Elettra Sincrotrone Trieste

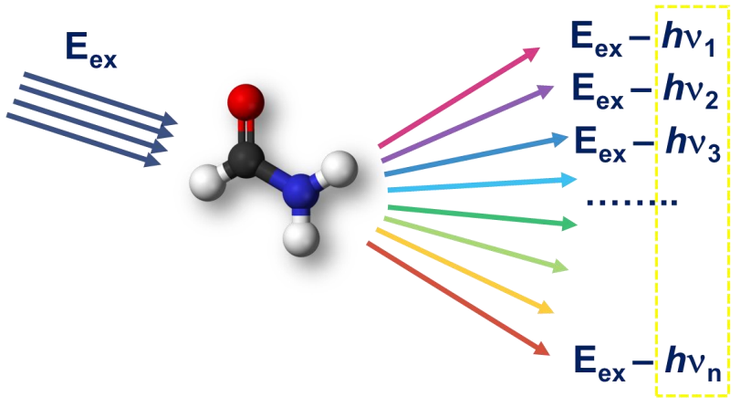
UV Resonance Raman for characterization of proteins

Barbara Rossi

Elettra Sincrotrone Trieste
barbara.rossi@elettra.eu

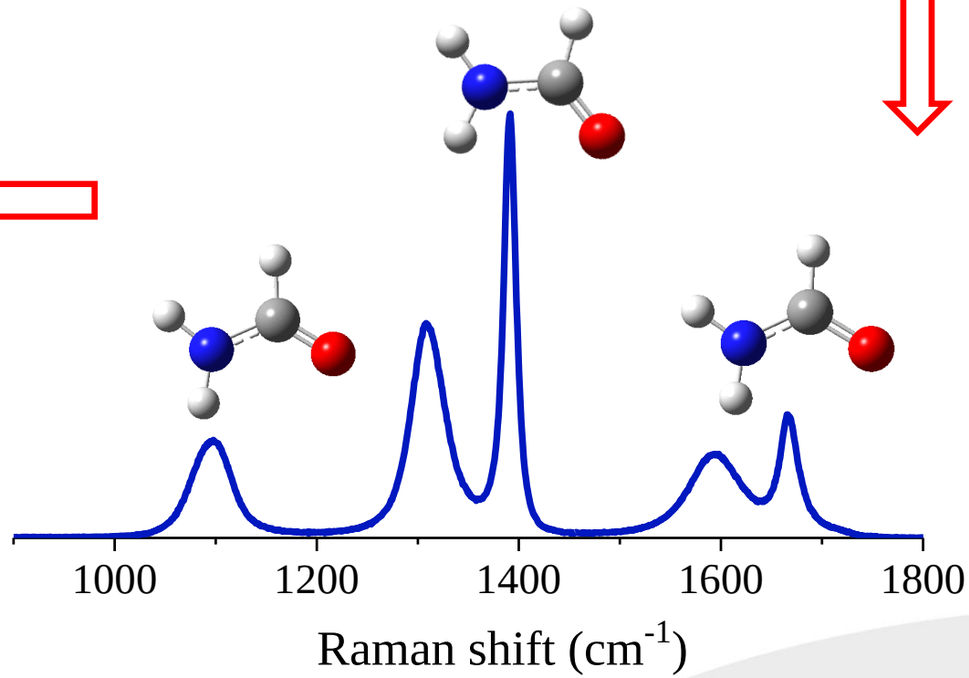
Raman spectroscopy

Sir C. V. Raman
Physics Nobel prize (1930)



shift in photon energy due to excitation/deactivation of molecular vibrations

$$I(\theta)_{av} = B(\nu_0 \pm \nu)^4 I_0 \left(\frac{\partial \alpha_{xx}}{\partial Q_1}\right)_0^2 \sin^2\theta$$



Molecular vibrational spectrum ⇐ Molecular structure

Raman spectroscopy provides a ***non-invasive*** way to characterize ***structure*** and ***dynamics*** of molecular systems

Limitations:
-> ***Weak effect*** (10^{-6} respect to elastic scattering)
-> ***Fluorescence*** can interfere

UV Resonance Raman (UVRR)

Raman signal intensities can be **selectively** enhanced by resonance by factor of up to 10^8 when **excitation wavelength ~ electronic transition**

$$I_{if} = \sigma^R I_{exc} N W(\Omega)$$

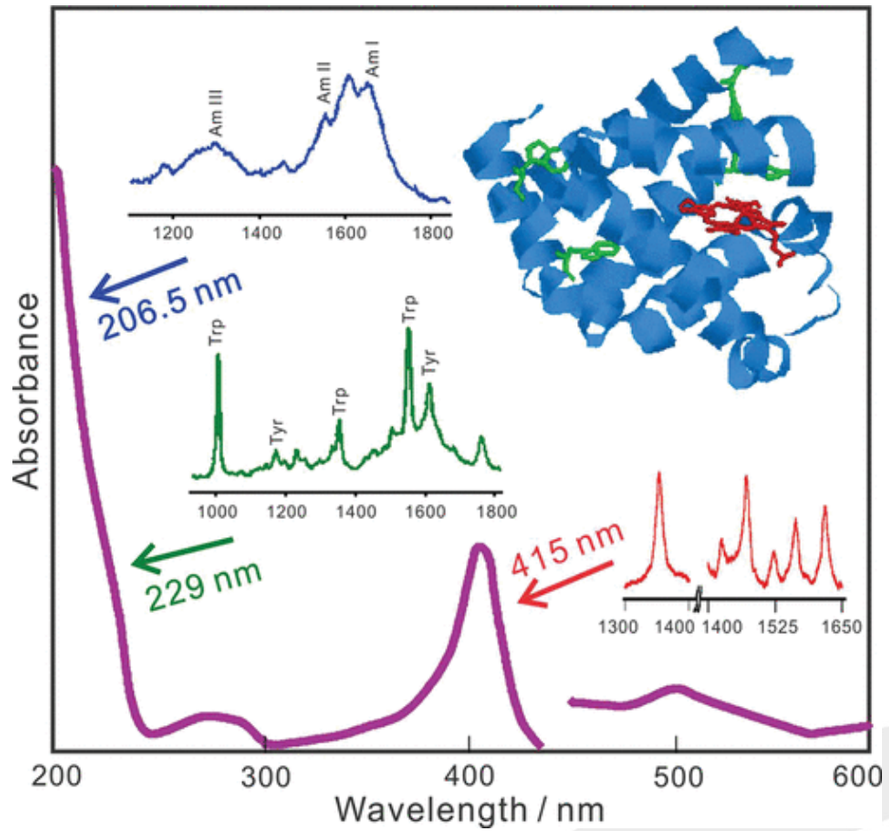
- I_{exc}: Instrumental efficiency
- N: Number of molecules
- σ^R: differential Raman cross section integrated over the Raman peak bandwidth

$$I_{Raman} \propto {}^{1}/_{\lambda^4} \rightarrow \textbf{sensitivity}$$

$$I_{Raman\,R} \propto \sigma^R \rightarrow \textbf{selectivity}$$

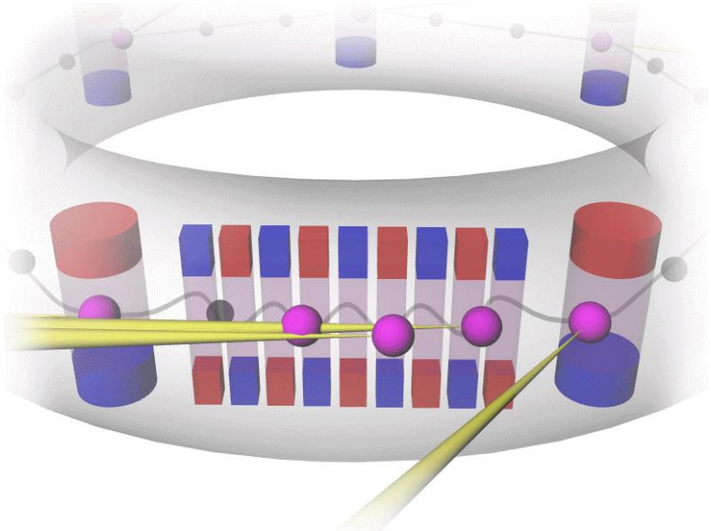
It requires tunable light sources in UV range!

Oladepo et al., Chem. Rev. 2012, 112, 5, 2604–2628



Why Synchrotron Radiation (SR)?

Synchrotron radiation sources use the emission produced by *relativistic electron bunches*



SR is a light source that irradiates all “colors”

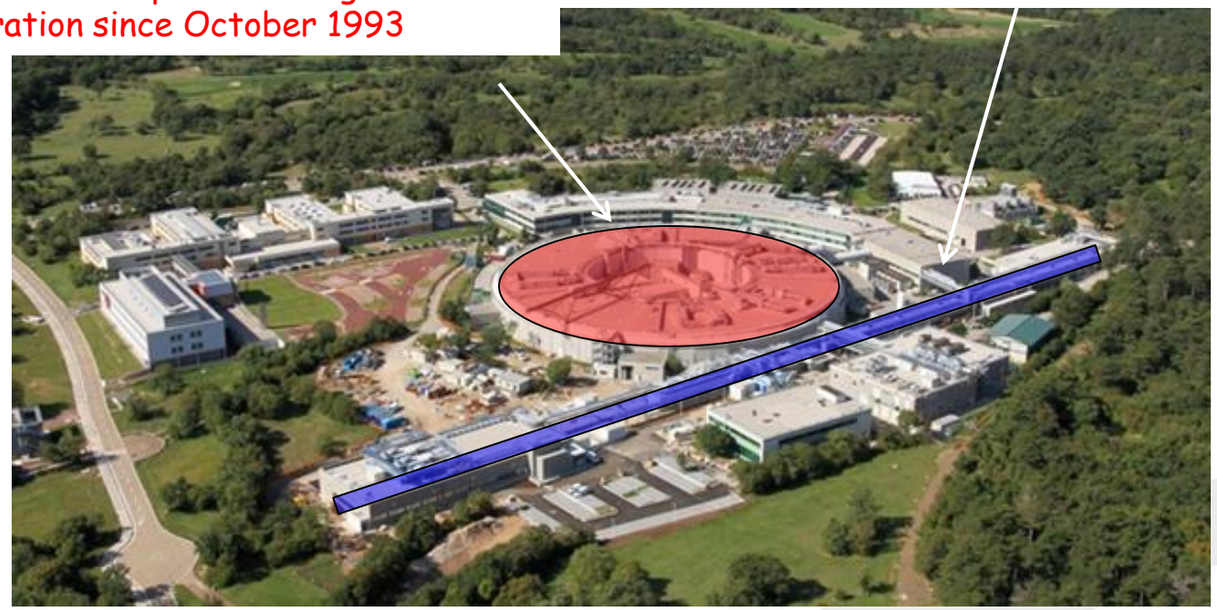
These sources allow a ***full tunability*** of the radiation over a very broad spectrum from ***IR to hard X-ray***

Elettra synchrotron light source

From IR to keV photon energies
in operation since October 1993

FERMI Free Electron Laser

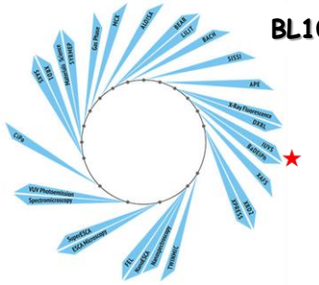
Pulsed source 100 fs, 10-1000 eV



Multi-wavelengths UVRR at IUVS@Elettra

Elettra Sincrotrone Trieste

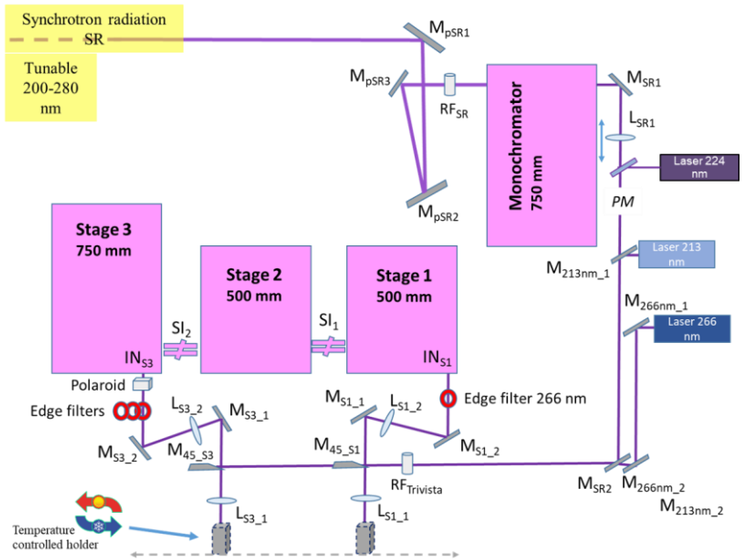
Present….

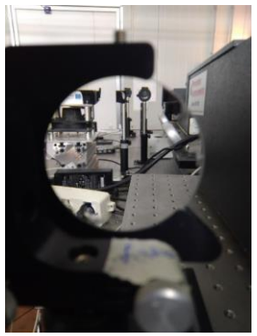


BL10.2-IUVS

Macro UVRR with SR (**200-270 nm**) and **lasers** (213, 224 and 266 nm)

Operando and **in situ** experiments, ideal for liquids and bulk samples

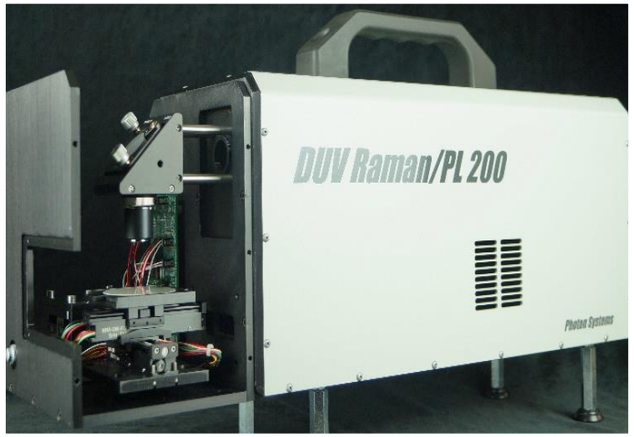


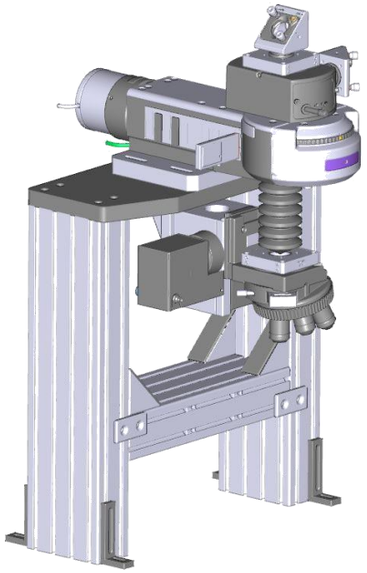


…and (near) future

PRP
Pathogen Readiness Platform for CERIC-ERIC Upgrade

Micro-UVRR station mainly dedicated for cytology and histology with micrometric lateral resolution





Fully Integrated DUV Raman micro- and macro spectrometer (248 nm excitation)
Portable instrument for in situ, real time and daily light measurements

UVRR is a sensitive molecular probe for biological species

- ✓ Non destructive technique
- ✓ Ideal tool for ***highly dilute samples***
- ✓ Possibility of ***operando*** and ***in situ*** experiments

Nucleic acids (1-10 μM)
Nucleus bases ≈ 230-280 nm

300 nm

Proteins (1-2 mg/ml)
Amide bonds ≈ 200 nm
Aromatic residues ≈ 230 nm

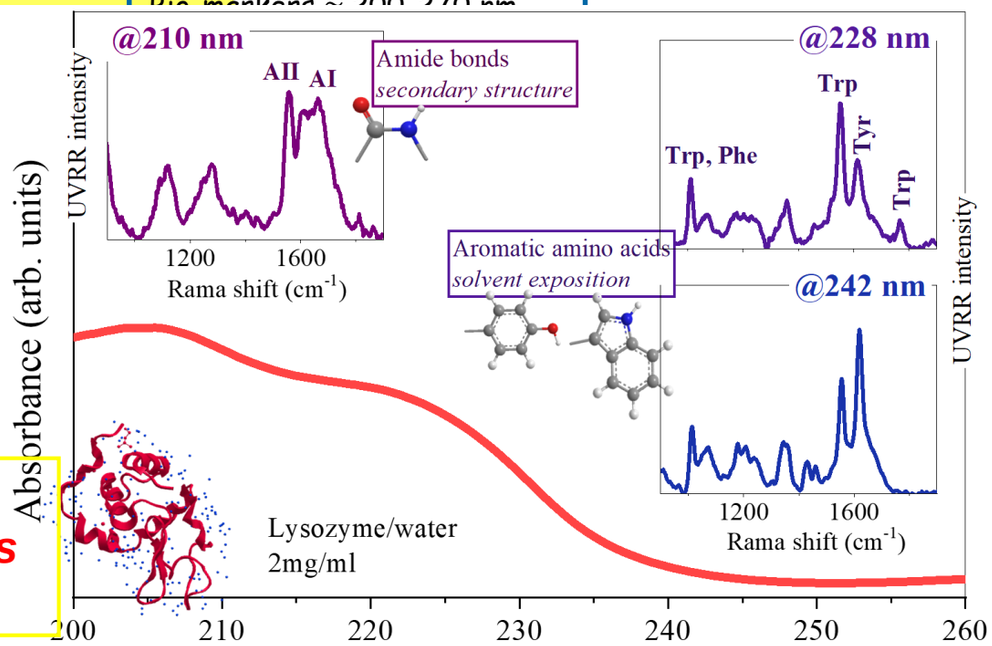
Poly-peptides
Amide bonds ≈ 200 nm

Viruses (10^9 viruses/ml)

150 nm

proline-based peptides
Imide Pro bond ≈ 210 nm

Water
A^1B_1 <- X^1A_1 ≈ 166 nm



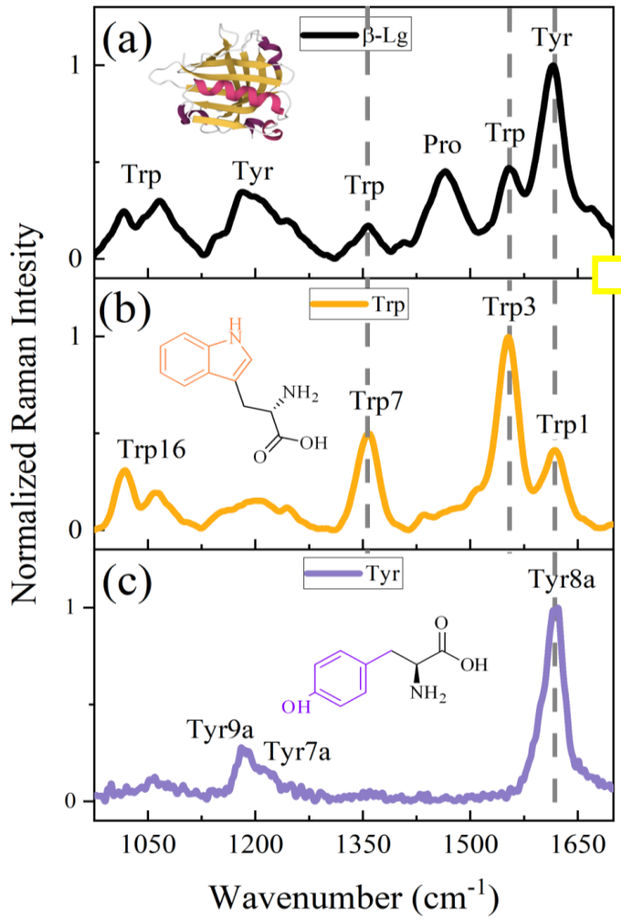
An appropriate tuning of the excitation wavelength allows to separate ***the signals from selected molecular portions***

BR et al.., Synchrotron based UV Resonant Raman scattering for material science, Molecular and Laser Spectroscopy, Elesevier (2020), Chapter 13

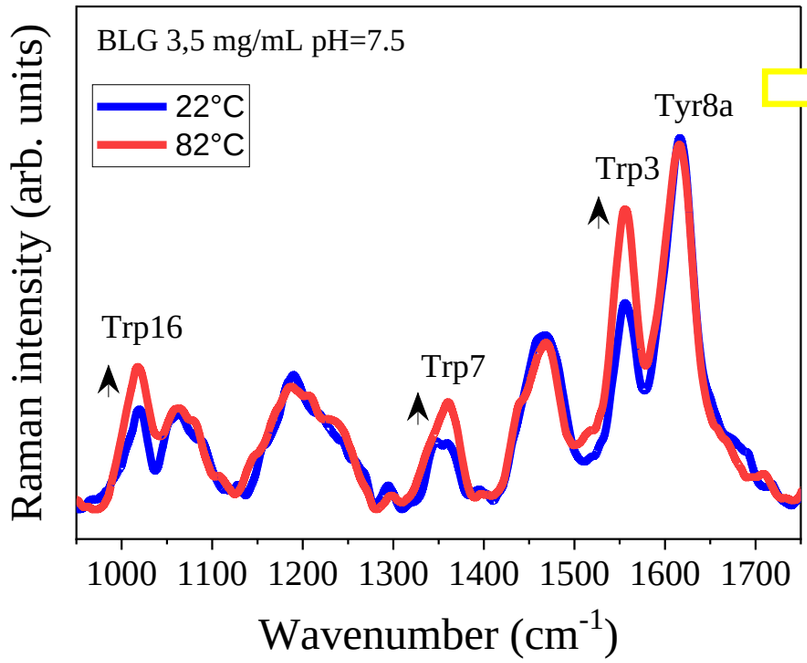
UVRR markers for structure and environment of amino acid side chains

S. Venturi et al., Int. J. Biol. Macromol. 2023, 242, 124621

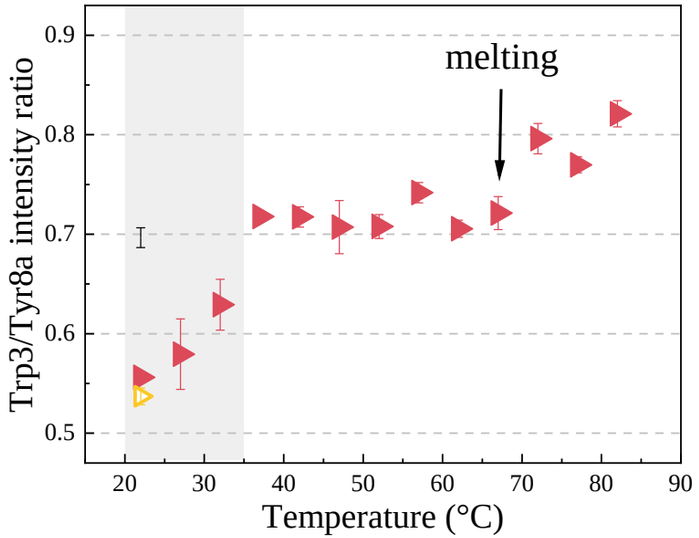
Exc. 226 nm



UVRR intensity of Trp and Tyr is influenced by their surrounding *chemical environment*



Conformational variations before the protein melting transition

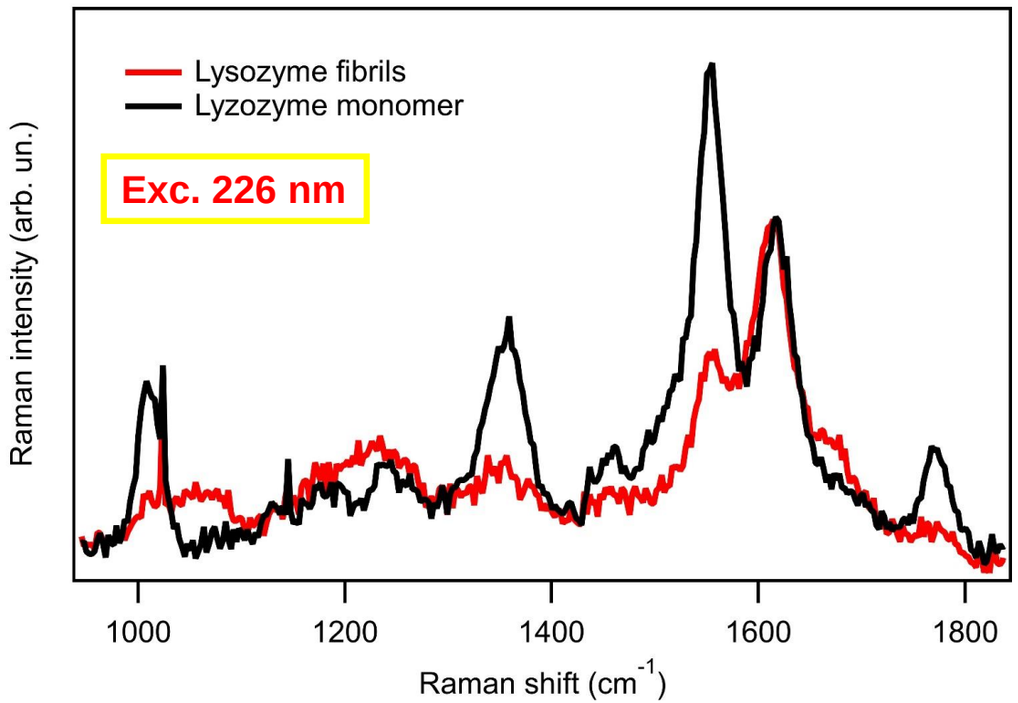


Increase in *water exposure* of Trp from change in the observed Raman cross sections

UVRR as a tool to probe tertiary structure

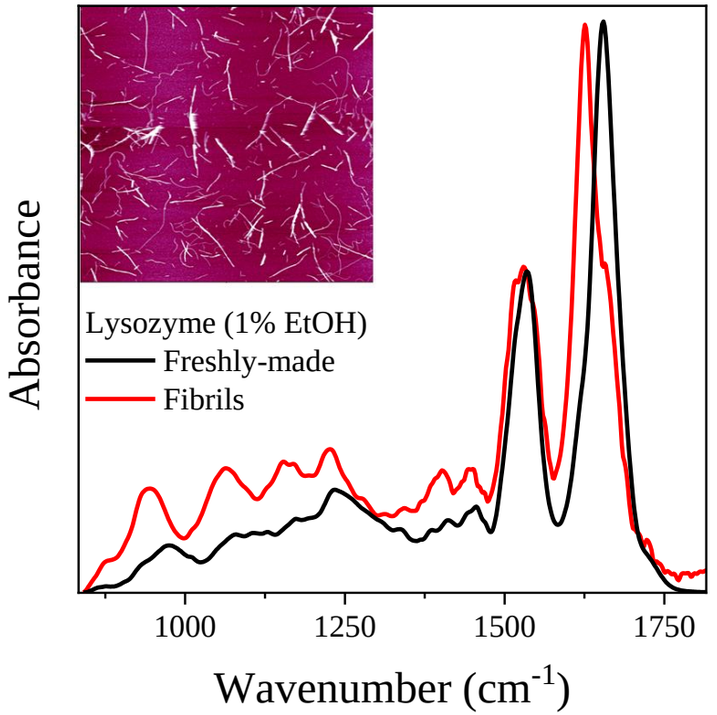
M. Pachetti et al. , Biophysical Journal 120, 4575–4589 (2021)

Modifications in the proteins aggregation state induce changes on the UVRR spectra



Modifications in the *tertiary* structure can be monitored by UVRR

FTIR spectra

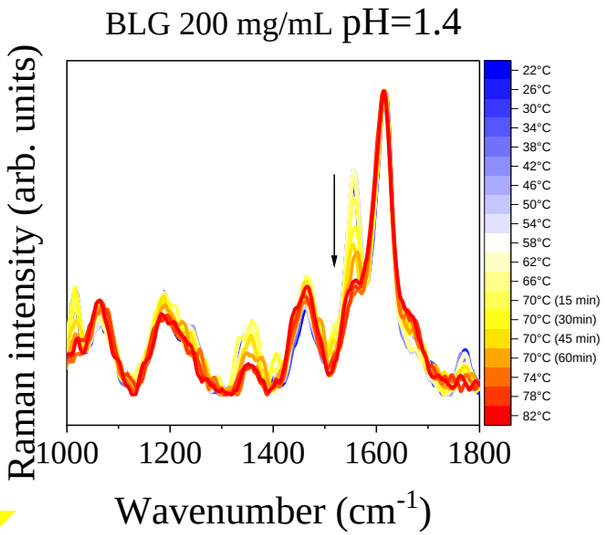


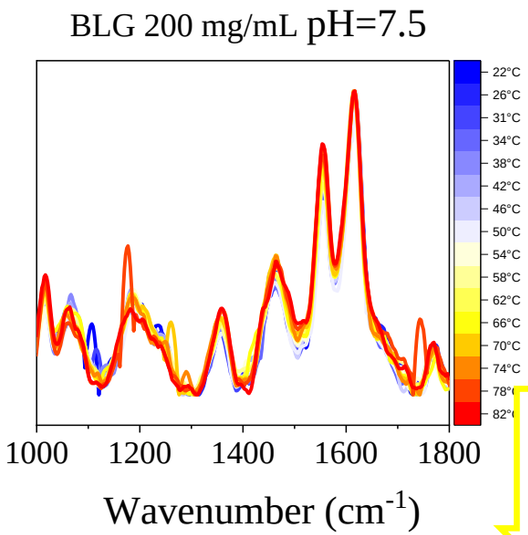
Modifications in the *secondary* structure can be monitored by FTIR

UVRR can discriminate different aggregation paths

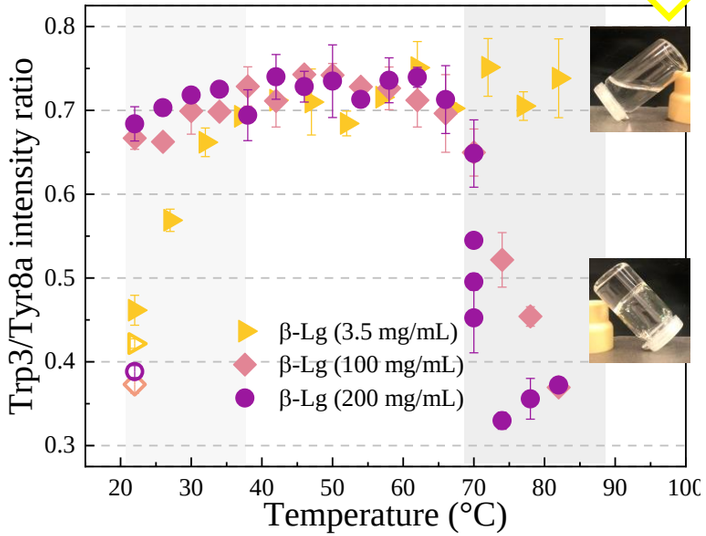
S. Venturi et al., Int. J. Biol. Macromol. 2023, 242, 124621

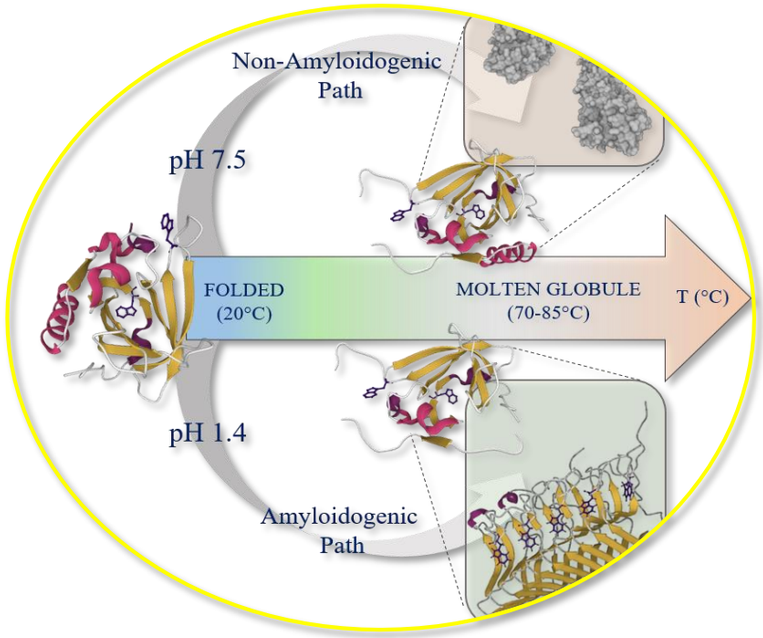
Amyloid-like-aggregates at acid pH
Formation of protein gels

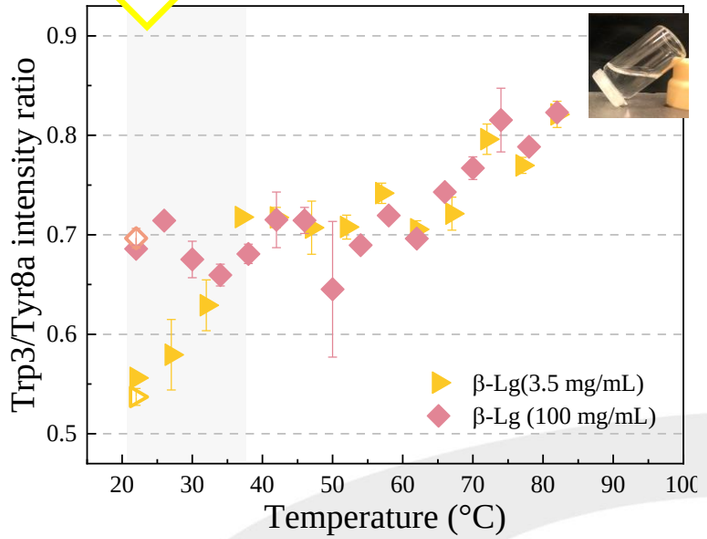




Transition from the folded to the molten globule state at basic pH







Unlocking the power of UVRR: amides in aqueous solution

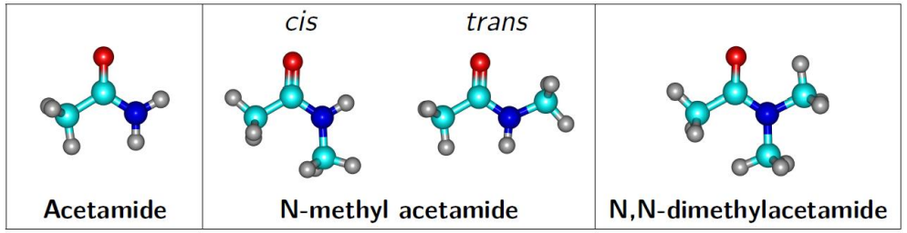
Solvation effects on spectroscopic features of amide and peptides

QM/FQ Protocol for RR simulations

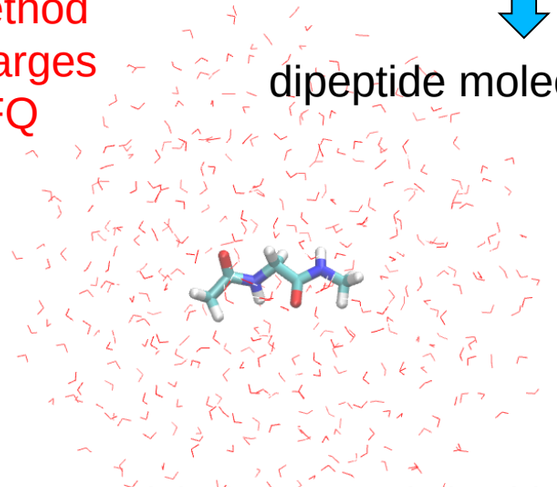
Polarizable QM/MM method based on fluctuating charges (FQ) known as QM/FQ

Test reliability of the model trough comparison with UVRR experimental spectra

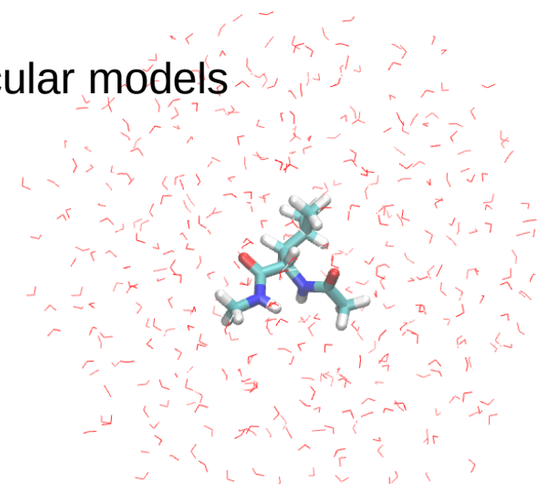
Small amides



dipeptide molecular models



N-acetylglycine- N-methylamide (NAGMA)



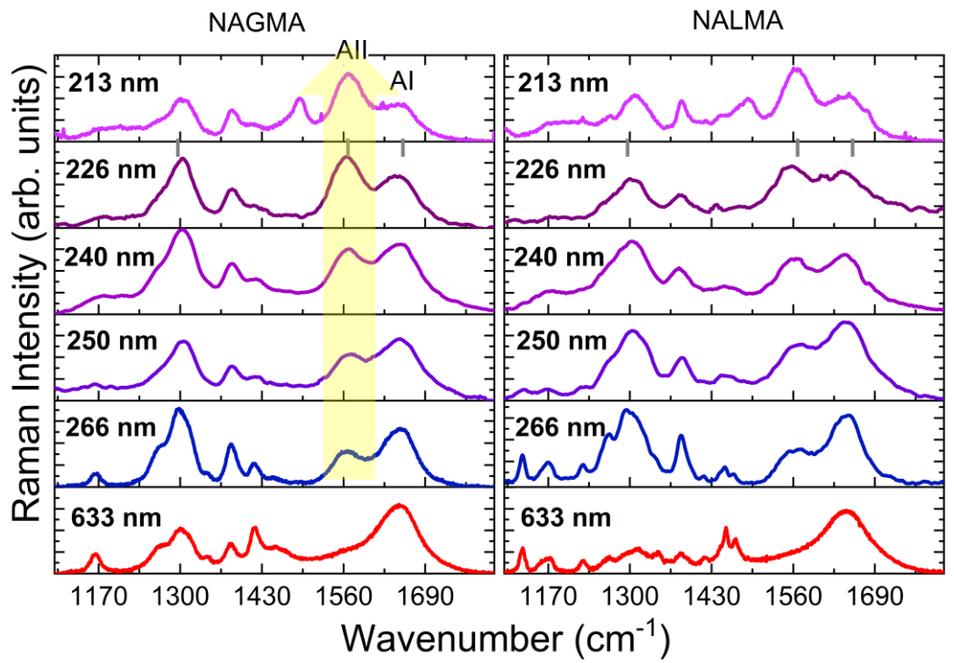
N-acetylalanine-N-methylamide (NALMA)

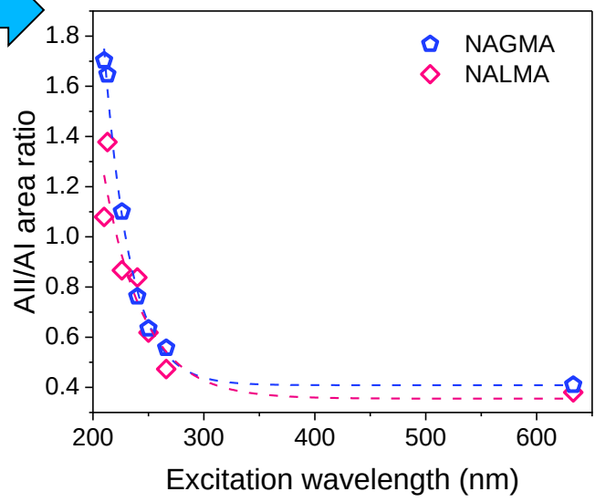
S. Gómez et al. J. Mol. Liq. 2022, 346, 117841
S. Gómez et al., J. Phys. Chem. Lett. 2022, 13, 6200−6207

UV Raman selective enhancement of Amide II signal

Selective enhancement of Amide II signal is observed even in pre-resonance conditions for di- and tri-peptides

Di-peptides





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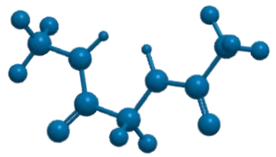
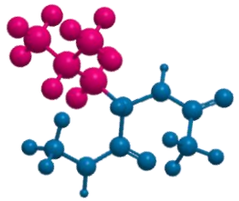


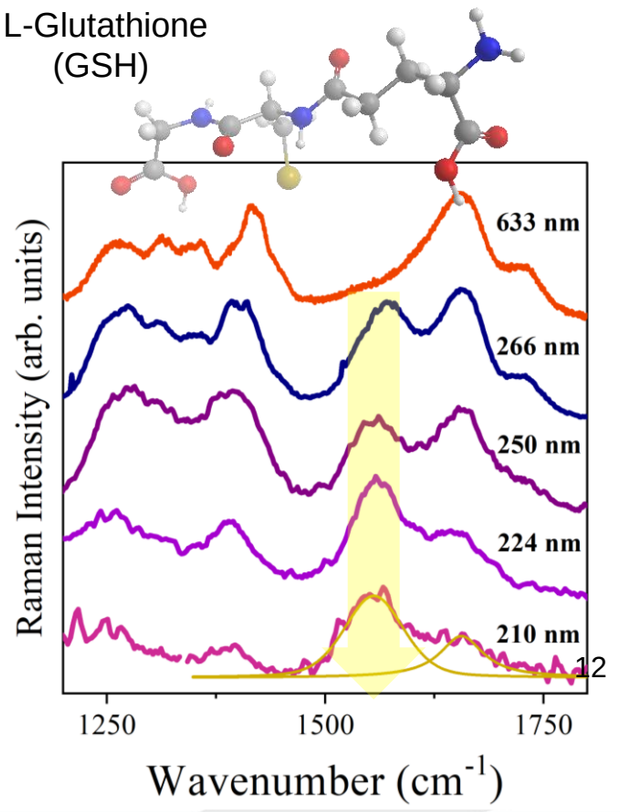
N-acetylalanine-N-methylamide (NALMA)



The effect is observed only in hydrated peptides!

Tri-peptides

L-Glutathione (GSH)



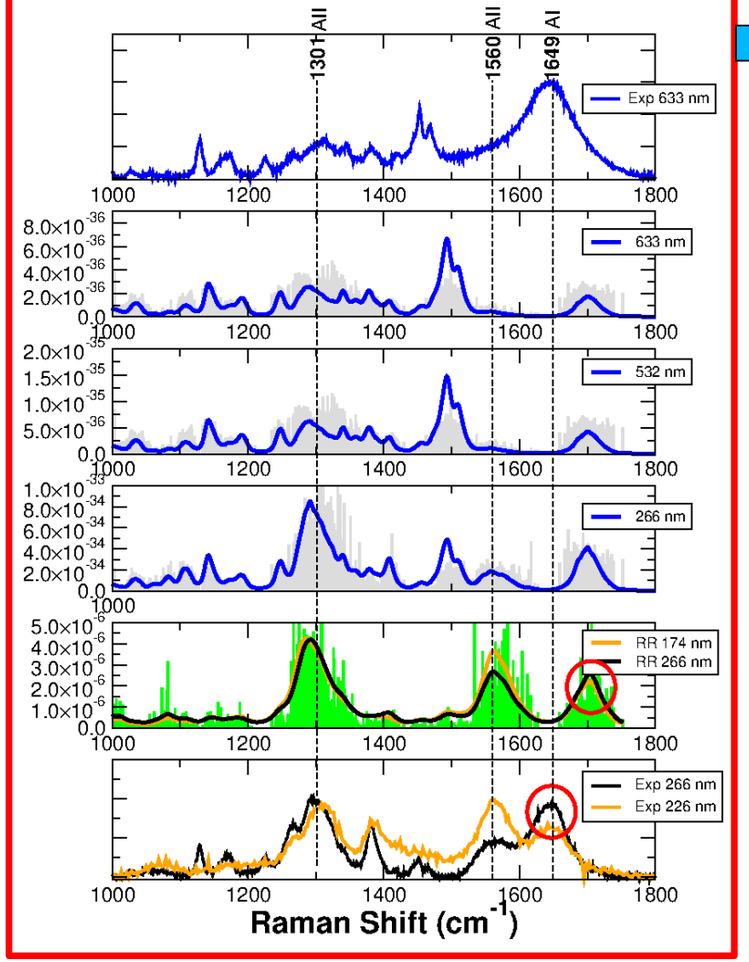
S. Gómez et al., J. Phys. Chem. Lett. 2022, 13, 6200−6207
S. Catalini et al., J. Mol. Liq. 2019, 283, 537-547

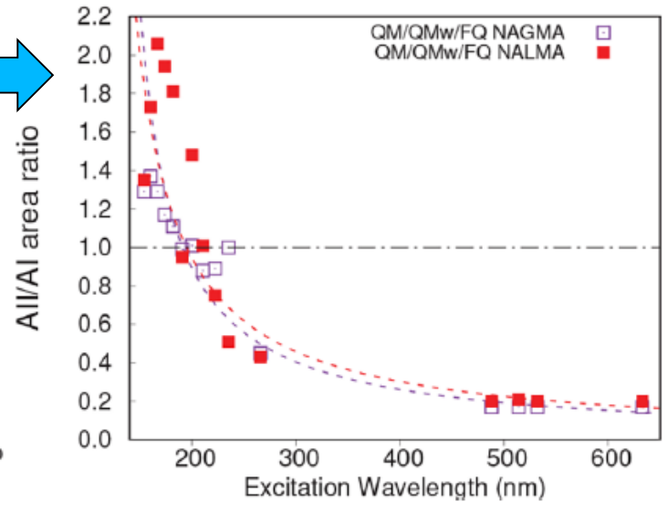
Amide Spectral Fingerprints are Hydrogen Bonding-Mediated

S. Gómez et al. J. Mol. Liq. 2022, 346, 117841
S. Gómez et al., J. Phys. Chem. Lett. 2022, 13, 6200−6207

polarizable QM/MM approach

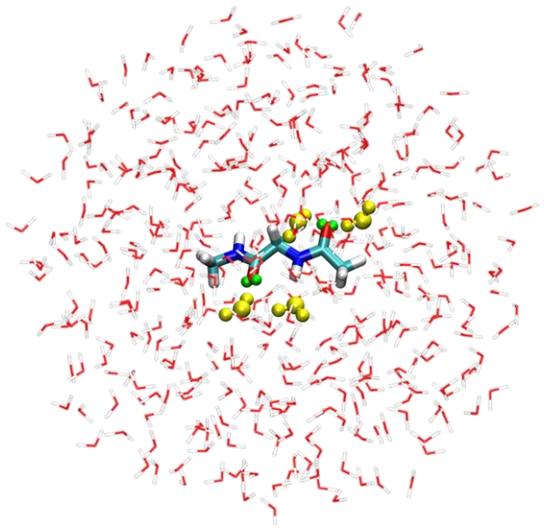
Simulations





The selective enhancement of the amides signals is **hydrogen bonding-induced**

-> linked to the effect that water molecules exert on the C=O, and N−H, C−N vibrations



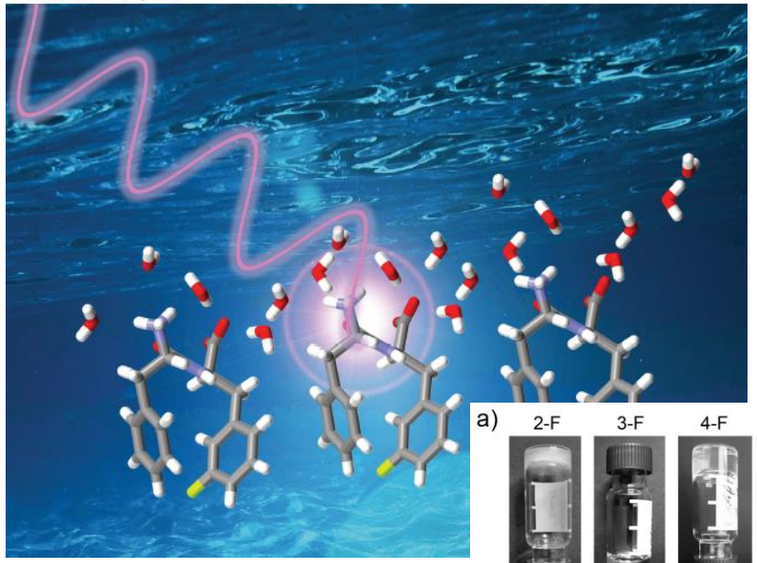
The inclusion of ***explicit water molecules*** can reproduces the experimentally observed enhancement of AII signal

-> quantum effects must be present in any modelling of the solute−solvent interactions of RR spectroscopy

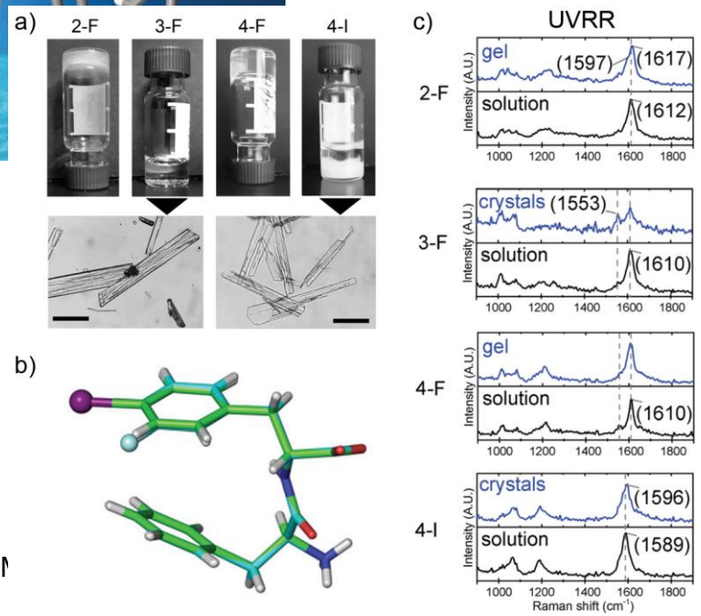
Self-assembly of small peptides

Soft Matter

rsc.li/soft-matter-journal



E. Scarel et al., Soft Matter, Vol. 18 - 11, 2129-2136 (2022)



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A Double-Walled Tetrahedron with Ag^{I}_{4} Vertices Binds Different Guests in Distinct Sites**

Samuel E. Clark, Dr. Andrew W. Heard, Dr. Charlie T. McTernan, Dr. Tanya K. Ronson, Dr. Barbara Rossi, Petr Rozhin, Prof. Silvia Marchesan, Prof. Jonathan R. Nitschke

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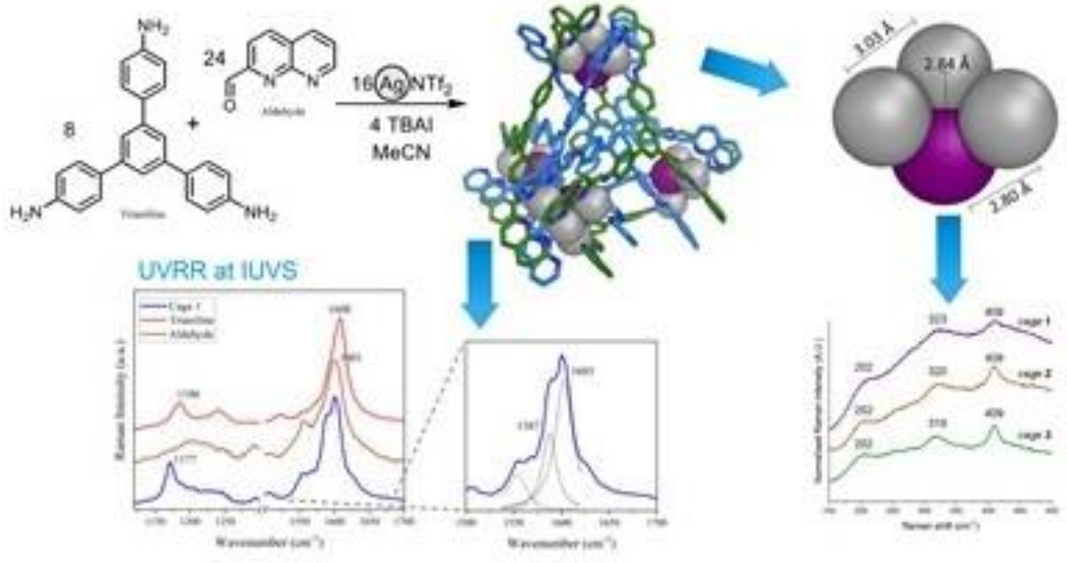

IUVS beamline staff and collaborators


Barbara Rossi


Claudio Masciovecchio


Alessandro Gessini


Francesco D'Amico


Mariagrazia Tortora


Fatima Matroodi


Sara Catalini
(visiting of University of Perugia)


Andrea Mele
(Visiting of Politecnico of Milano)

Giancarlo Franzese
Gionni Marchetti
(University of Barcellona)

Alberto Martinez Serra
Marco Monopoli
(Royal College of Surgeons)

C. Cappelli
S. Gomez
(SNS Pisa)

Sara Venturi
Paolo Foggi
(LENS, Firenze)

Yukiro Ozaky
Hidetoshi Sato
Kosuke Hashimoto
Yusuke Morisawa
(Knsei University)

Silvia Marchesan
(University of Trieste)

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Thanks for your kind attention!

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