Fisica del DNA e delle BIOMOLECOLE

ALESSANDRO PACIARONI

LUCIA COMEZ

ALESSANDRA LUCHINI

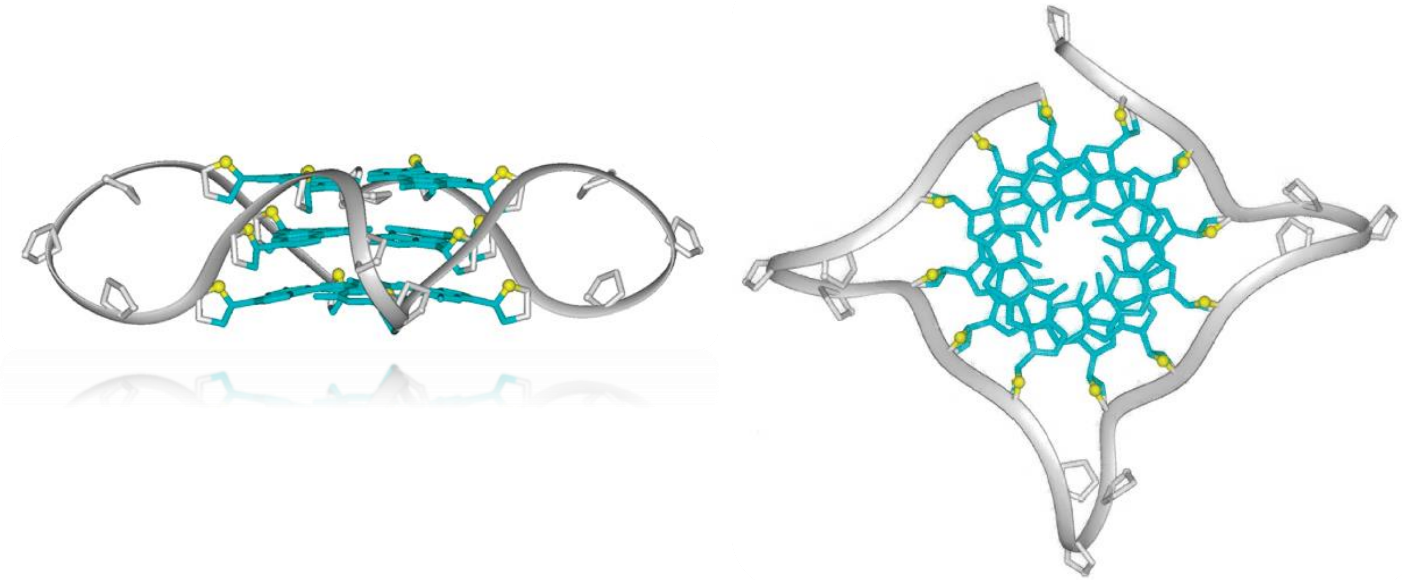
ANDREA ORECCHINI

ISTITUTO OFFICINA DEI MATERIALI

A.D. 1308 unipg UNIVERSITÀ DEGLI STUDI DI PERUGIA

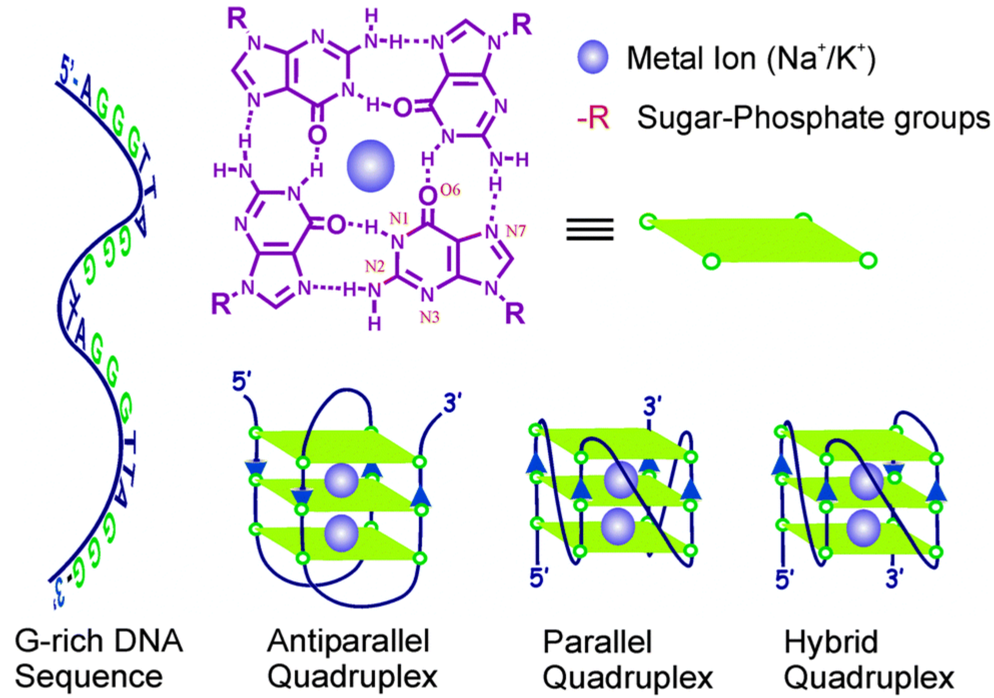
M. Trapella, R. Malaspina, B. Romano, S. Stefani, L. Bertini, I. Mosca, V. Libera, C. Petrillo

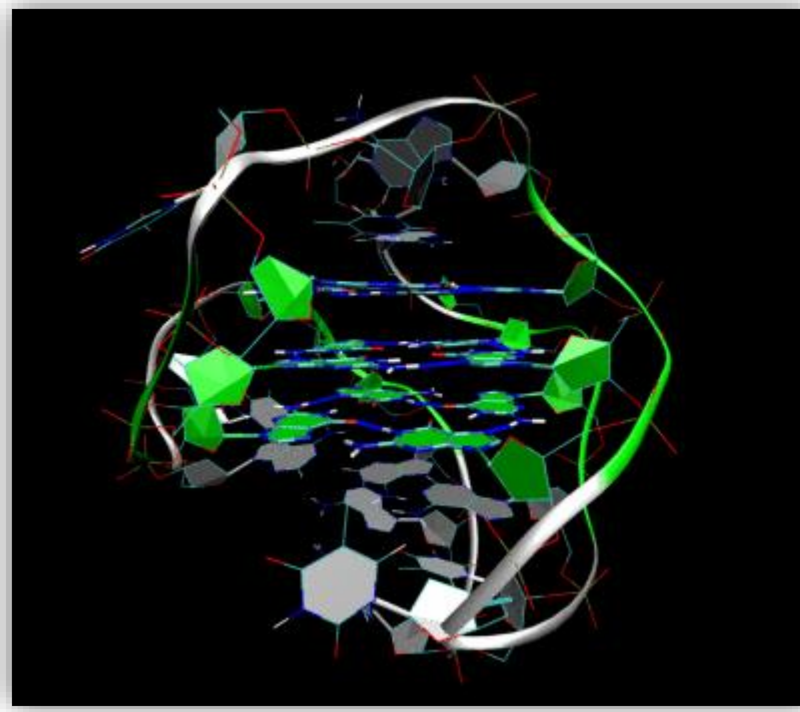
The nano-world of G-quadruplexes



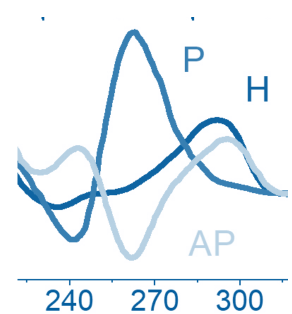
Non-canonical DNA structures

G-quadruplex structures: nanoDNA

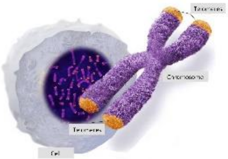




- Oligonucleotides, guanine-rich sequences (tens of nucleotides).
- G-quadruplexes are polymorphic, versatile and controllable "bricks".
- Their stability depends on temperature, pH, ligands.



G-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[1], David Tannahill[1], John McCafferty[2] and Shankar Balasubramanian[1,3]*

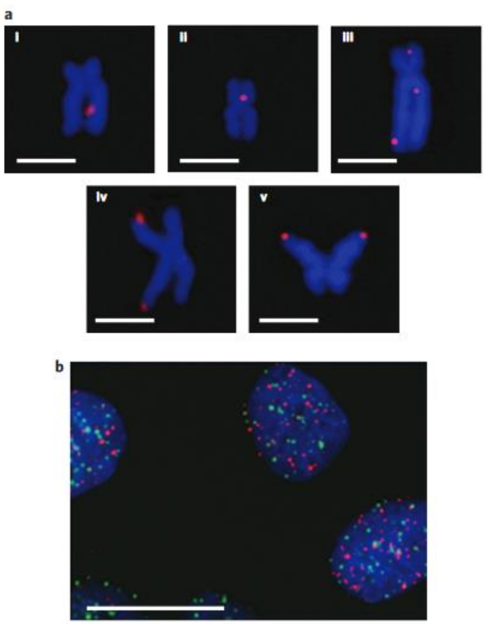
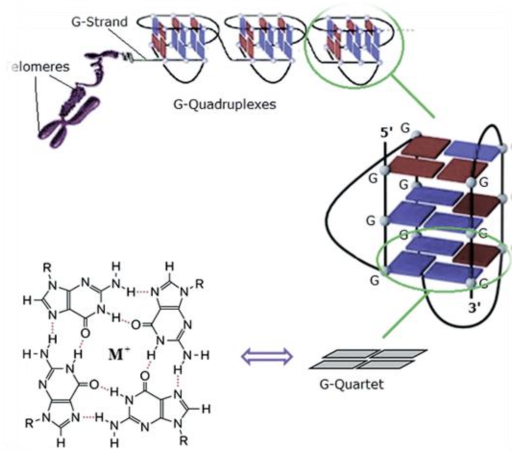
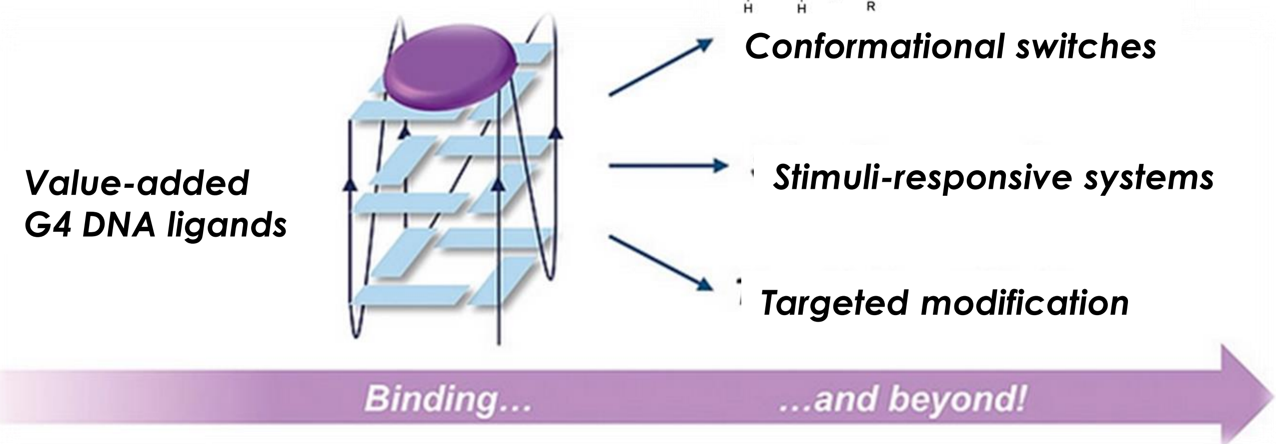


Figure 3 | Localization of G-quadruplex structures in chromosomes.

Human telomeric DNA consists of tandem repeats of the sequence d(TTAGGG)





A Multi-technique biophysical method

G-quadruplex characterization

In-house techniques:

- UV-Vis absorption (ABS)
- Circular Dichroism (CD)

Large scale facilities:

- Small Angle Scattering (SAS)
- UV-Resonant Raman (UVRR)

Stat/Comput Methods

- Singular Value Decomposition (SVD)
- Extremely coarse-grained simulations (ECGS)

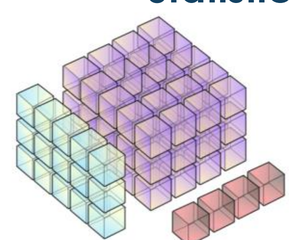
In-house techniques

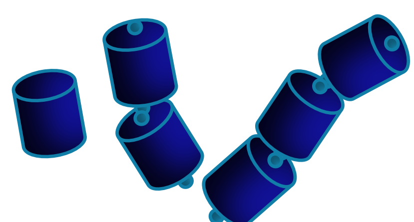
Large scale facilities

ELETTRA Sincrotrone, *Trieste, Italy*

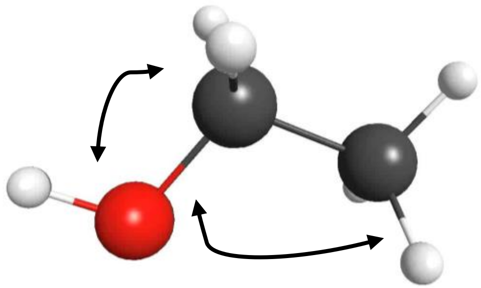
SAXSPACE *Juelich*, **MLZ** *Munich, Germany*
ESRF, ILL, *Grenoble, France*
DIAMOND, ISIS, *UK*

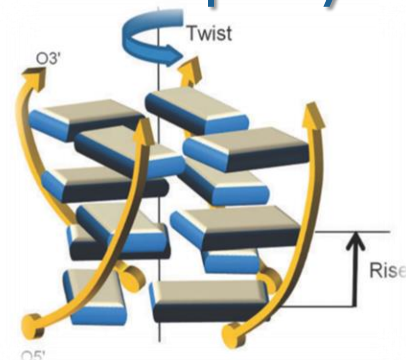
Statistical & Computational Methods

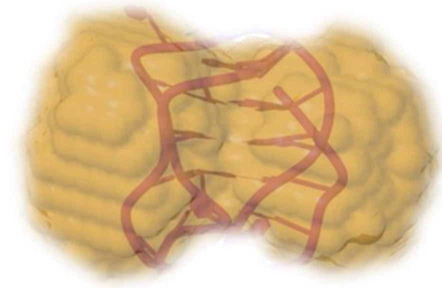




Multi-technique biophysical method







UV-Resonant Raman Spectroscopy

-Vibrations

-Stacking

Circular Dichroism

-Conformation

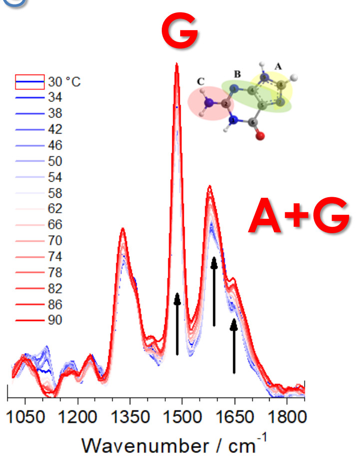
-Secondary structure

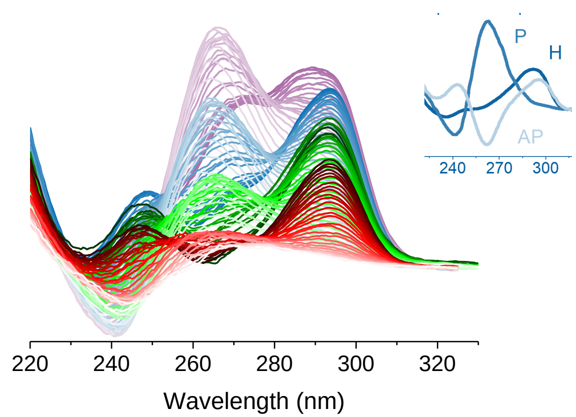
Small angle techniques

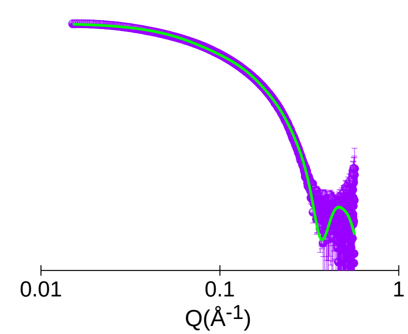
-Size

-Shape

-Low-res models







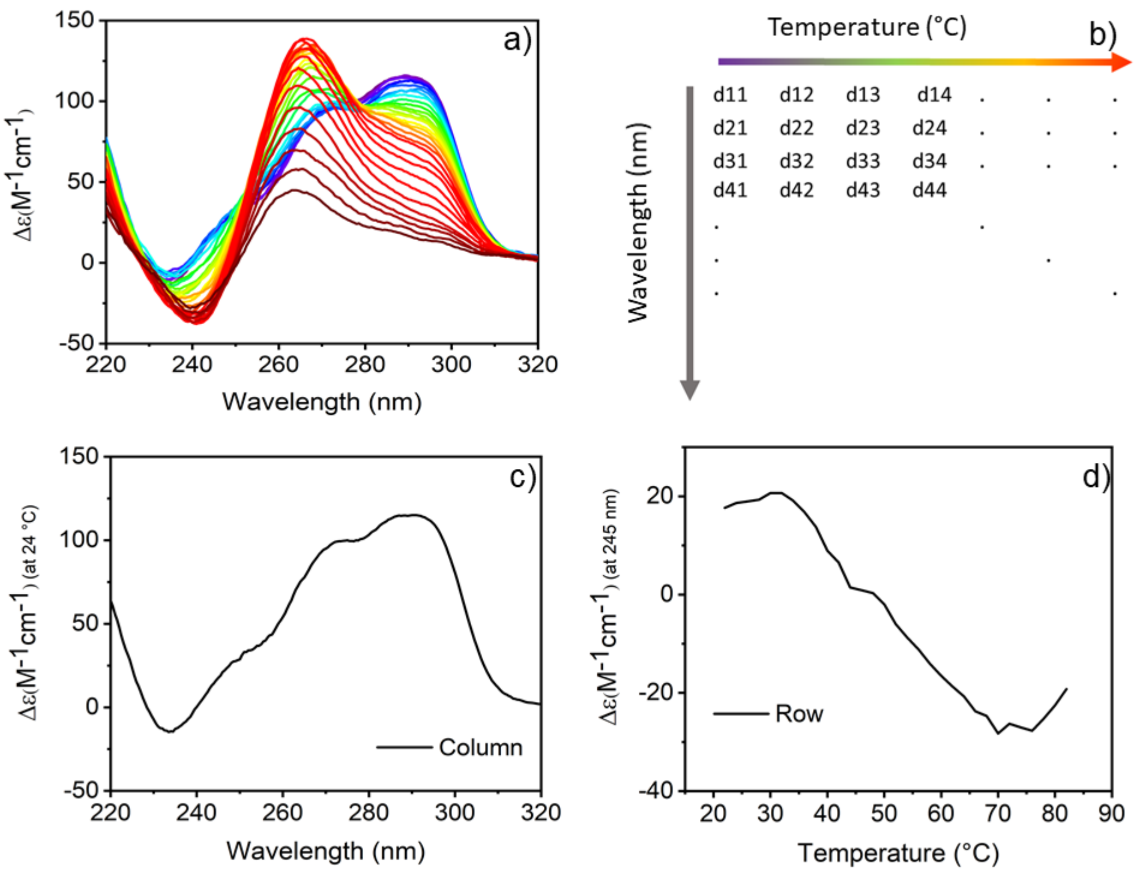
SVD: multivariate statistical analysis

G-quadruplex characterization

Singular Value Decomposition (SVD)

- ✓ Characterization of the significant species of the unfolding process
- ✓ Thermodynamic parameters

SVD is a rigorous and model-free analytical tool to evaluate the **number of significant spectral species** required to account for the changes in CD.

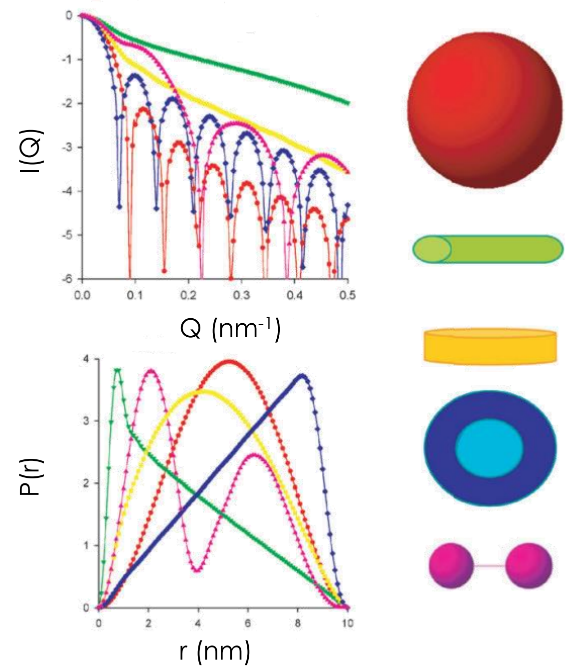


SAS: shape, size

G-quadruplex characterization

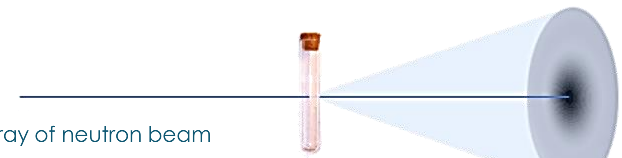
Small Angle Scattering (SAS)

- ✓ **Solution**
- ✓ **Quaternary structure changes**



Low-resolution structural information
on the overall shape

To determine the shape/size, SAXS/SANS experiments typically require a homogeneous dilute solution of macromolecules in a near physiological buffer without special additives



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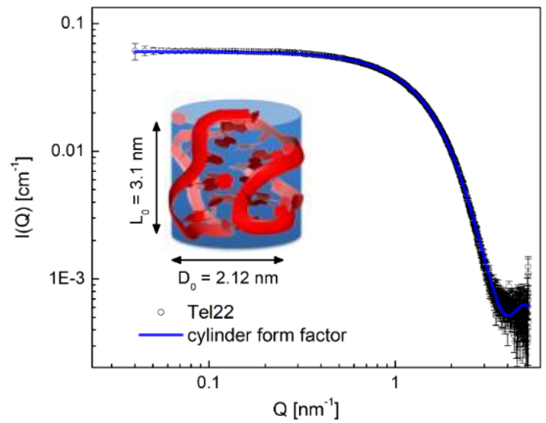


*Reports on Progress in Physics 66.10, 1735, **2003***

Extremely coarse graining simulations

G-quadruplex Multimerization

Circular dichroism (CD)
- ✓ Solution
- ✓ Secondary structure changes

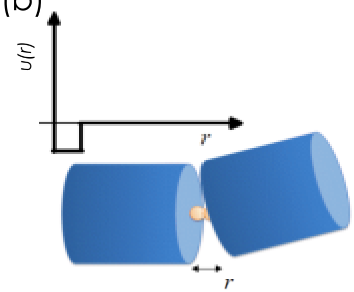
Small Angle Scattering (SAS)
- ✓ Solution
- ✓ Quaternary structure changes
- ✓ Multimerization

EGCS
- ✓ Solution
- ✓ Quaternary structure changes
- ✓ Multimerization
- ✓ Polydispersity
- ✓ Stacking properties

➤ Simulation model for the G4 unit

(a)

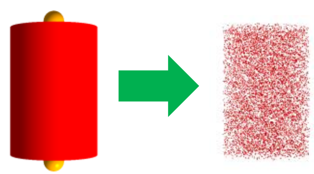
(b)



$D = D_0\,K \quad L = \frac{L_0}{K} \quad D_0 = 2.12\,nm \quad L_0 = 3.1\,nm$

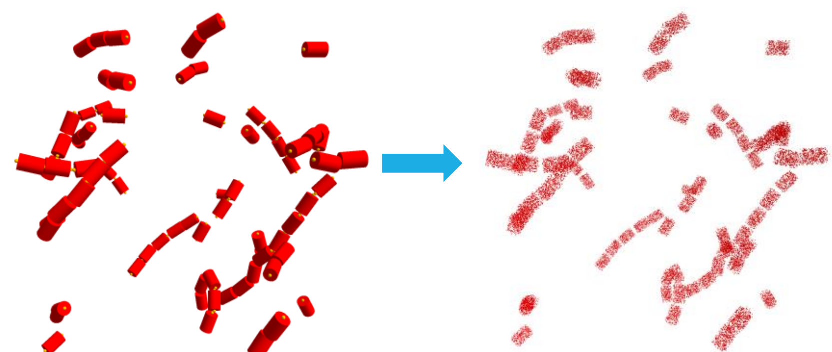
(a) Each G4 is modeled as a HC with diameter *D* and length *L*.
(b) Each HC is decorated with two attractive sites at the basis. Sites belonging to different cylinders interact through the SW potential *u(r)*.
The stacking interaction between the Tel22 units was varied through the effective temperature $T^* = k_BT/u_0$ (where u_0 is the binding energy of the HC)

➤ Numerical Static Structure Factor



Each hard cylinder is replaced with a random set of scattering points inside it

Hard Cylinders   Scattering Points



From the equilibrated configurations, we can obtain

$$I(Q)=S(Q)\cdot P_{cyl}(Q)$$

Experiments meet ECG simulations

Multimers

Small Angle Scattering (SAS)
- ✓ Solution
- ✓ Quaternary structure changes
- ✓ Multimerization

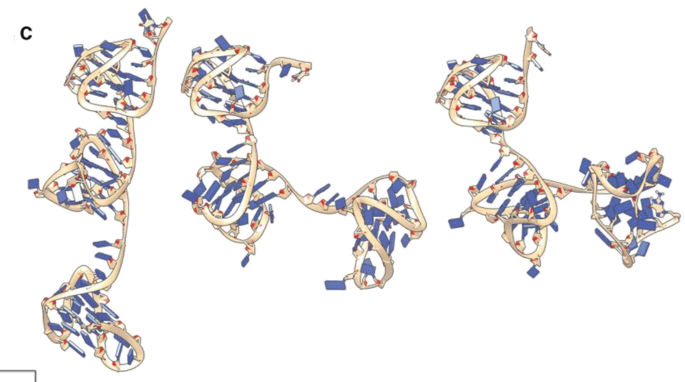
Monsen et al. NAR 2021

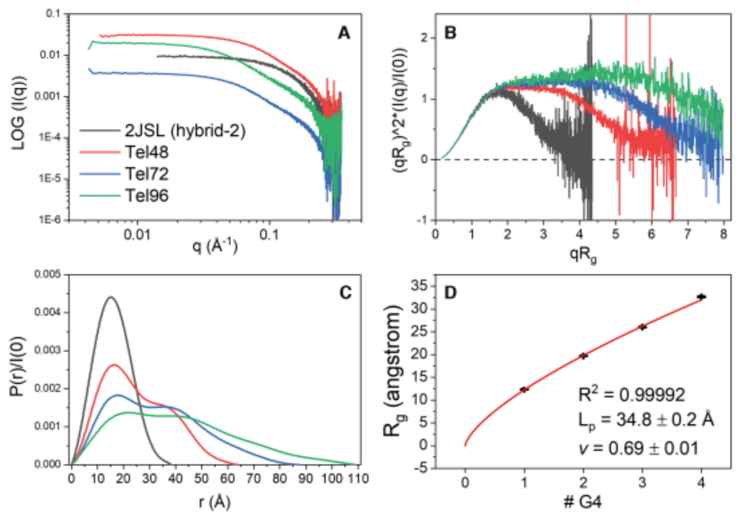
EGCS
- ✓ Solution
- ✓ Quaternary structure changes
- ✓ Multimerization
- ✓ Flexibility

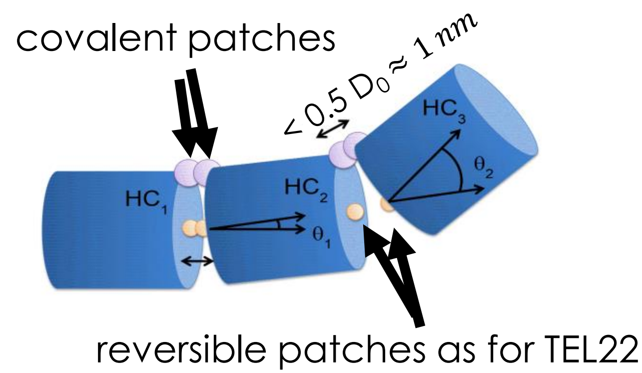
➢ Higher-order human telomere G-quadruplex multimers

ECG modeling of TEL72

Flexibility of the structures







Monsen et al data @ https://www.sasbdb.org/

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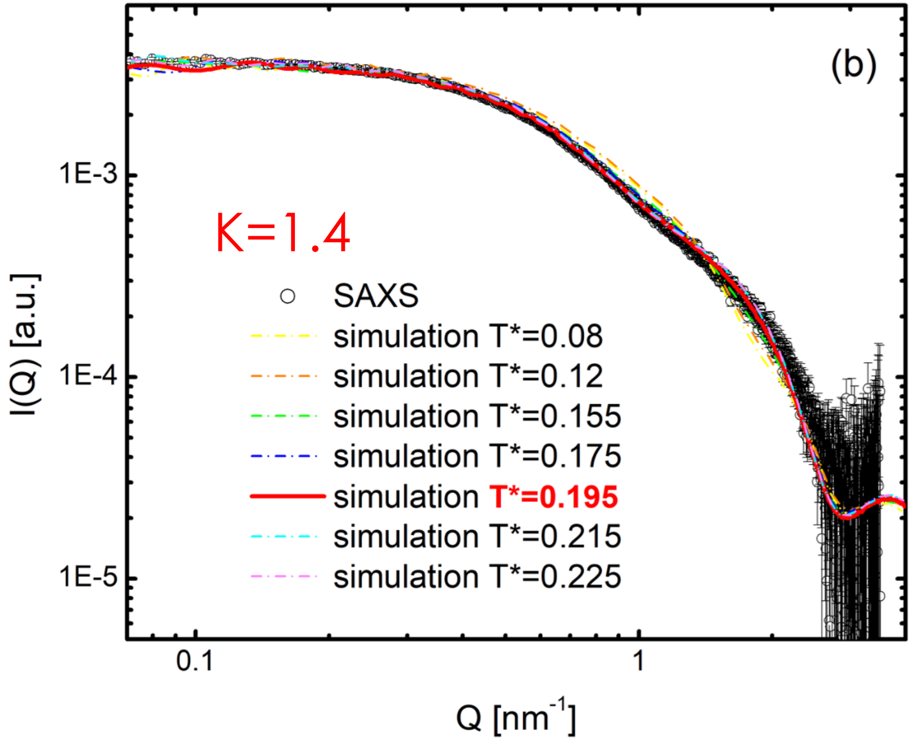
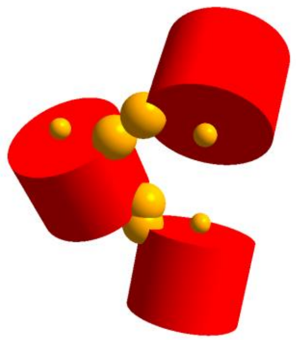
The importance of the Flexibility



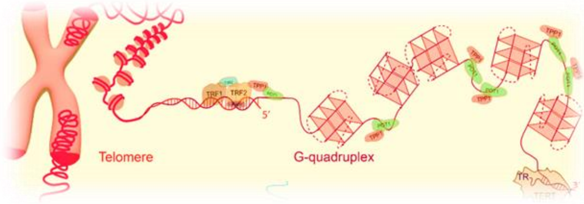


Monsen et al data @ https://www.sasbdb.org/

Thesis Projects

Experiments and Modelling of The:

- Thermal unfolding pathway of quadruplex structures
- Ligand-induced stabilization/destabilization of quadruplex structures
- Higher-order human telomere G-quadruplex multimers



comez@iom.cnr.it
alessandro.paciaroni@unipg.it

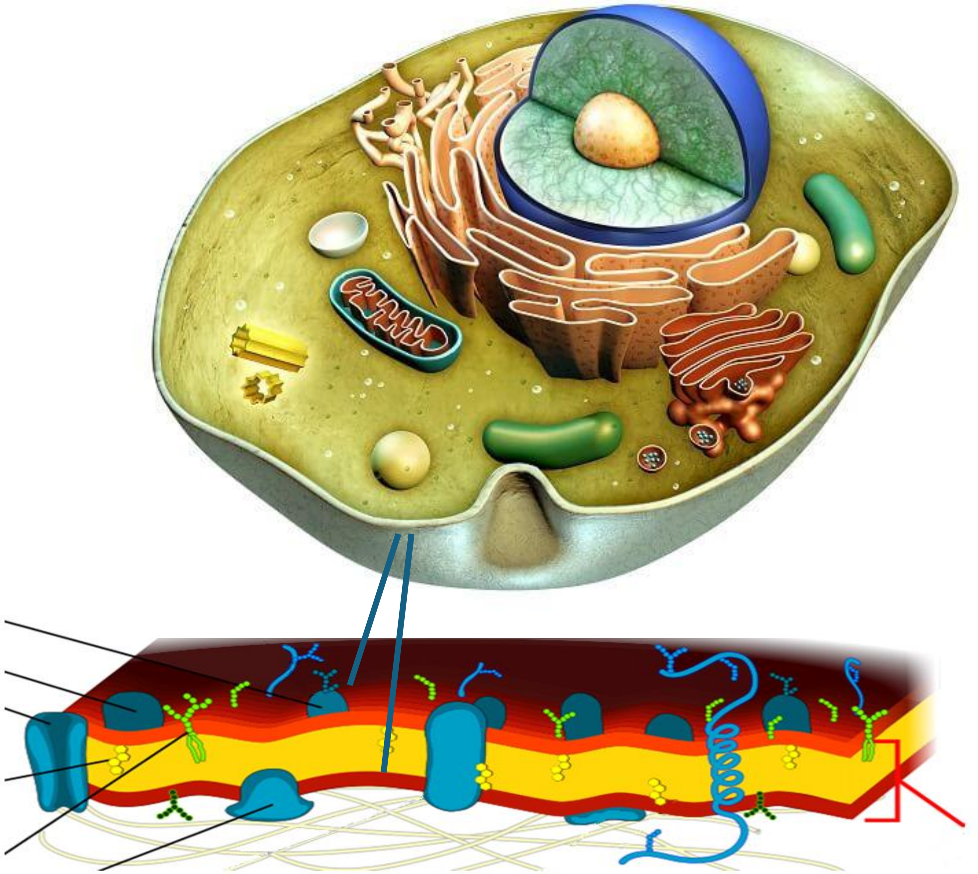
Modelling the structure and dynamics of Biological membranes

Biological membranes

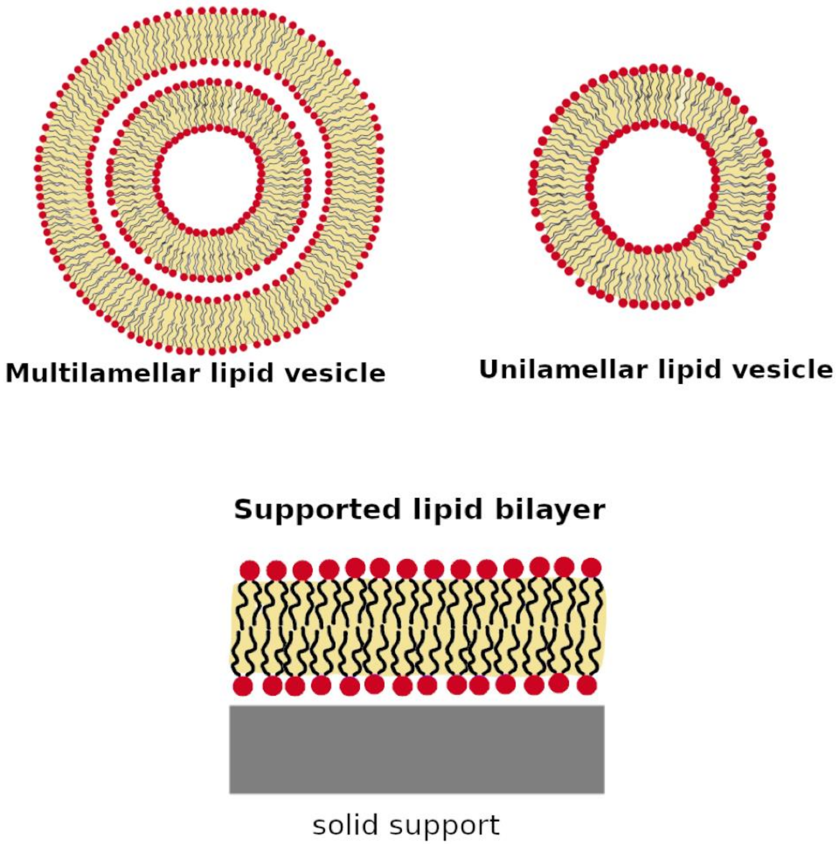
Biological membranes are very complex systems including hundreds of different lipids and proteins that are self-assembled within a double layer structure, i.e. the lipid bilayer

Challenges

- They cannot be directly extracted from cells
- Their compositional complexity limits the application of biophysical methods



Models of Biological membranes

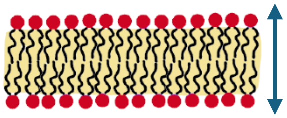


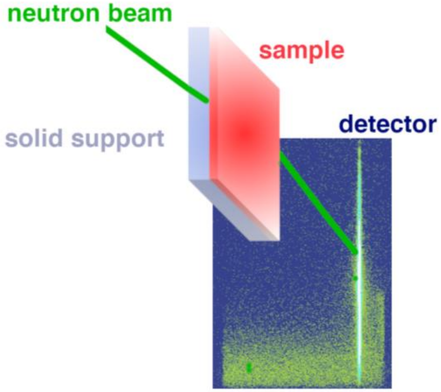
Overcoming the challenges

- Membrane models are simplified systems, which can be easily prepared in the lab
- They can be produced in different formats according to the biophysical technique to be used for their characterisation
- They provide information about structure and dynamics very close to native biological membranes

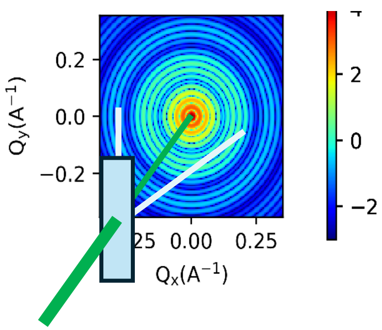
Neutron scattering techniques

Structure



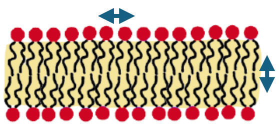


1. Neutron reflectometry

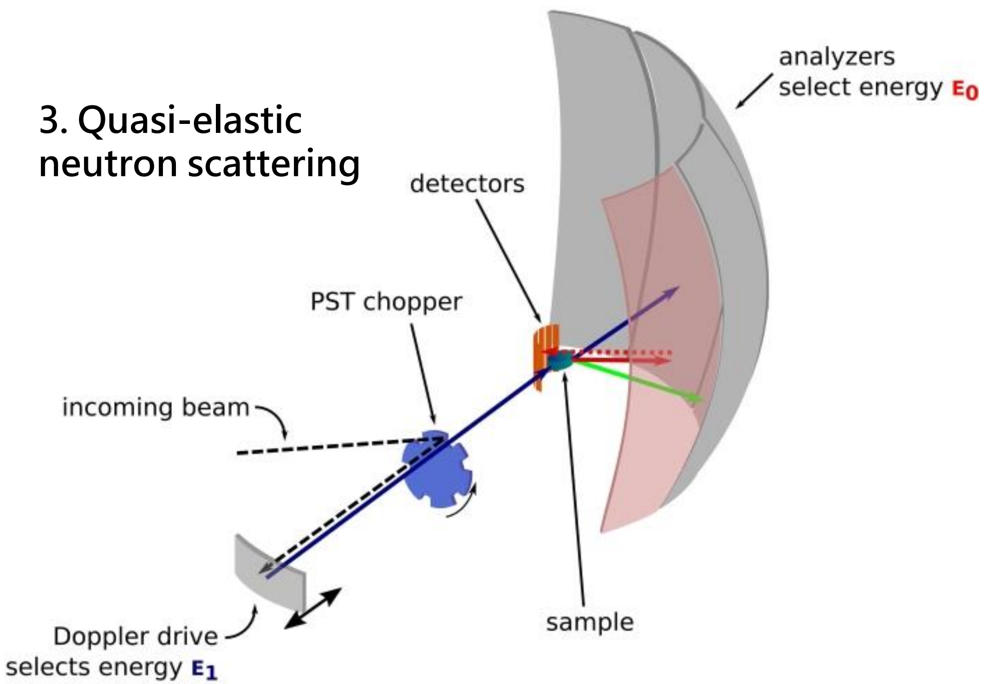


2. Small angle neutron scattering

Dynamics

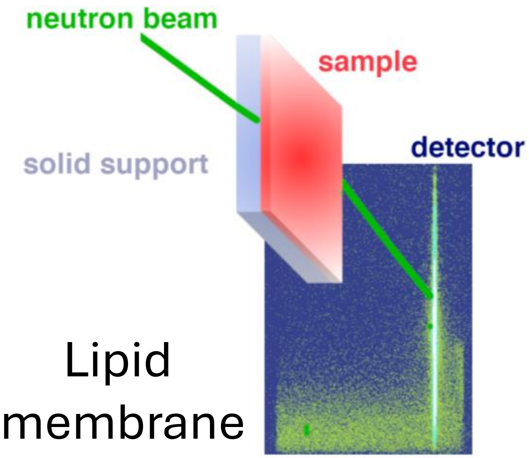


3. Quasi-elastic neutron scattering

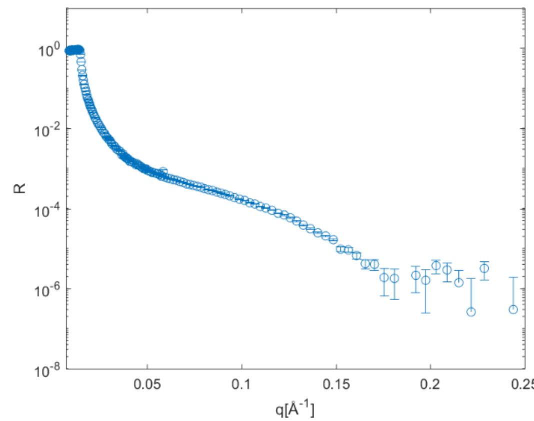


Neutron scattering techniques

Example for neutron reflectometry, but generic workflow for neutron scattering experiments

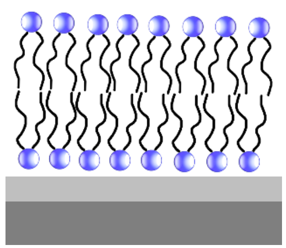


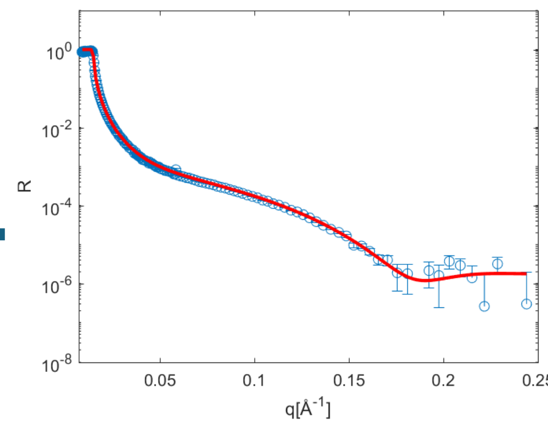
2D detector image is elaborated in a 1D curve



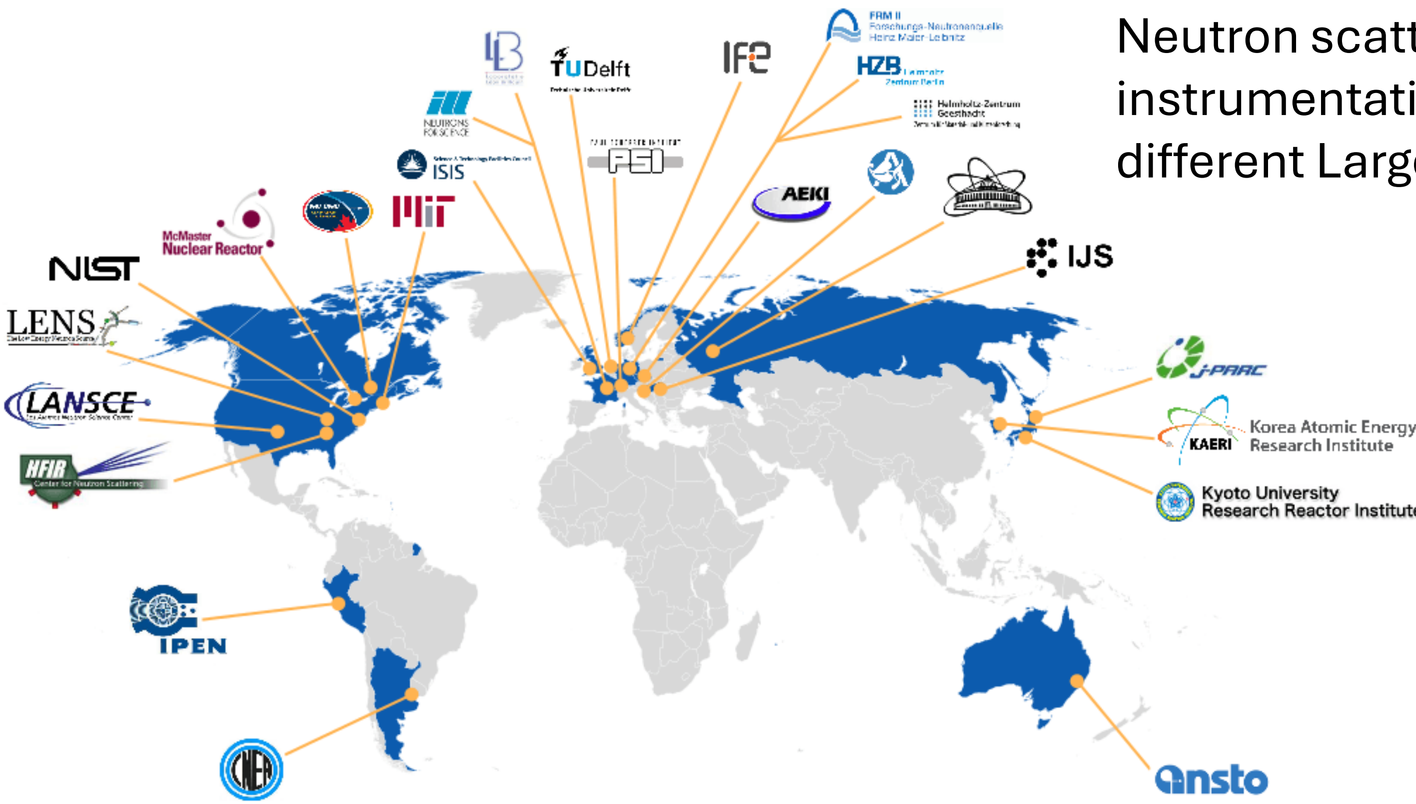
Data modelling

Lipid membrane



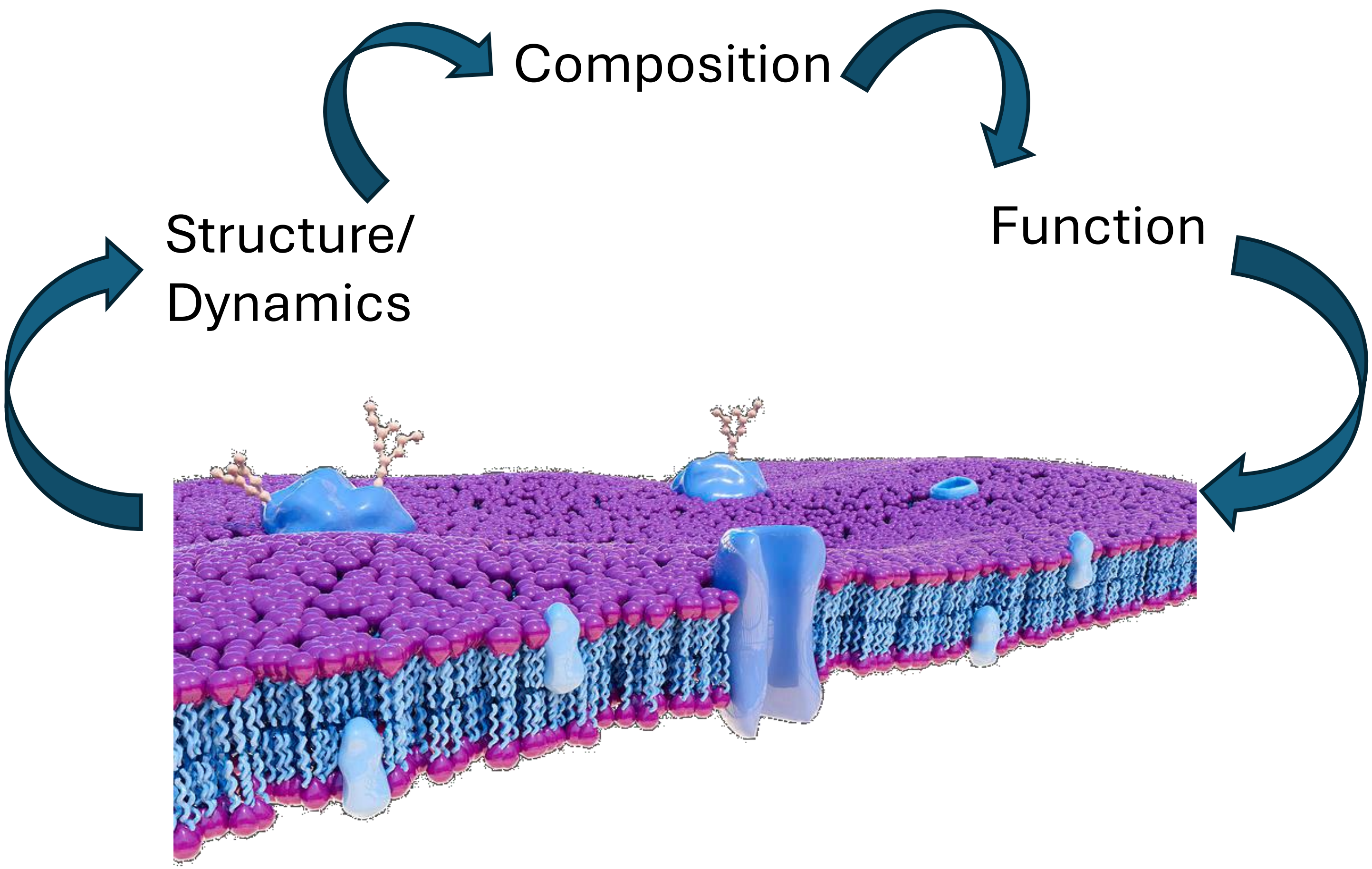


Neutron scattering techniques

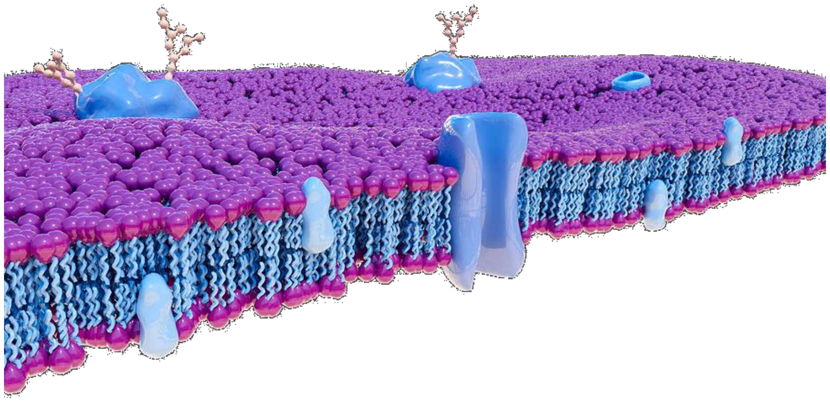


Neutron scattering instrumentation is available at different Large Scale Facilities

Understanding biological membranes



Thesis project



1. Modelling the structure of biological membranes – neutron reflectometry, small angle neutron scattering (and lab techniques)

2. Modelling the dynamics of biological membranes – quasi-elastic neutron scattering (and complementary lab techniques)

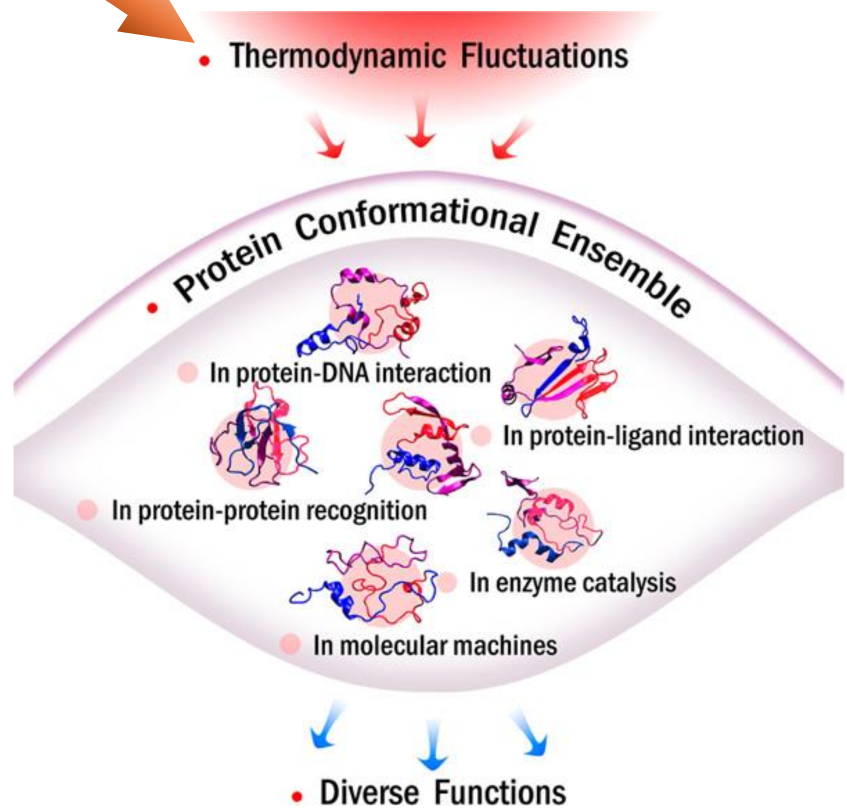
The relation between Function and Dynamics of Proteins

New experimental techniques in advanced Neutron Scattering

Protein Dynamics

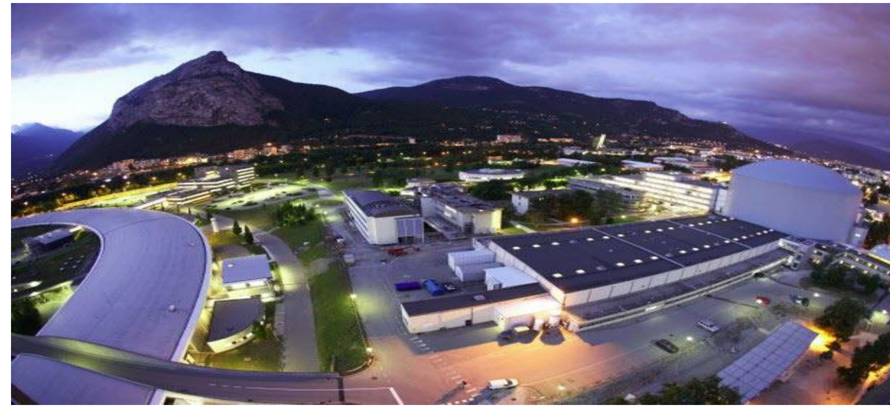
Protein Structure

Protein Sequence



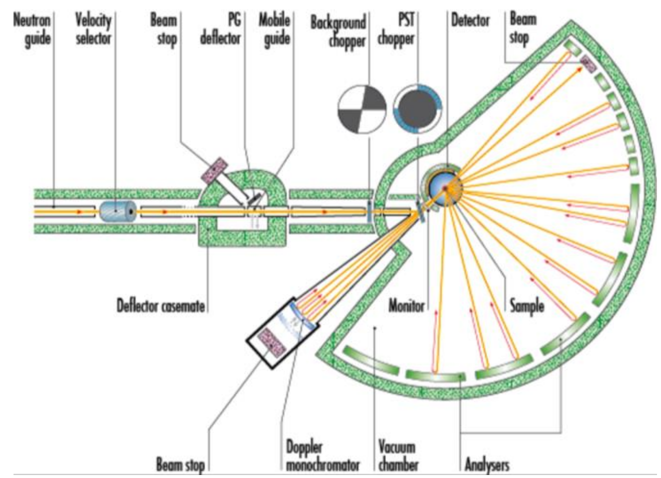
Institut Laue-Langevin

The world-leading neutron facility
in Grenoble (France)



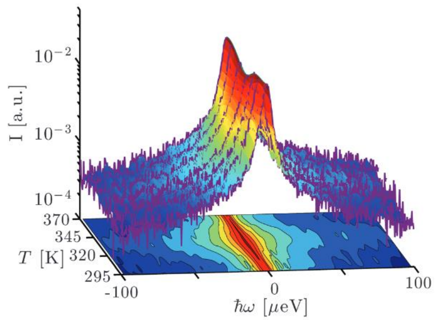
The neutron spectrometer IN16B

The world-leading back-scattering spectrometer



Thesis projects

- Suited for Master degree
- Internship at the Institut Laue-Langevin
- In collaboration with Tubingen University



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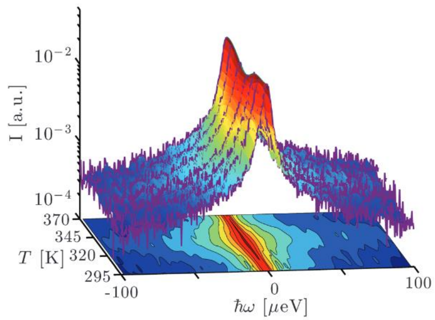

Quasi-Elastic Neutron Scattering coupled to Fixed-Window Scans on:

Thesis projects

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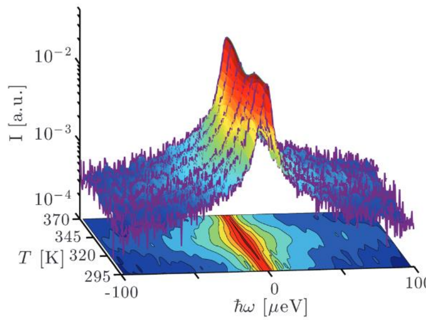
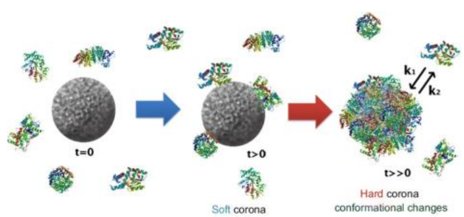
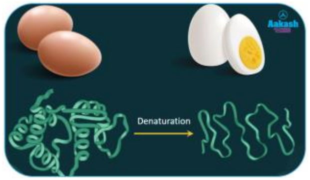


Quasi-Elastic Neutron Scattering coupled to Fixed-Window Scans on:

1. Protein Dynamics during kinetics



2. Protein Dynamics during denaturing



3. Protein polydispersity: proteins meet colloid physics