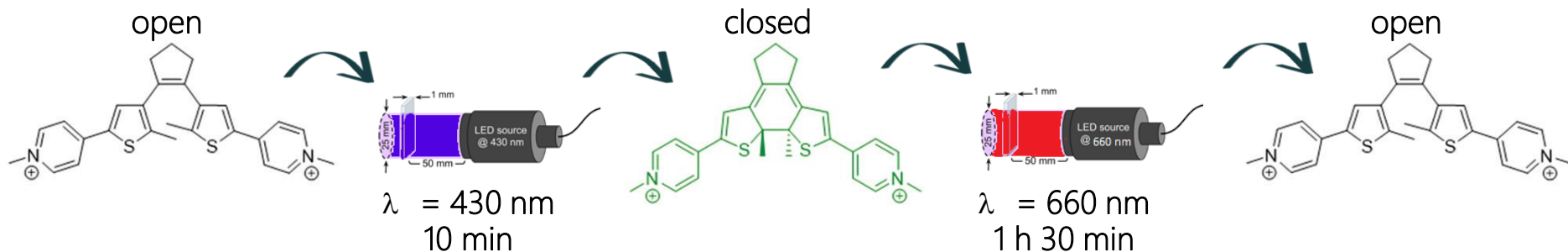
DOI: 10.1039/d0cc01581d

## Visible-light photoswitching of ligand binding mode suggests G-quadruplex DNA as a target for photopharmacology†

Michael P. O'Hagan,<sup>a</sup> Javier Ramos-Soriano,<sup>b</sup> Susanta Haldar,<sup>ab</sup> Sadiyah Sheikh,<sup>a</sup> Juan C. Morales,<sup>c</sup> Adrian J. Mulholland<sup>abd</sup> and M. Carmen Galan<sup>bc\*</sup>



# Photoswitching of the ligand

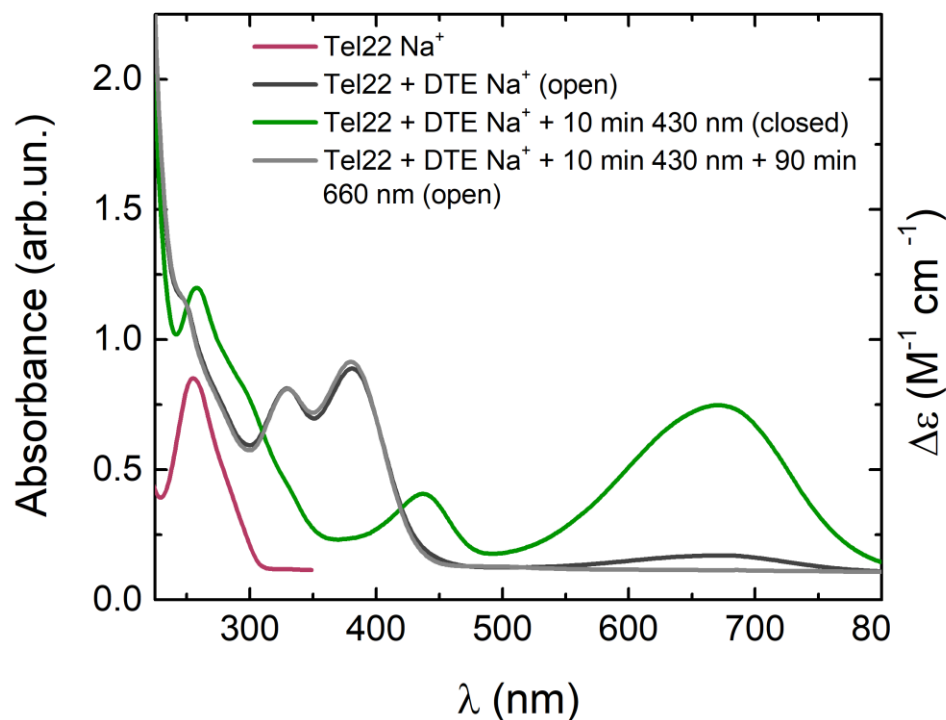




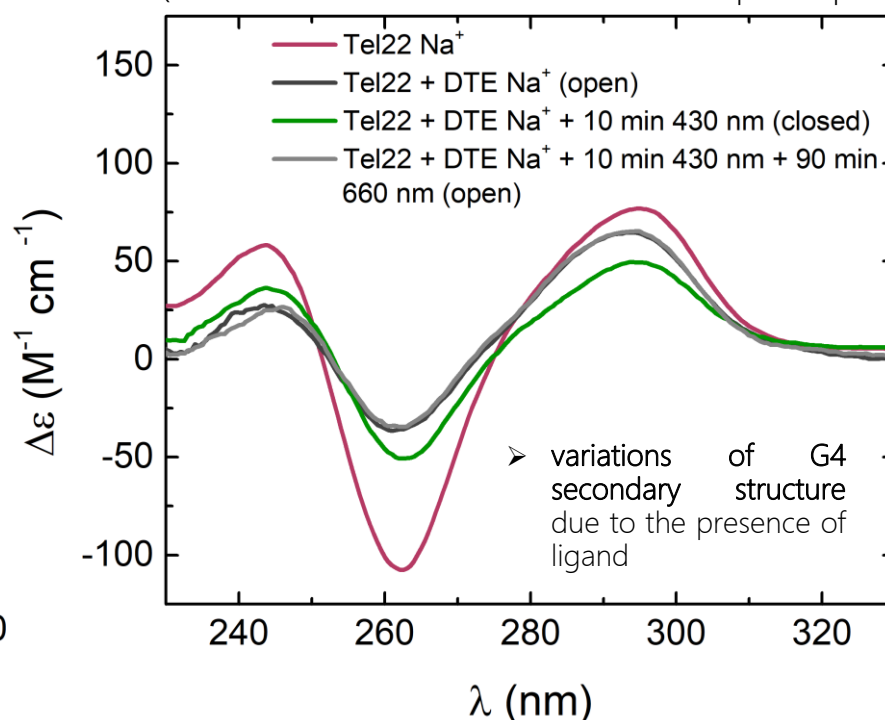
# Photoswitching of the complex: G4+ligand

- sample preparation: Tel22 30  $\mu\text{M}$  + DTE 60  $\mu\text{M}$  (1:2 molar ratio)
- same illumination conditions: 430 nm laser for 10 minutes, 660 nm laser for 90 minutes

UV-Visible absorption spectroscopy

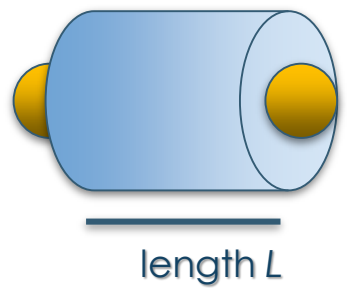
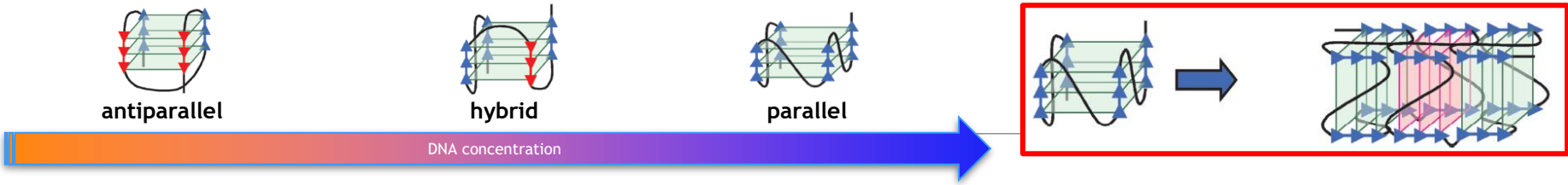


Circular Dichroism  
(normalized to concentration and optical path)



Experiments on different G4s and under different conditions  
BUG @ ESRF SAXS in ten days (P. Pernot, G. Schirò)

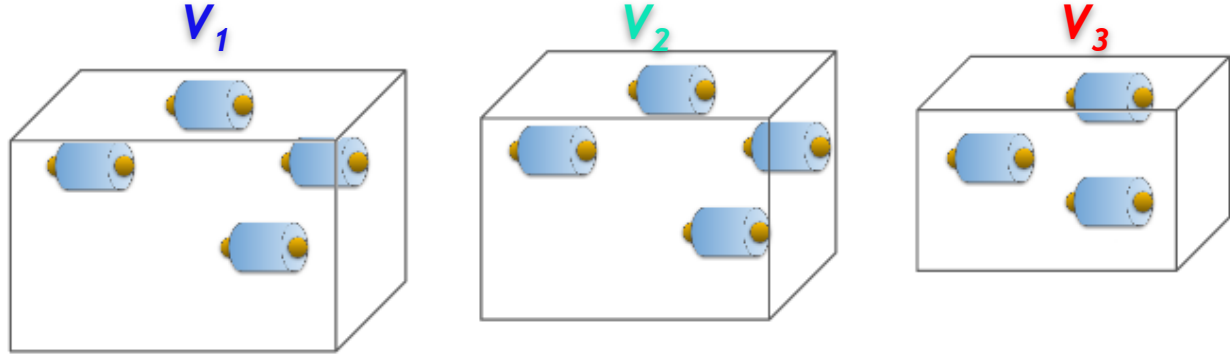
# Self-assembled G-quadruplex nanostructures: CD, Simulations & SAXS techniques



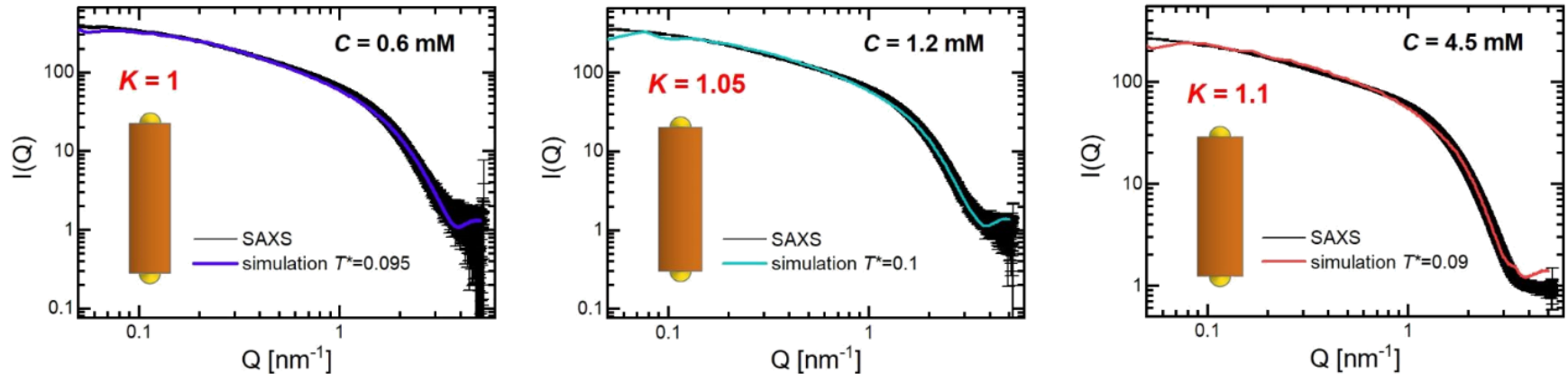
diameter  $D$       G-quadruplex: **patchy hard cylinder**

already utilized for modeling the **reversible self-assembly aggregation** of DNA (see, for instance, C. De Michele, T. Bellini and F. Sciortino, *Macromolecules* 2012, 45, 1090-1106)

**Monte Carlo simulations** in the **NVT** ensemble: at fixed  $N$



# Self-assembled G-quadruplex nanostructures: CD, Simulations & SAXS techniques



**Accordance** between simulated and measured structure factors

The variation in the cylinder shape is in agreement with an **increase in hybrid/parallel G4 population**

**We can derive:**

- average length of the aggregates
- polydispersity
- energy of aggregation

# People

V. Libera, L. Bertini, B.P. Rosi, F. Ripanti, C. Fasolato, A. Orecchini, S. Corezzi, F. Sacchetti, C. Petrillo, A. Paciaroni Dipartimento di fisica e geologia



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A. Macchiarulo Dipartimento di scienze farmaceutiche

P. Postorino, C. De Michele, Roma



SAPIENZA  
UNIVERSITÀ DI ROMA

F. Spinozzi, ANCONA



C. Sissi, UNIPD



G. Schirò, CNRS, IBS



M. Webba da Silva



C. Galan's group



V. Rathi



AMITY INSTITUTE OF MOLECULAR MEDICINE  
& STEM CELL RESEARCH



**CERIC**

Central European  
Research Infrastructure  
Consortium



**Large Scale Facility: ESRF, ILL,  
SOLEIL, MLZ, ISIS, ELETTRA**

# Funded Projects

- **Interaction of cationic porphyrins and G-quadruplex DNA.** Collaboration with IBS-CNRS France (Short Term Mobility action)
- **Photoinduced conformational changes in DNA G-quadruplex complexed with photosensitive ligands** PhD Project CNR-UNIPG-NBI
- **Light-switchable G-quadruplex Conformation by interaction with photosensitive ligands LOGIC** Fondi Ricerca di Base Ateneo UNIPG

## Submitted Projects

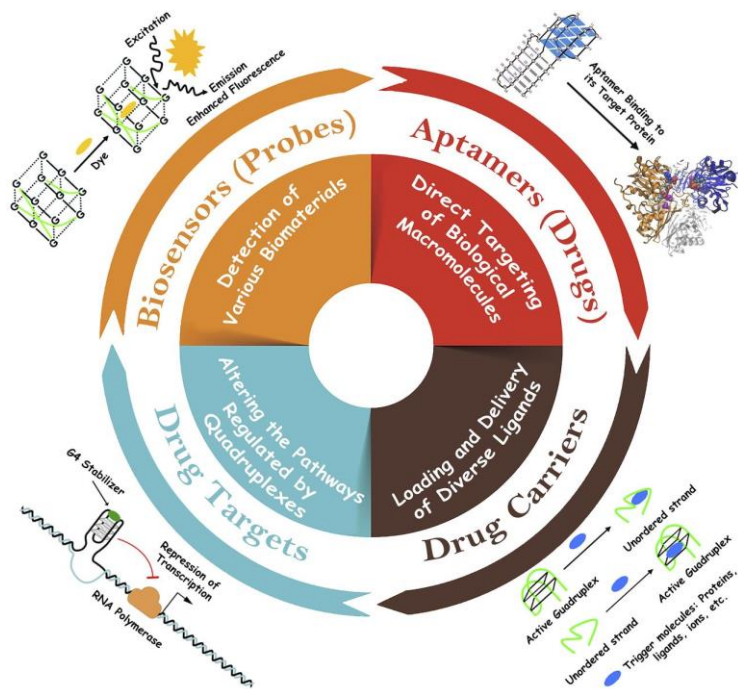
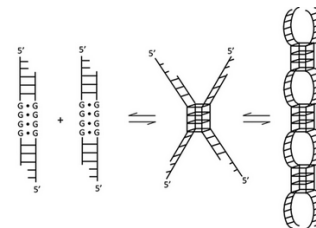
### **INDO-ITALIAN EXECUTIVE PROGRAMME OF COOPERATION IN SCIENTIFIC & TECHNOLOGICAL COOPERATION SIGNIFICANT BILATERAL PROJECT**

**INSIde the complexation of highly selective ligands with BCL-2 G-quadruplexes for anticancer tHerapeutiCS (INSIGHTS)**

**Partner Vinit Kumar** Amity Institute of Molecular Medicine and Stem Cell Research, Univ. Noida

# "Foresights"

- Unlocking G-quadruplexes as Antiviral Targets
- DNA Quadruple Helices in Nanotechnology
- G-quadruplex DNA for construction of biosensors



- Bioinformatics studies led to the conclusion that noncanonical nucleic acid secondary structures, such as G-quadruplexes, are cogent elements in the pathogenicity and viral proliferation of both RNA and DNA viruses.
- Quadruplexes in nanotechnologies, in which G4 and i-motif structures are incorporated into a variety of objects (wires, origamis, gels, micelles, etc.) and used for a diversity of applications, such as biosensing or therapeutics, that take advantage of the unique properties of these fascinating structures.
- Much of G4 DNA's relevance in biosensors is related to its ability to bind specific metal ions, dyes and porphyrins.

# "Foresights"

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Ampliare collaborazioni locali e non, creare piattaforme per selezione e test di farmaci, esperti in biosensoristica, creazione di nanoscaffolds stabili, nuova interfaccia con gruppi di medicina

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- Collaborazioni italiane (UNIPD, Roma Sapienza, ANCONA) e/o estere per la scrittura di progetti → PRIN, AIRC
- Collaborazioni internazionali → Azioni bilaterali (Italy-Japan; Italy-UK; Italy-France; Italy-India)
- Apertura a collaborazioni intra-ateneo → Progetti CRP per azioni sul territorio & ENTI
- C-LABS, INFRASTRUTTURE
- EU-PLATFORMS
- PNRR

