

## Elettra Sincrotrone Trieste





# Biophysics and beyond @ SISSI PhD Opportunities

SISSI (Synchrotron Infrared Source for Spectroscopy and Imaging) is the infrared beamline at Elettra. SISISI extracts the IR and visible components of synchrotron emission for performing spectroscopy, microspectroscopy and imaging. The applications cover a wide range of research fields, including surface and material science, biochemistry, forensics, microanalysis, geology, cell biology, biochemistry, biomedical

## diagnostics, microfluidics, high-pressures, time resolved IR, conservation science, chemical kinetics etc.

Federica Piccirilli, PhD in Physics Trieste University - Cycle XXIV

## SISSI-Mat Material Science at SISSI

F1

SISSI-Bio Chemical and Life Sciences at SISSI Paolo Zucchiatti, PhD in Nanotechnology Trieste University - Cycle XXXI

## **HIGH PRESSURE FTIR**

High pressure technologies are coupled to IR spectroscopy to study the thermodynamic stability of proteins. Diamond Anvil Cells are typically used. Pressure calibration is performed through the evaluation of the spectral position of ruby R1 and R2 flurescence lines around 690 nm.



## **BEAMLINE LAYOUT**



## **ENDSTATIONS**







## **Collective enhanced SRIR** Absorption Microcopy for protein conformational studies

Despite its potential for biomolecule characterization, FTIR microscopy (FTIRM) of sub-micromolar samples using conventional approaches is extremely challenging.

For increasing the technique sensitivity we take advantage from the electric field enhancement associated with the excitation of plasmonic resonances from ad-hoc shaped metallic structures by exploiting the collective resonant excitation of nanoantennas assemblies, a technique called Collective Enhanced IR Absorption (CEIRA).



CEIRA substrates are produced with state of the art nanotechnological approaches in collaboration with IIT (Istituto Italiano di Tecnologia), Plasmon Nanotechnolgy line (A. Toma and F. De Angelis)



### **Amyloid fibrils dissociation at high pressure**

Since high pressure favors the state that occupies the minimum available volume, proteins structural stability can be strongly affected by compression. Thus, proteins unfolding and oligomers and aggregates dissociation can occur and Intermediate states of folding can be stabilized as a result of electrostatic interactions and hydrophobic contacts destabilization.





## Vibrational dynamics of biomolecules in non-aqueous glassy matrices

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To provide a deeper understanding on the specific interactions that determine the vibrational dynamics and the collective motions of proteins embedded in glassy sugar matrices, systems very often used as excipient to bio-protect biopharmaceutical ingredients, is a fundamental step for improving their long-term storage and stability of their native structure.

## MIR and FIR T-dependent studies of lysozyme in sugar matrices

Absorbance spectra of glucose + lysozyme showing the amide bands (II and I) in the FIR Absorbance of glycerol + lysozyme in the 280K to 405 K T range. Deconvolution of Amide I band at different temperatures, 285K to 425K T range . The inset is an enlargement of the low frequency band. highlighting the  $\alpha$ -helix and a  $\beta$ -sheet components

**—●**— 278 K



(A) Scheme of a single array of gold od-shaped nanoantennas (50X50  $\mu$ m<sup>2</sup>) on CaF<sub>2</sub> substrate and two possible nanoantennas arrangements: (B) line-to-line geometry and (C) cross geometry.

(D) Focal Plane Array FTIR image of IR field enhancement of a single nanoantenna array with line-to-line geometry, with parallel (upper panel) and perpendicular (lower panel) IRSR light polarization ; (E) Focal Plane Array FTIR image of IR field enhancement of a single nanoantenna array with cross-geometry for both polarizations.

SR CEIRA microscopy offers the opportunity for addressing protein conformational studies in liquid-physiological environment at physiologically relevant protein concentrations.

### Protein anchoring



Target proteins are immobilized after surface functionalization by exploiting thiols chemistry and amide coupling immobilization protocol. This protocol allows the formation of a protein monolayer on the gold surface of the nanoantennas.

Reference spectra of

SR CEIRA measurements





aS amyloid fibrils, hallmark of Parkinson's disease, a pressure of 2-3 kbar.



### Contacts



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