



Infrared spectroscopy and nanoscopy: new frontiers in protein studies

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22-23 May 2023
Trieste, Italy



Outlook



- ✓ IR bio-spectroscopy at a glance: SISSI-Bio
 - Present space domain of SISSI-Bio
 - User-driven and collaborative Research at SISSI-Bio



- ✓ Infrared Spectroscopy of Proteins
 - Bridging biophysical and structural studies with PIR-SEIRA
 - Cellular Biology with IR microscopy
 - Protein studies at the nanoscale



- ✓ Future upgrade plans



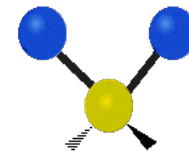
IR bio-spectroscopy at a glance: SISSI-Bio

SISSI – Synchrotron Infrared Source for Spectroscopy and Imaging

Infrared radiation probes the vibrational modes of covalently bonded molecules

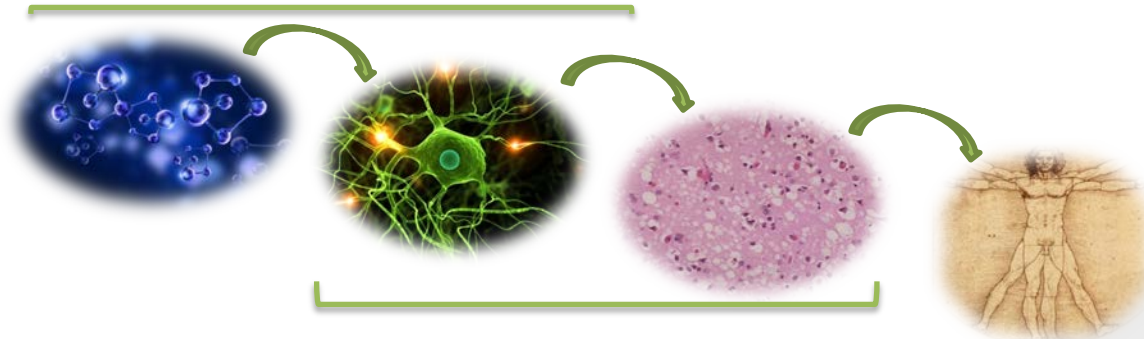
Qualitative Information: molecular composition of the sample

Quantitative Information: concentration of the sample constituents



Bio-spectroscopy is the spectroscopy of the **Biomolecules**

Samples conventionally studied by IR Spectroscopy



Samples conventionally studied by IR Microscopy and Nanoscopy





Present space domain of SSSI-Bio

Vibrational information on the sample at different level of detail, depending on instrumentation and IR source (IR conventional, IR-Synchrotron Radiation, IR laser)

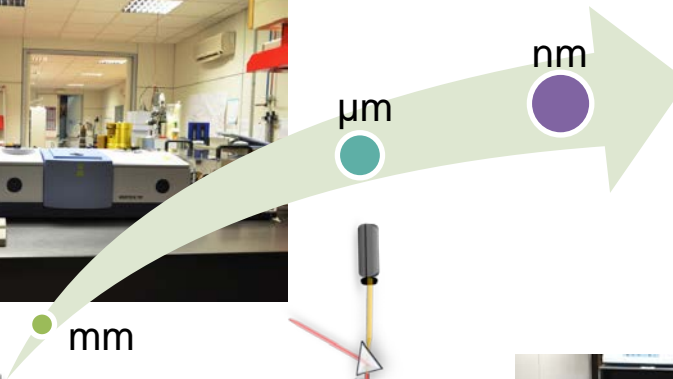


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NFA nanoscience foundries
and fine analysis
research & innovation actions

CERIC

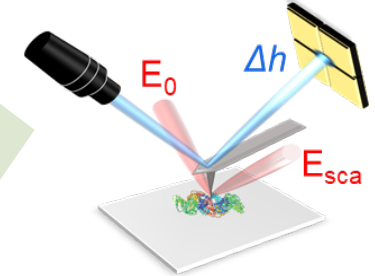
IR spectroscopy



mm

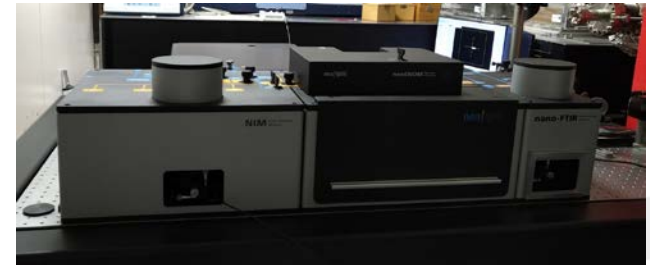
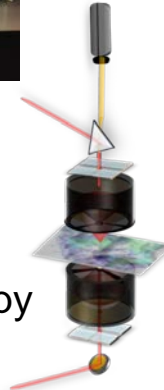
μm

nm



Near field
IR nanoscopy

Far Field
IR microscopy



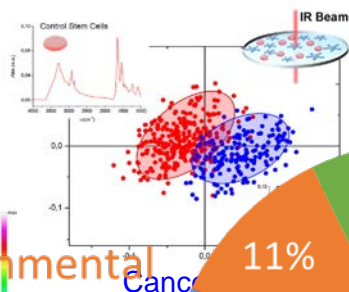
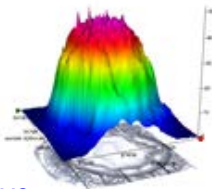


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User driven and collaborative Research at SSI-Bio

(Dry) Cell and tissue mapping From diagnostic to prognostic purposes

Fertility
&
Sterility



Neurodegenerative
disorders
Rare diseases



Environmental
Sciences

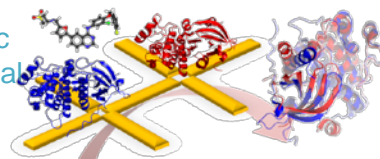
Cancer
research

(Near-)physiological conditions experiments

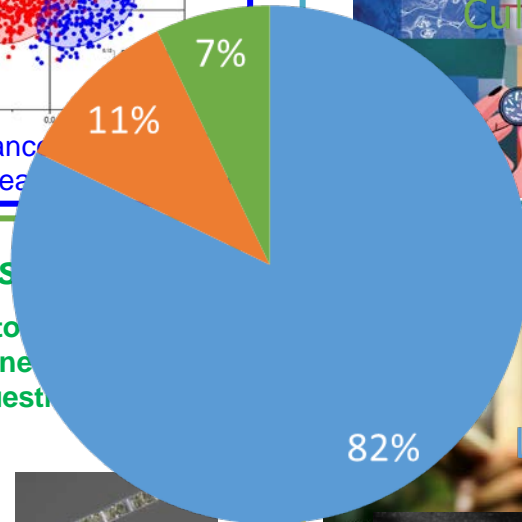
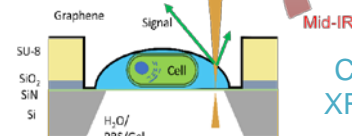
Cell Dynamics



IR plasmonic
for bio-physical
studies

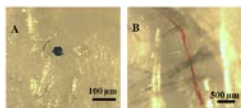


Chemical Science (including
Cultural Heritage)

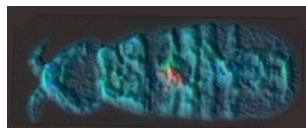
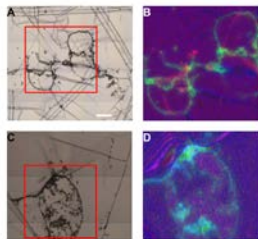


Environmental Sciences

Microplastics in Antarctic



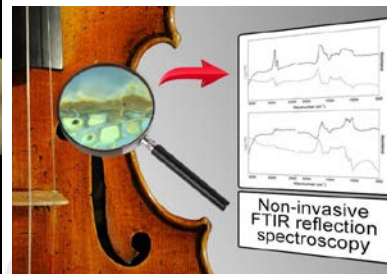
Carbon Storage
Pollution, Biomineralization
Carbon Sequestration



Cultural Heritage

Paleoarchaeology

Bowed string
instruments



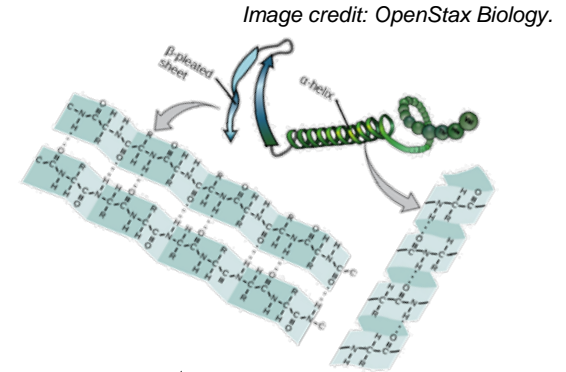
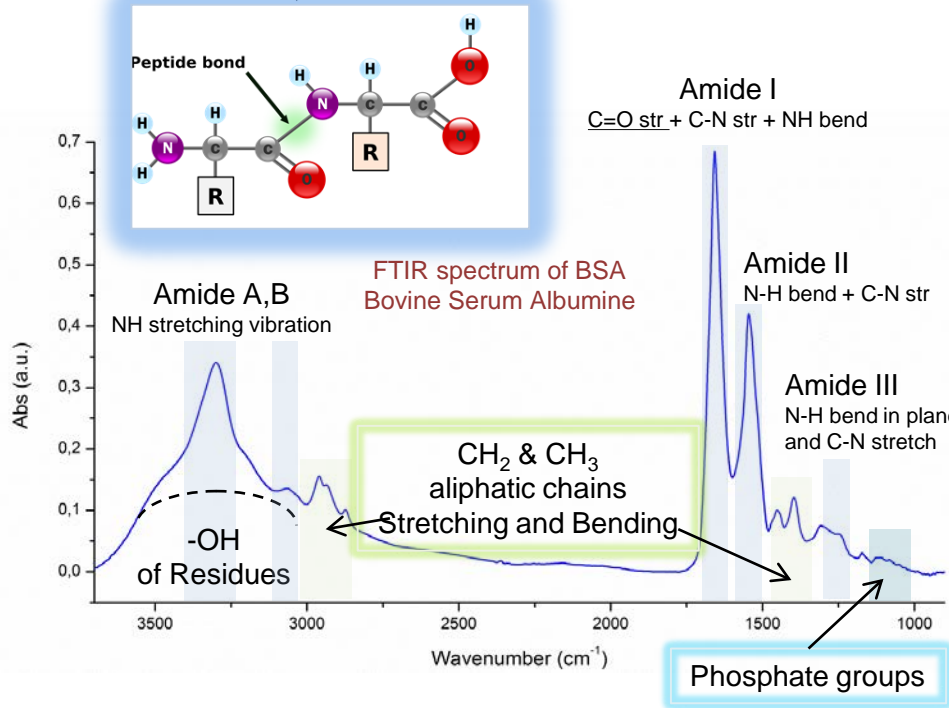
Life Sciences

Early

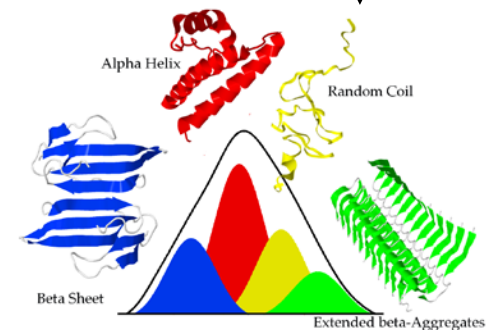




Infrared Spectroscopy of Proteins



Different H-bonding networks for different peak positions



Amide I band is particularly sensitive to protein secondary structure, and conventionally employed for protein conformational studies





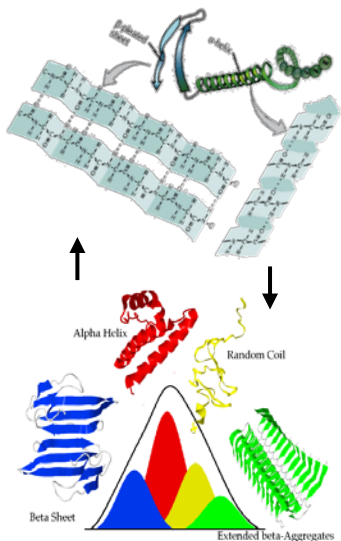
Bridging biophysical and structural studies with PIR-SEIRA

Drug-Receptor Binding studies is a key step in drug-discovery

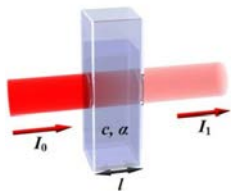
- Existing biophysical methods are fast, unexpensive, requires minimal sample preparation but they are blind to the protein structure
- Structural determination methods (MX, NMR, cryo-EM) are extremely sensitive to receptor structure and its modification upon binding, but they are usually slow, expensive and may require large material amount

Does exist a method able to bridge biophysical and structural protein studies?

FTIR spectroscopy is sensitive to protein secondary structural elements



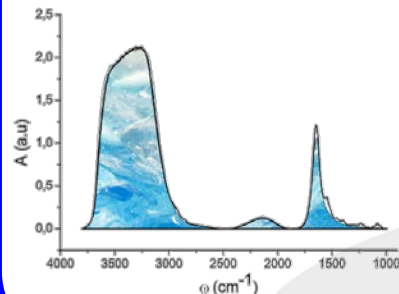
Sensitivity Limit



Requires extremely high concentrations, in the mM regime

These concentrations are often not-relevant in physiological conditions

Water Barrier



Water bands overlaps with the vibrational features of biomolecules
Requires dried samples or D_2O , that are not compatible with biomolecules

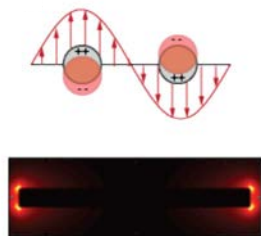


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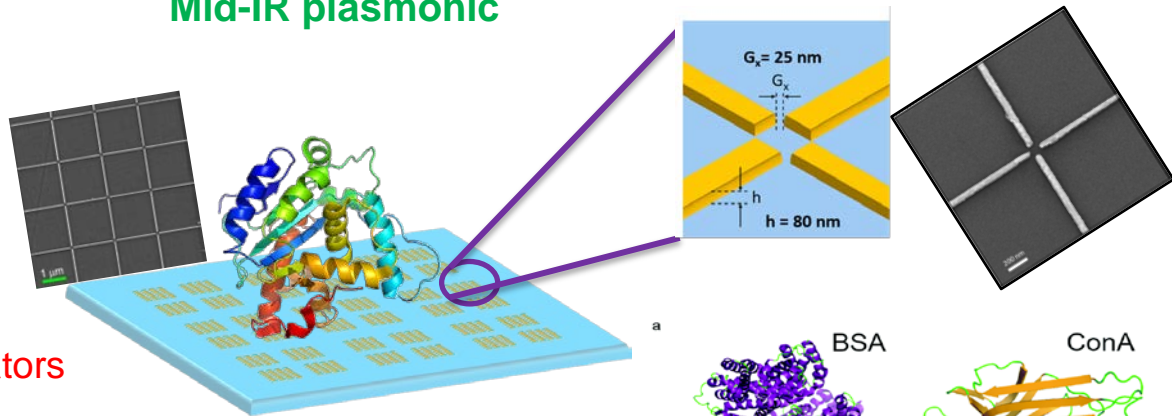


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Bridging biophysical and structural studies with PIR-SEIRA

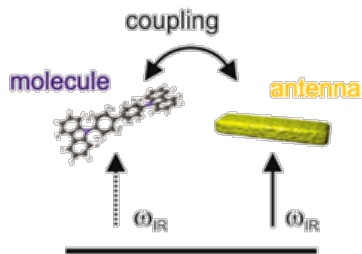


Mid-IR plasmonic



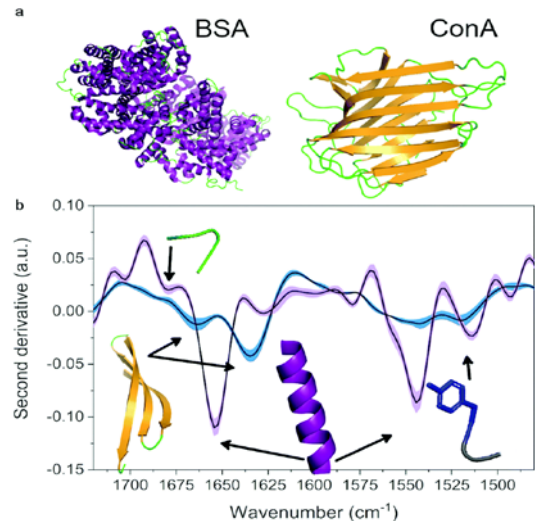
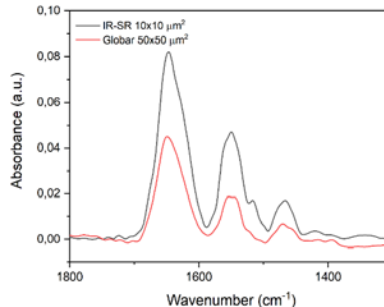
Optical nano-resonators

- Focus light into nanoscale volumes
- Enable strong light-matter interaction
- Ideal to achieve ultra-high sensitivity



Signal
enhancement of
several order
of
magnitude
can
be
achieved

Micrometric sampling with IRSR





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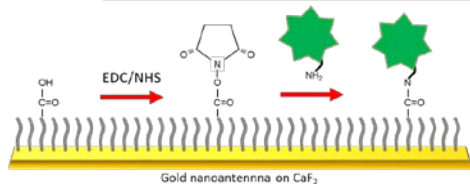
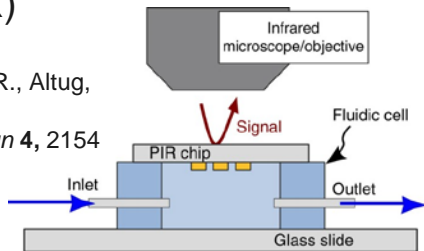


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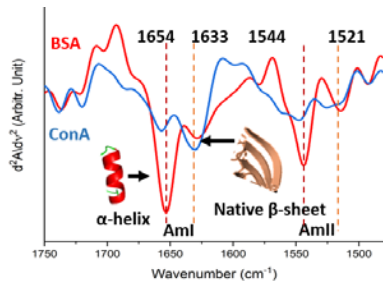
The case of EGFR-KD / Lapatinib binding

Plasmonic internal reflection (PIR)

Adato, R., Altug,
H. *Nat Commun* 4, 2154
(2013)



Without water subtraction!

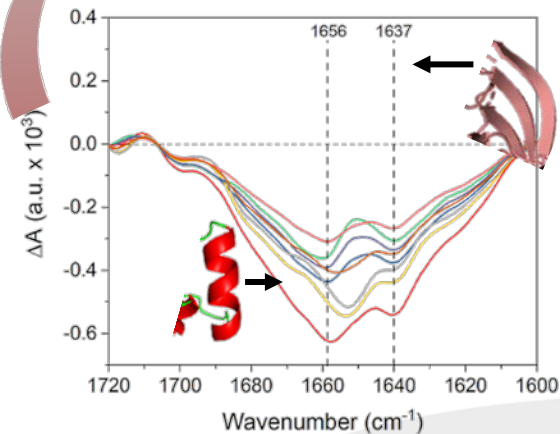
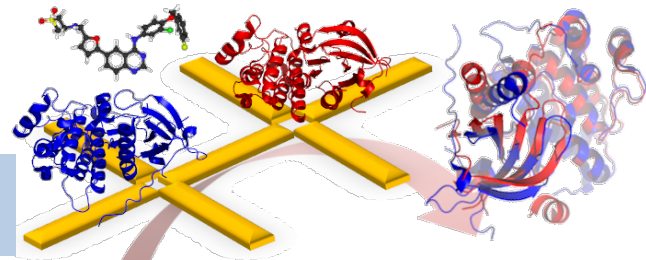


Kinase domain of Epidermal Growth Factor Receptor (EGFR-KD)

Receptor for members of the epidermal growth factor family (EGF family) of extracellular protein ligands.

Mutations that lead to EGFR overexpression (upregulation) or over-activity have been associated with squamous-cell carcinoma of the lung (80% of cases).

Drug-target for anticancer therapies (Lapatinib, Gefatinib etc...).



EGFR-KD	Lapatinib/EGFR-KD
36% helices (both alpha and 3_{10})	32% helices (both alpha and 3_{10})
15% beta sheet strands	14% beta sheet strands.

Zucchiatti P, Birarda G, Cerea A, Semrau MS, Hubarevich A, Storici P, De Angelis F, Toma A, Vaccari L. **Binding of tyrosine kinase inhibitor to epidermal growth factor receptor: surface-enhanced infrared absorption microscopy reveals subtle protein secondary structure variations.** *Nanoscale*. 2021 13(16):7667-7677



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Santa Maria
della Misericordia
di Udine

Cellular Biology with IR microscopy

IR Microscopy and Cancer Research

Glioblastoma Cancer Stem Cells Theory

CSCs not removed by surgery are responsible for tumor recurrence; they are resistant to standard radio- and chemo-therapies.

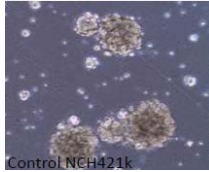
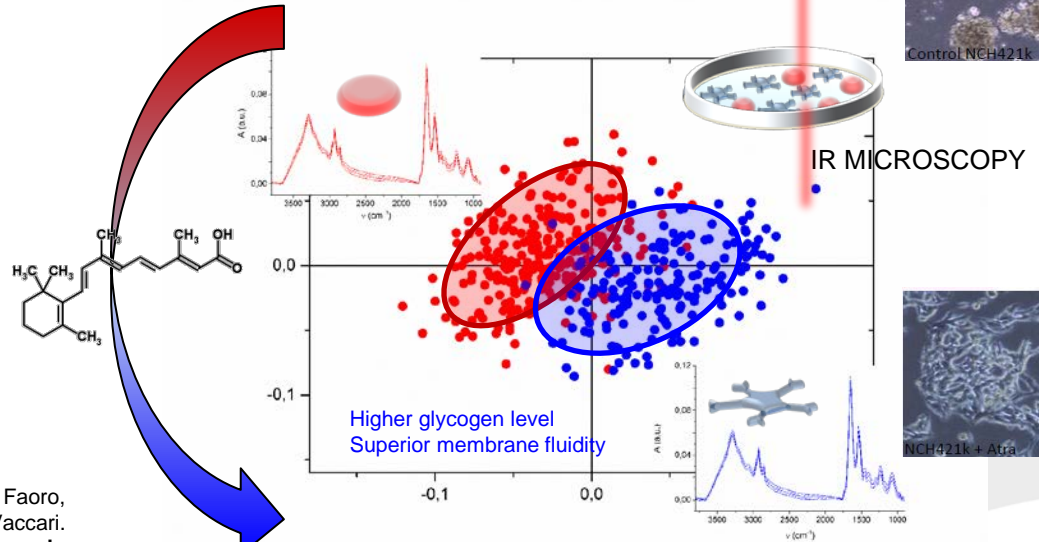
Inducing differentiation of stem cells is the most promising therapeutic approach nowadays under investigation.

Fast methods for monitor the presence/abundance of stem cells and the efficiency of stem-differentiating agent are needed

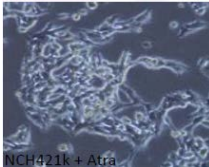
Saša Kenig, Diana Bedolla, Giovanni Birarda, Valentina Faoro, Elisa Mitri, Alessandro Vindigni, Paola Storici, Lisa Vaccari. **Fourier transform infrared microspectroscopy reveals biochemical changes associated with glioma stem cell differentiation.** *Biophysical Chemistry* **2015** 207, 90-96

NCH421K Human Stem Cell Like Glioblastoma cell line

CONTROL STEM CELLS



Control NCH421k



NCH421k + ATRA

ATRA-DIFFERENTIATED CELLS



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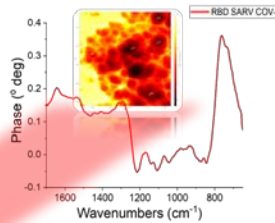
Protein studies at the nanoscale



ALUMINUM ADJUVANTS FOR SARS-COV-2-VACCINES



IR LIGHT

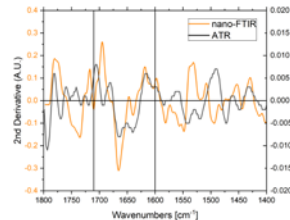
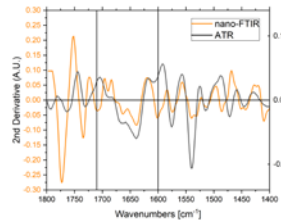


Vaccine adjuvant

SARS COV-2 Receptor binding protein

RBD/AI NR 1:1
Whitelight Image

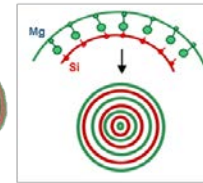
RBD/AI NR 3:1
Whitelight Image



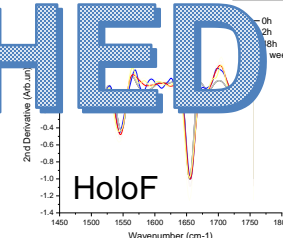
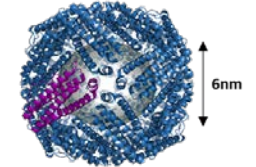
UNIVERSITÀ
DEGLI STUDI
DI TRIESTE

PROTEIN-ASBESTOS FIBER INTERACTION IN ASBESTOS PATHOGENECY

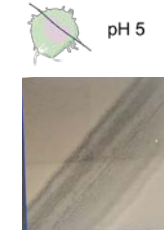
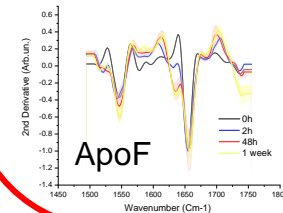
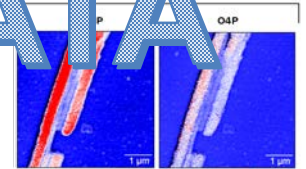
Chrysotile fiber



+ Ferritin [ApoF and HoloF]



Phagosome
68 mM KCL, 37° C



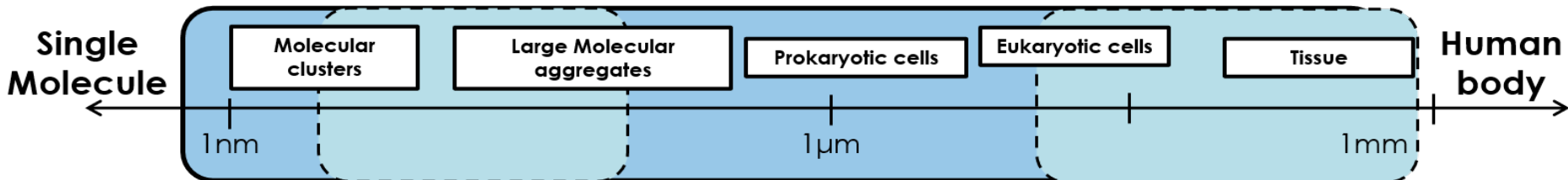
UNPUBLISHED DATA





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Future upgrade pans



Activity 5.5

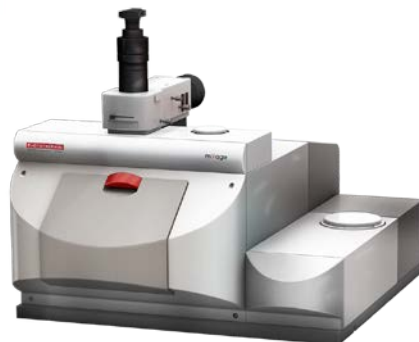
SISSI-Bio&TeraFERMI

FIR-THz low-energy molecular vibrations for understanding how motility and flexibility of key infection mediators influence virus lethality and infectivity



Activity 5.3

Two independent user-dedicated measurement station with nanometric spatial resolution, with IRSR and conventional sources



Activity 5.1

Two independent user-dedicated measurement station for FTIR histology and cytology with cellular spatial resolution, with IRSR and conventional sources



Finanziato
dall'Unione europea
NextGenerationEU



Ministero
dell'Università
e della Ricerca



Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA

AREA
SCIENCE PARK



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IR-THz macro-group



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Thank for your attention



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