ISTITUTO DI FISICA APPLICATA NELLO CARRARA

Nano Innovation 2020 Rome, 15-18 September
Conference & Exhibition

Label-free plasmon-enhanced Raman detection of biomarkers in neurodegenerative disorders

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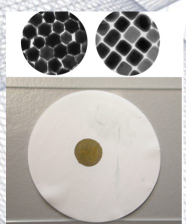
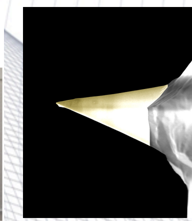
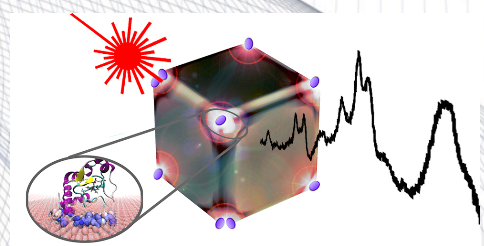
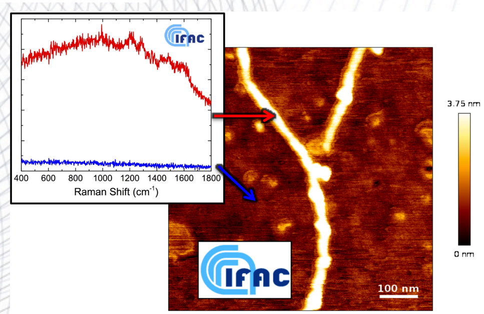




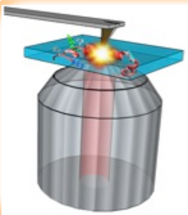




DESWEAT Project – Participants

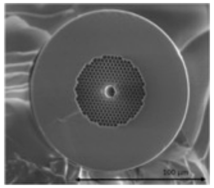
NanoBioSpectroscopy Lab.
@ bnlab IFAC-CNR

bnlab.ifac.cnr.it/index.php/research- topics/nano-biospectroscopy



TERS spectroscopy of biomolecules

Small 2018, Materials 2019

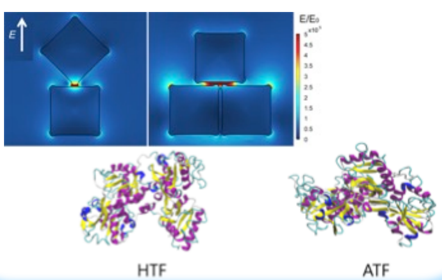


Fiber-enhanced Raman spectroscopy

J Biomed Opt 2020 (in press)

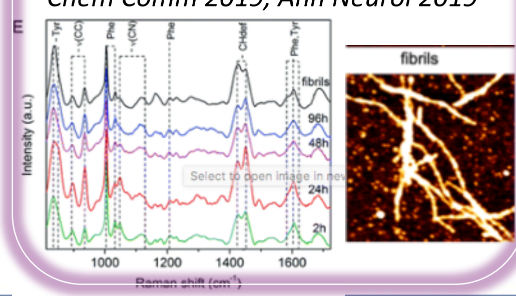
Theoretical simulations (FEM, MD)

Nanoscale 2015, ACS Appl Mater Interfaces 2016



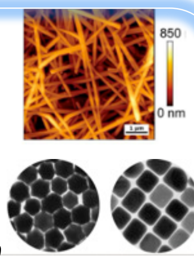
Optical detection in neurological diseases

Chem Comm 2019, Ann Neurol 2019



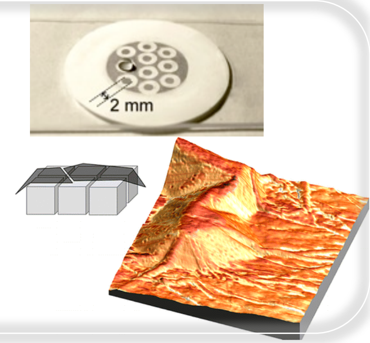
Noble metal NPs synthesis (AgNWs, AgNCs, AuNSs, others)

Nanoscale 2015, ChemNanoMat 2019



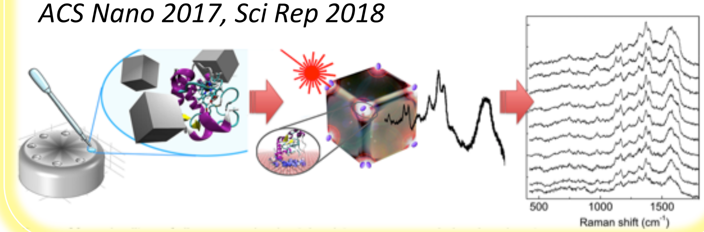
Fabrication of 2D/3D substrates for optical sensing (NPs, hybrid graphene coated)

Beilst J Nanotechnol 2016
RSC Advances 2020



Development of optical methods for biomedical analysis in physiological environments, in cells

ACS Nano 2017, Sci Rep 2018

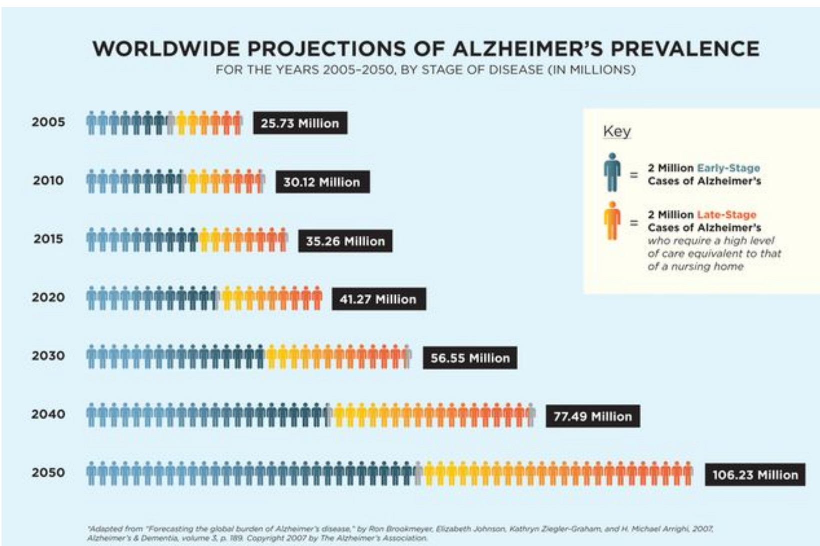


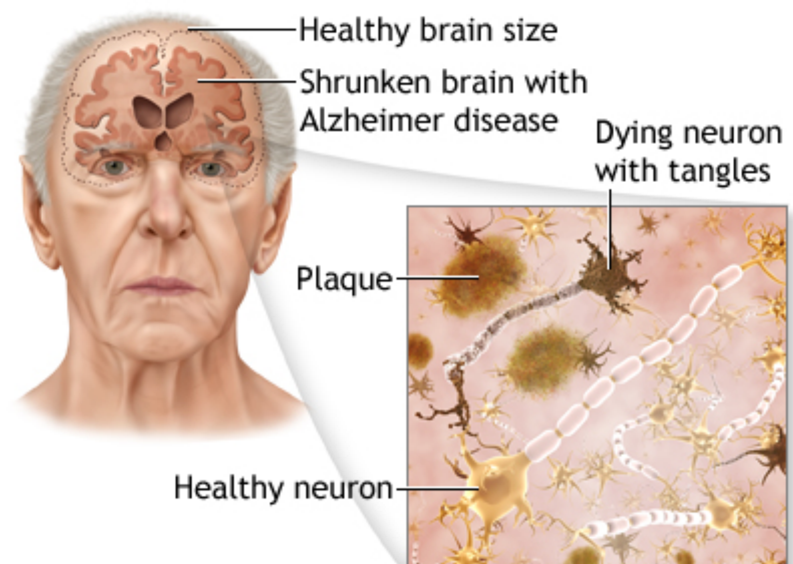
Outline

- Alzheimer’s disease (AD): current diagnostic strategies and their limits
- Amyloid Oligomers for early diagnosis of the Alzheimer’s disease
- Raman & Plasmon Enhanced spectroscopy
- Identification and Characterization of Amyloid Oligomers
- Conclusions

Alzheimer’s Disease (AD)

- **Progressive neurodegeneration** of the brain
- Cognitive disabilities, memory loss, challenges with daily tasks
- AD: up to **70% of all dementia** cases
- **5th major cause of death** by the next 20 years
- **Early diagnosis is vital** since there are already medications in the maket that may slow progress of disease. So earlier could allow to maintain a better level of brain activity

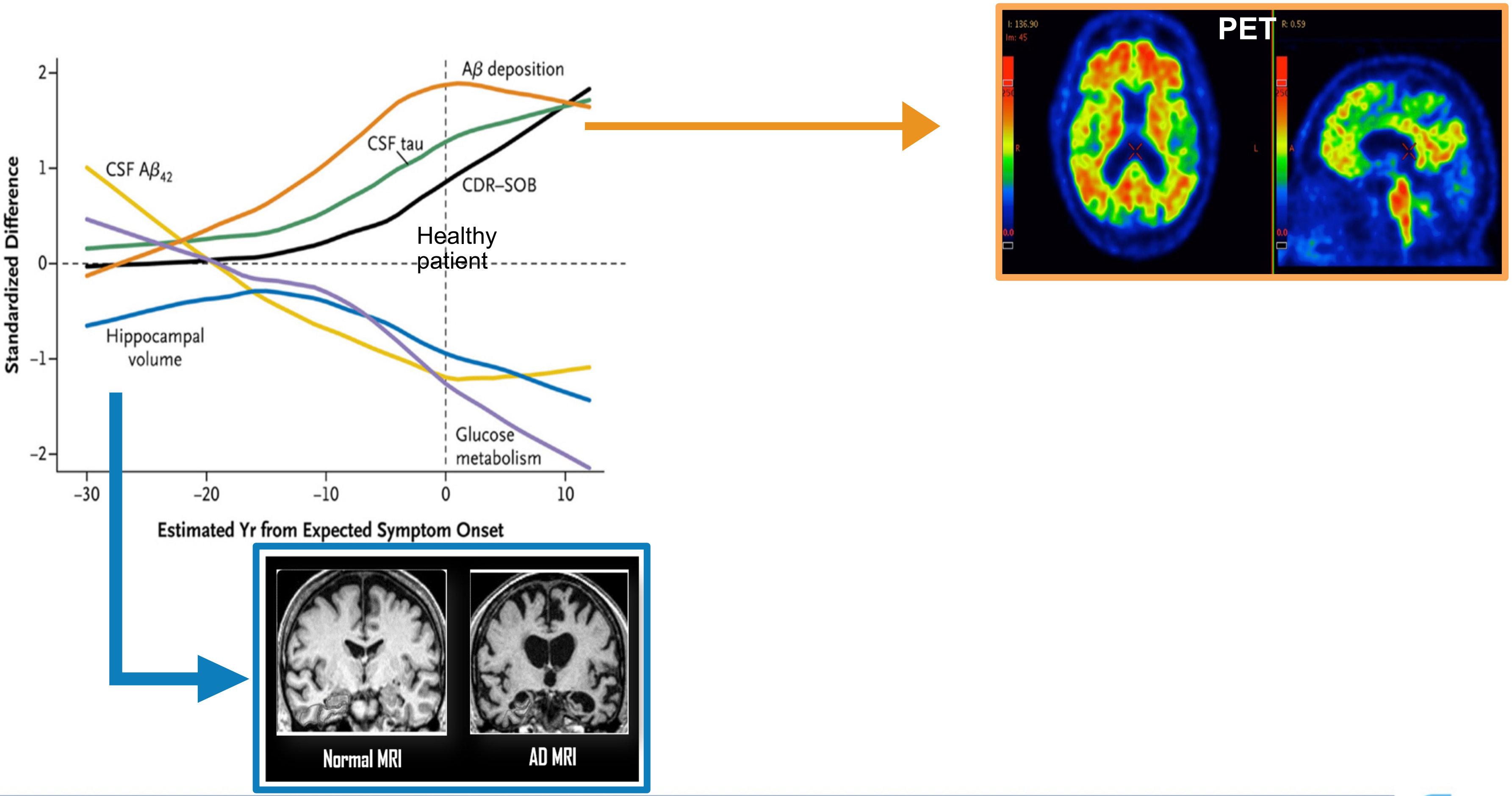




Alternative diagnostic strategies of AD

The most widely accepted and validated predictors of AD are:

1. Brain atrophy by Magnetic Resonance Imaging (MRI)
2. Amyloid β deposition by Positron Emission Tomography (PET)
3. Molecular biomarker detection in Cerebrospinal Fluid (CSF)



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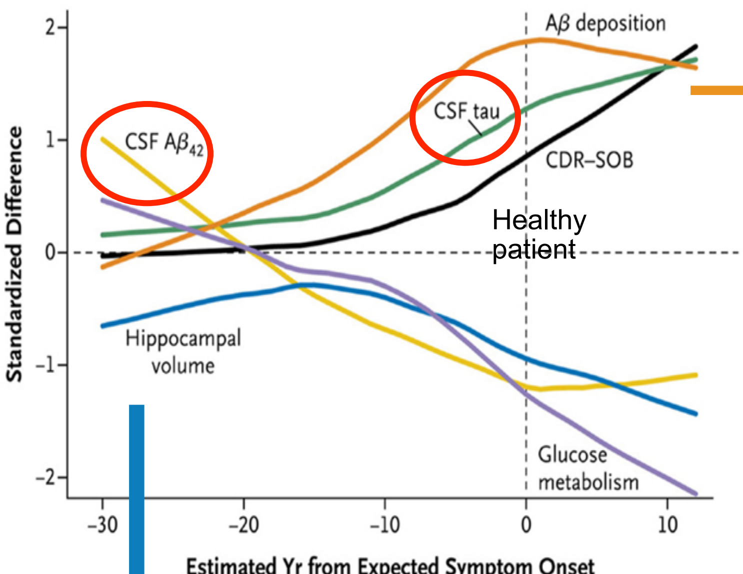
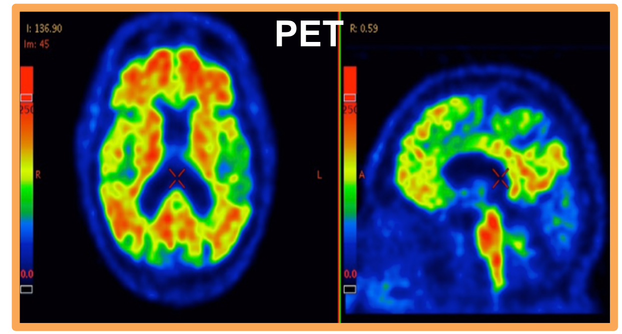
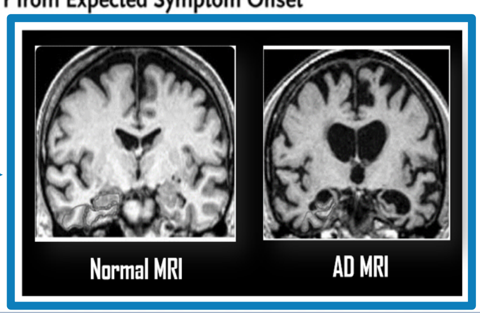
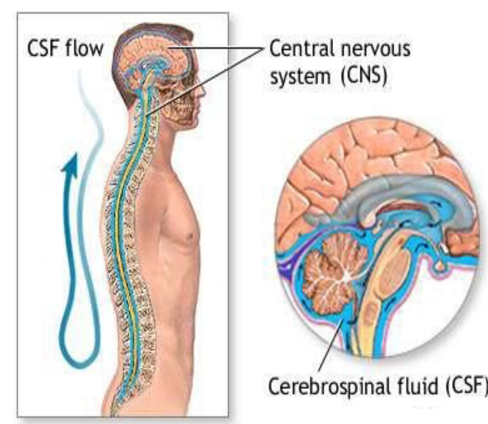






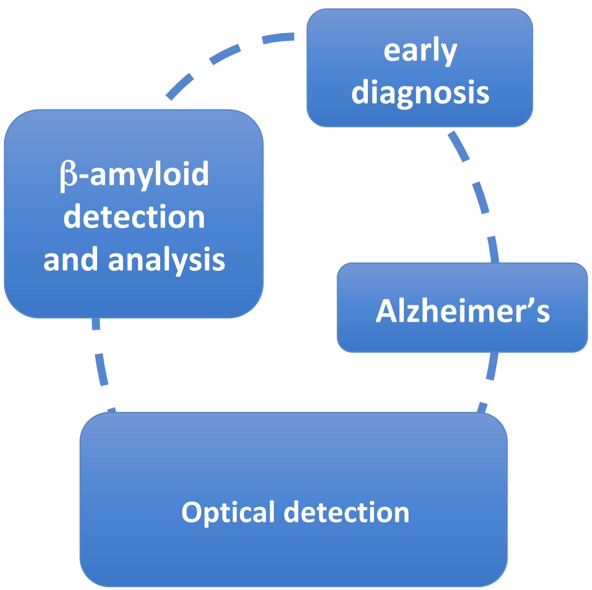
CSF in direct contact with the brain and reflects molecular changes occurring inside the brain



Aβ oligomers for early Alzheimer's disease diagnosis

- Misfolding of β-amyloid peptide and its oligomerization are early molecular events that lead to AD.

- Recent evidences suggest that trace-amounts of misfolded Aβ might be found in peripheral tissues, like CSF, of patients in the very early stages of AD.

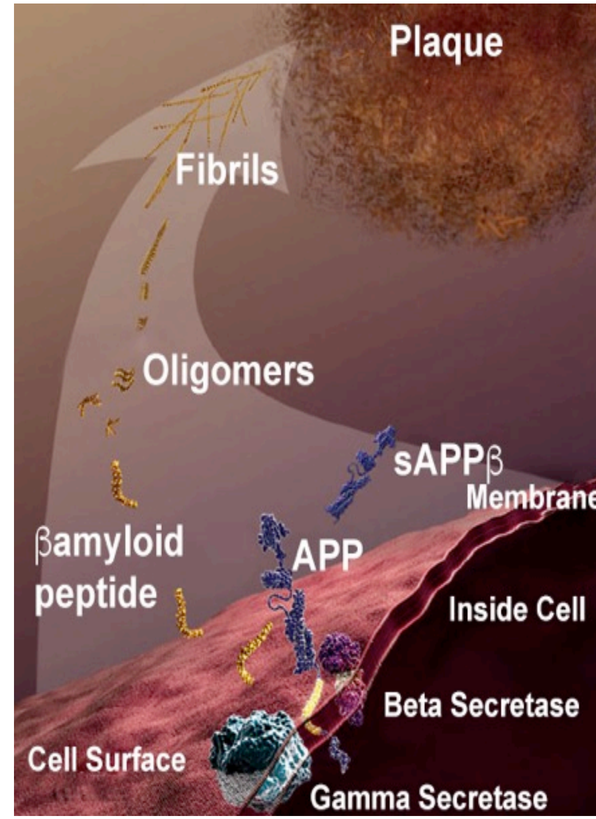


European Commission | Horizon 2020 European Union funding for Research & Innovation

EuroNanoMed3

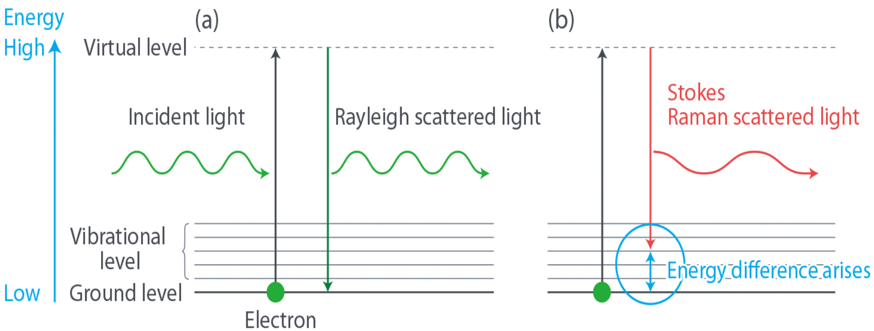
SPEEDY Project:
Recognition of Aβ oligomers in CSF and other biofluids by optical techniques (2018/2021)

- IRCCS Foundation "Carlo Besta" Neurological Institute, Italy
- Tel-Aviv University
- SME: 3D-nano



Raman & Plasmon Enhanced Raman Spectroscopy

Raman spectroscopy provide information on molecular vibrations (**fingerprint of molecules**) exploiting the inelastic scattered light by the sample under a Visible (or NIR) laser irradiation.



- Molecular identification, structural changes, folding states, etc.
- **Raman effect is very weak**: only a small portion (0.000001%) of the scattered radiation has frequencies different from that of the incident beam

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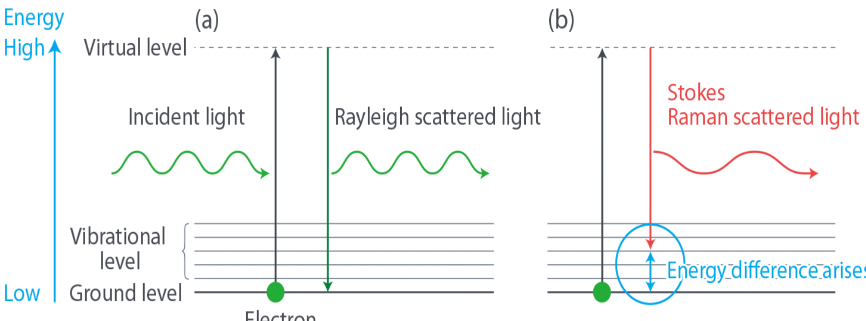

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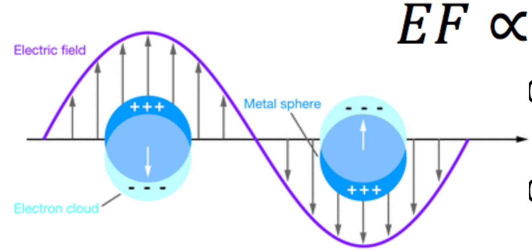
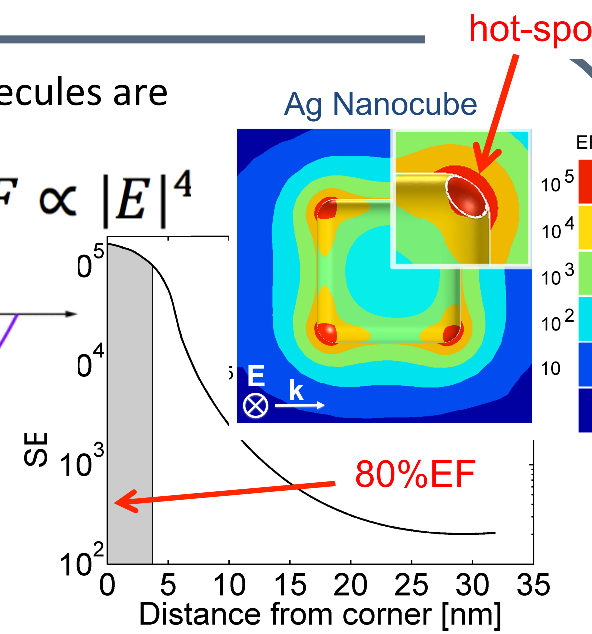
- Plasmonic NPs **can increase the Raman signal** up to 10^{14} once molecules are adsorbed on their surface (typical enhancement = 10^4- 10^7)

Upon light excitation of a metal nanoparticle the electric field of the radiation drives the **conduction electrons** into **collective oscillations on the particle surface** (Localized Surface Plasmon Resonances- LSPRs)

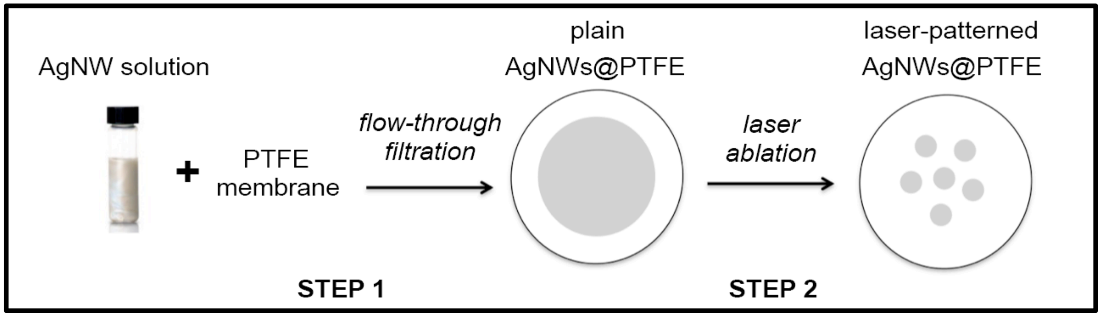


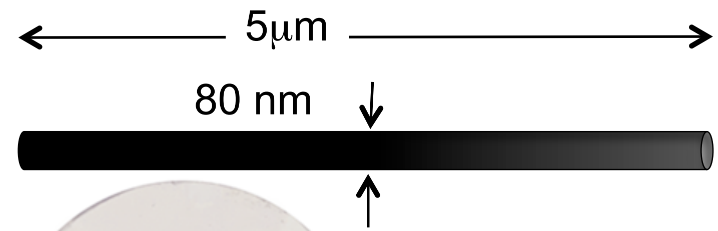
$$EF \propto |E|^4$$

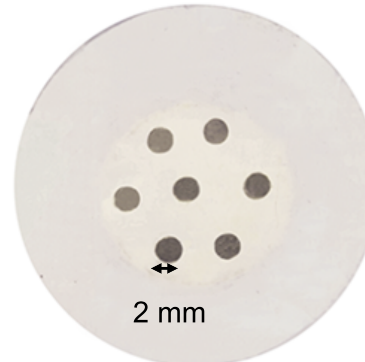


Enhancement of the local electromagnetic field (MAX at the nanoparticle surface and rapidly falls off with distance)

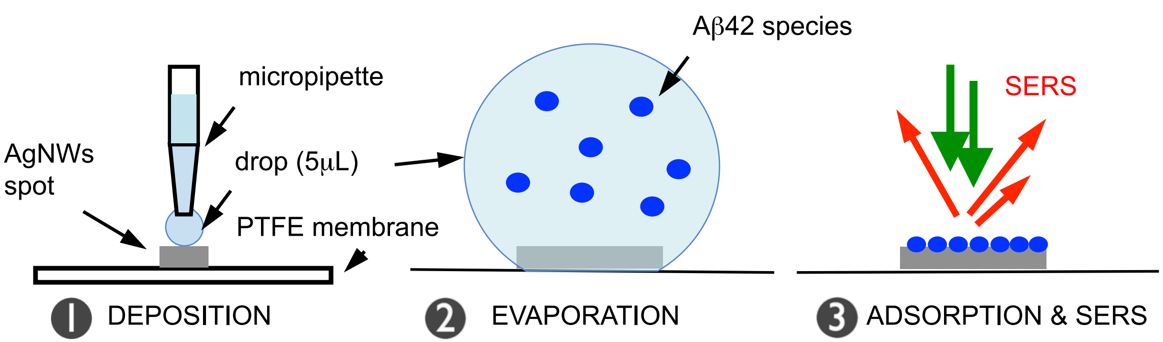
AgNWs@PTFE membranes for SERS detection of Aβ







Optical image of a SERS chip showing 2 mm in diameter SERS-active spots



Banchelli M et al, ***ChemNanoMat*****.** ***2019****, 5, 1036-43*

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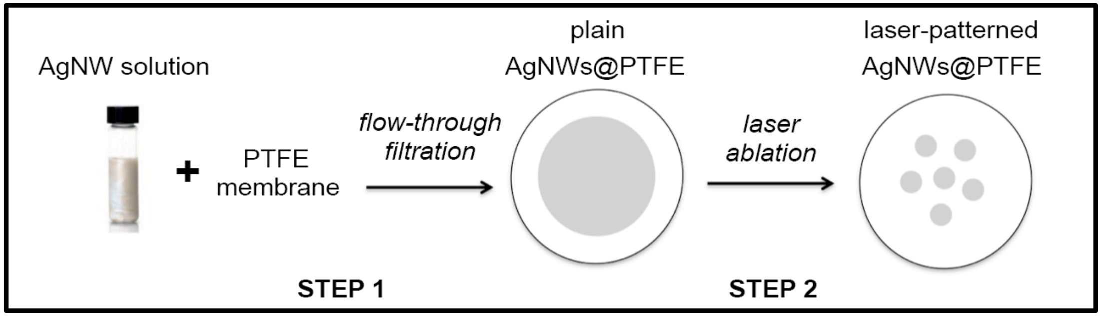
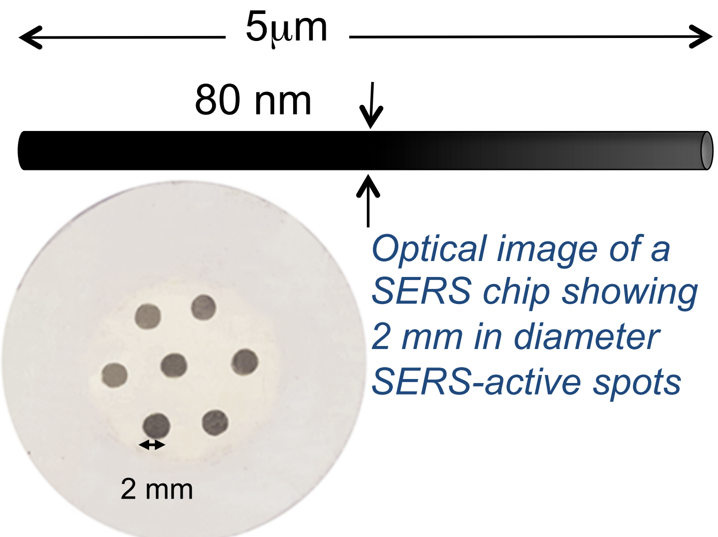
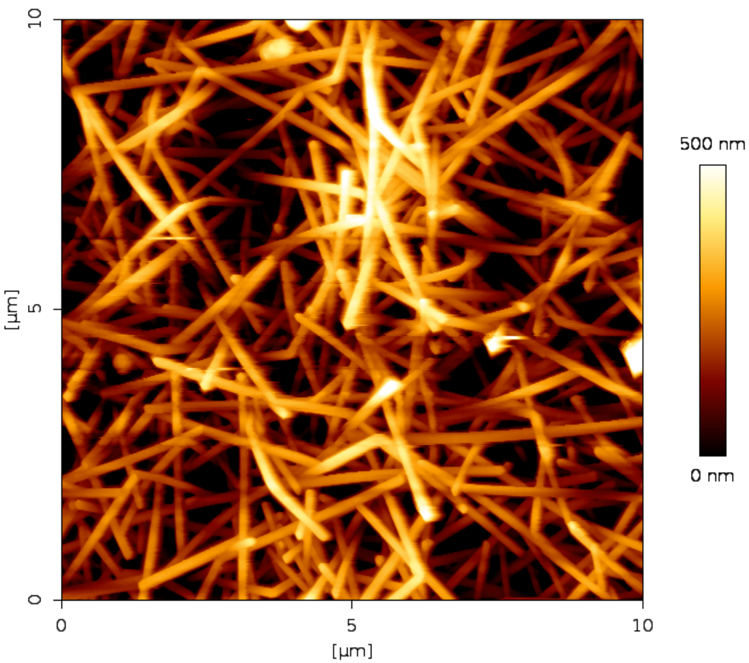
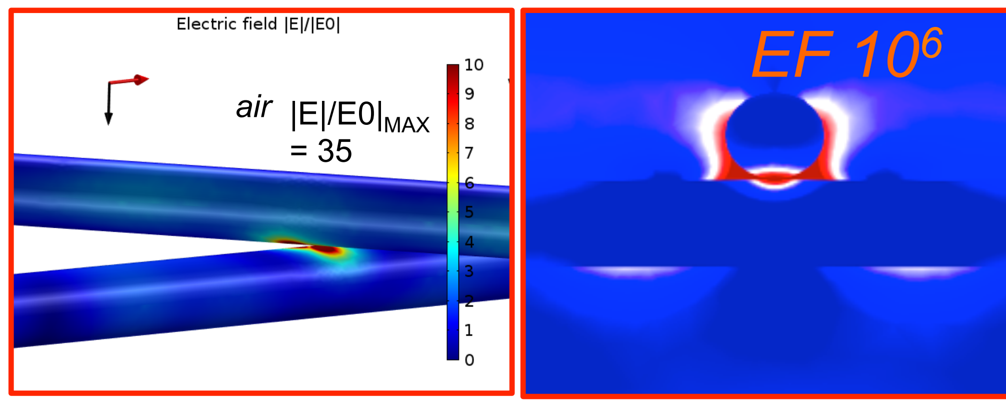






*Banchelli M et al, **ChemNanoMat**. **2019**, 5, 1036-43*



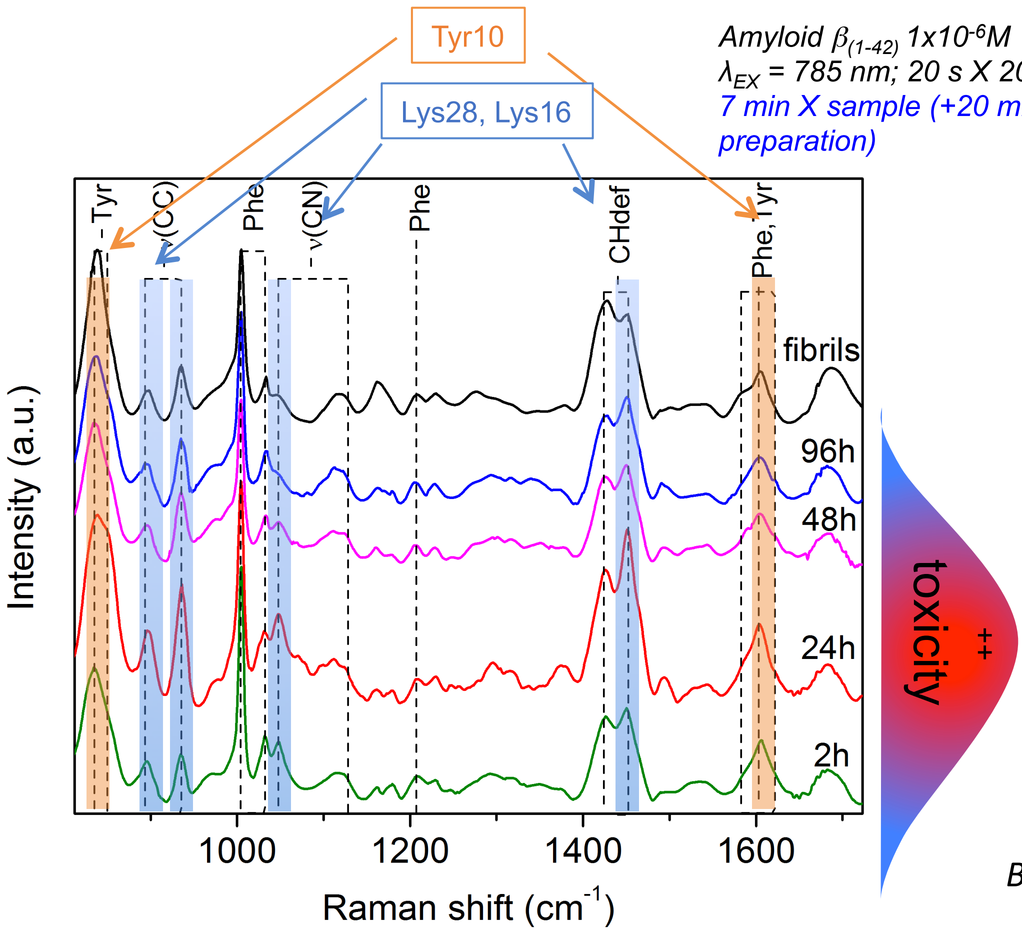
- **Effective interstitial SERS hotspots** (10/μm²) generated in the gaps between crossed AgNWs

AgNWs@PTFE membranes for SERS detection of Aβ

Amyloid $\beta_{(1-42)}$ monomer → *Hexafluoroisopropanol* / *NaOH, PBS days 0-4* → *Amyloid $\beta_{(1-42)}$ toxic oligomers and non-toxic prefibrillar aggregates*

Ladiwala et al J Biol Chem 287 24765 (2012)

Amyloid $\beta_{(1-42)}$ $1x10^{-6}M$
λ_{EX} = 785 nm; 20 s X 20 points = 7 min X sample (+20 min sample preparation)



Biomarker detection at different aggregation stages

- Rapid procedure
- Nice signals at $1x10^{-6}M$!
- Tracking amyloid conformation

(and toxicity) by following ***897, 935, 1047,1460 cm^{-1} of Lys*** and ***850,1605 cm^{-1} of Tyr***

*Banchelli M et al, **RSC Adv 2020**, 10 21907*

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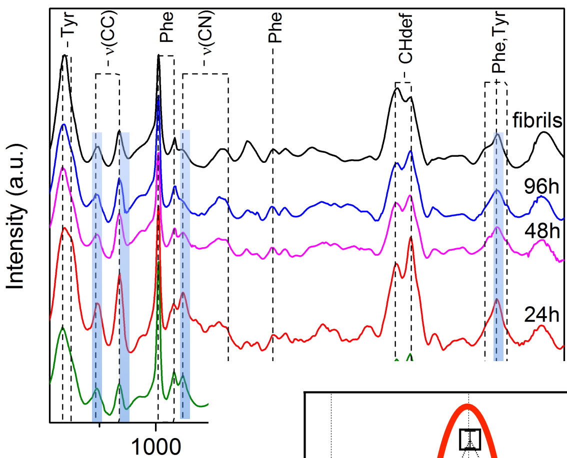

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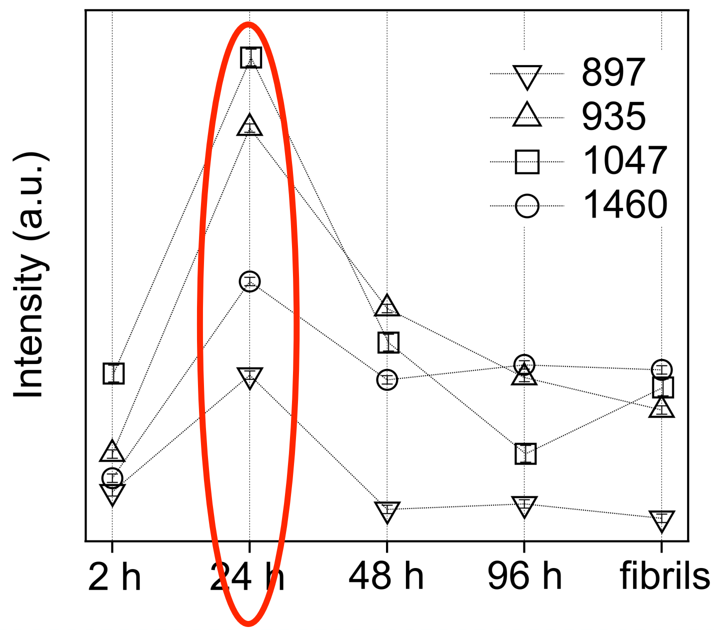
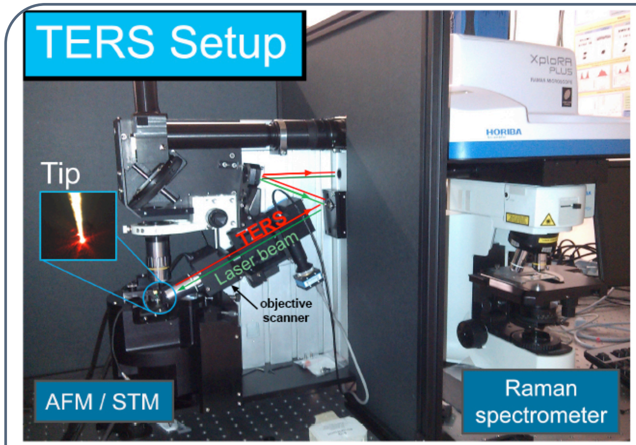


- Characteristic "toxic fingerprint" for the 24h - most neurotoxic species
- Neurotoxic stages can be identified from a body fluid sample and promptly diagnosed?
- Preliminary screening of potential/suspected AD patients

*Banchelli M et al, **RSC Adv 2020**, 10 21907*

Tip-Enhanced Raman Spectroscopy (TERS) discrimination of toxic vs non-toxic amyloid species

- TERS combines scanning probe microscopy (SPM) with optical (Raman) spectroscopy, conferring **chemical specificity to SPM and nano-resolution to Raman**



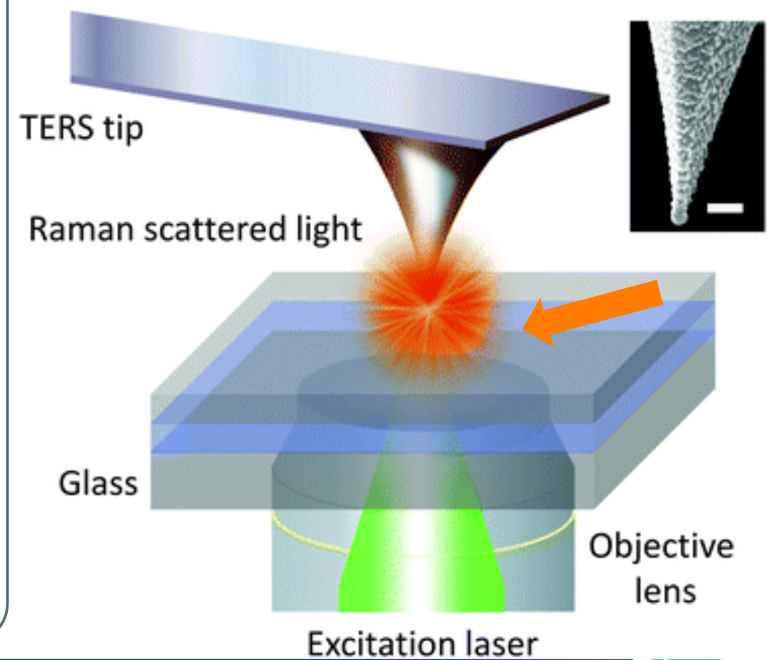
Horiba Xplora Plus Raman micro-spectrometer
AIST NT – AFM equipped with STM module
Reflection Mode-Side illumination
Excitation Wavelength @638nm

Dr. P. Gucciardi -IPCF – CNR Messina (Italy)

JPK NanoWizard Sense SPM + Renishaw InVia Raman Spectrometer
Transmission Mode-Bottom Illumination
Excitation Wavelength @632nm

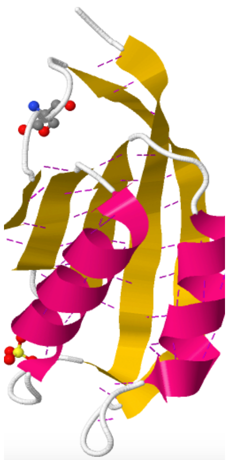
Dr. P. Matteini -IFAC – CNR Sesto Fiorentino (Italy)

In **TERS** the enhancing substrate is reduced to a **single local 'hot spot'** at the end of a very sharp tip, which can be accurately positioned on the sample surface by SPM



HypF-N as β-amyloid olig. Model

The N-terminal domain of the *Escherichia coli* protein HypF (HypF-N) is a small stably folded α/β protein with 91-residues (10 kDa).
It forms toxic oligomers and/or amyloid-like fibrils similar to those associated with Alzheimer's disease.



HypF-N
Monomer
(50 μM)

TOXIC OLIG – TYPE A
50 mM acetate buffer, 12% (v/v) trifluoroethanol (TFE)
2 mM DTT, **pH 5.5**

- **2 distinct types of stable oligomers**
- **Same polypeptide sequence**
- **Similar morphological properties**
- **Different abilities to cause cellular dysfunction**

NON-TOXIC OLIG - TYPE B
20 mM trifluoroacetic acid (TFA) 330 mM NaCl, **pH 1.7**

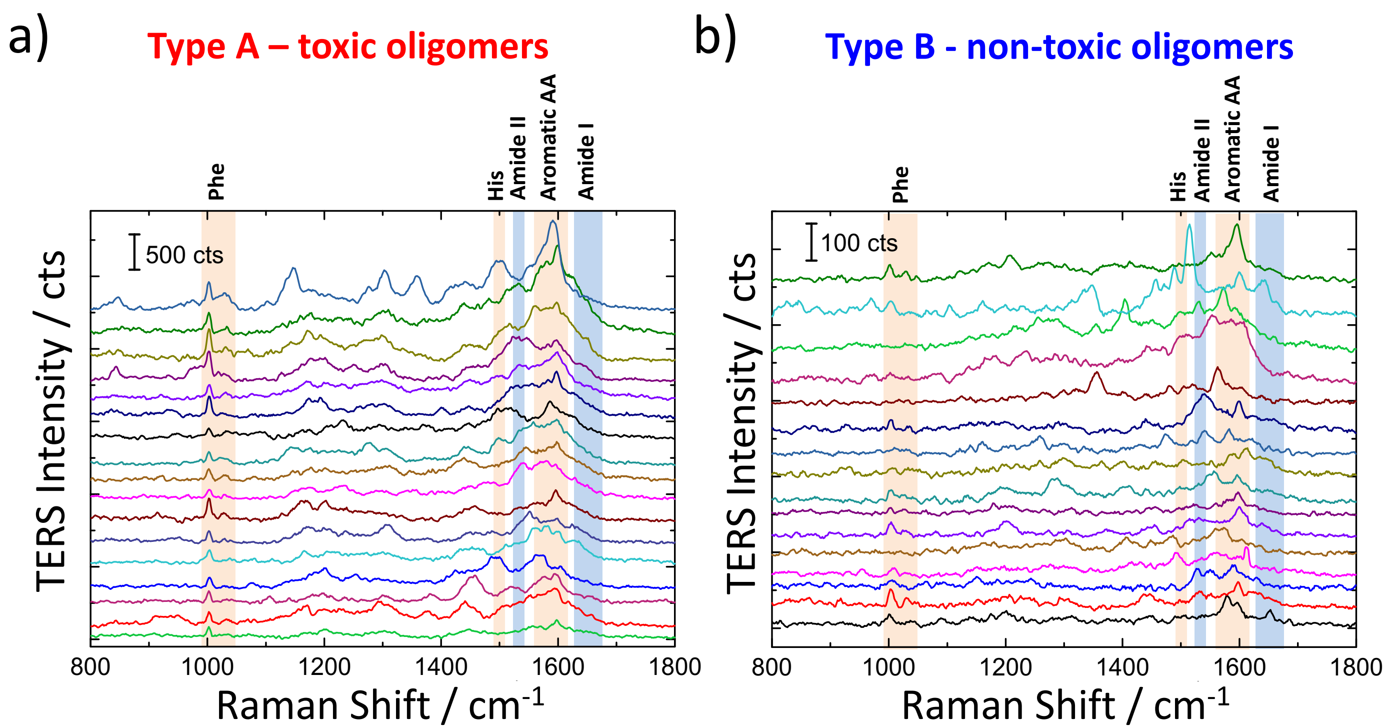
C. Capitini et al ***ChemComm*** *54 8637 (2018)*

TERS spectra

*C. D'Andrea et al **Small** 14 1800890 (2018)*

TERS spectra

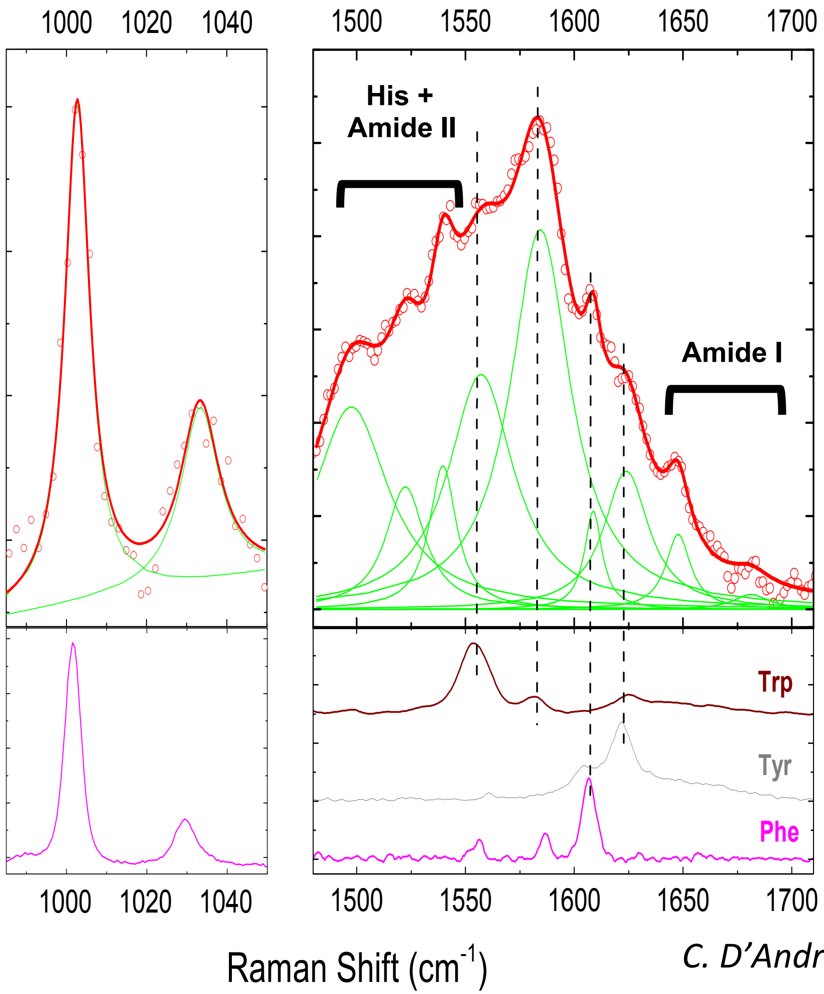
- High signal variations are consequential to the molecular complexity inspected at the nanoscale
- An immediate discrimination between Type A and B is not trivial
- A post-processing data analysis is required (fitting curves + statistical analysis)



Samples prepared incubating a gold commercial substrate in the 1µM Oligs solution for 16h, then rinsing in water and drying in air.
TERS maps: 64 points, 200nm x 200nm, step 25nm - λ_{exc} = 638nm – Power 2.51mW – Time 1s - Grat 1200 gr/mm

TERS spectra analysis

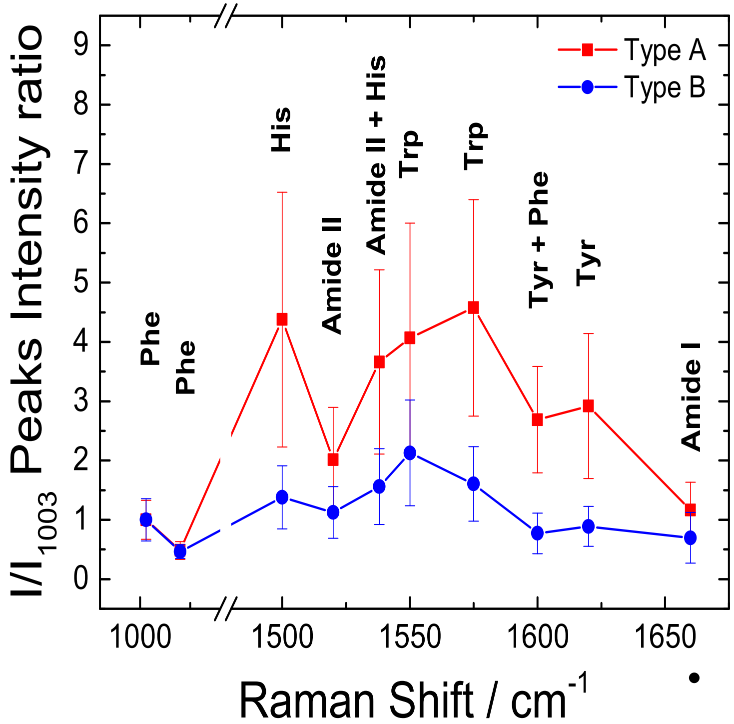
Spectra deconvolution through Lorentzian multi-peak fit



C. D'Andrea et al ***Small*** *14 1800890 (2018)*

TERS spectra analysis

Intensities were normalized to Phe mode at 1003 cm^{-1} to rule out local fluctuations of the molecular density and EF of the sample.

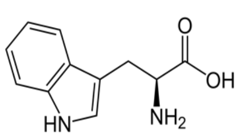


Amide I and **Amide II** modes intensities overlap (≈ Phe)

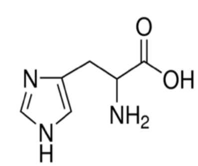
Histidine (**His**) and Aromatic Amino Acids (**Trp**, **Tyr**) are ≈ 4 times more intense for **type A (toxic)** oligomers

Outer exposition of these AA on the surface of type A oligomers where TERS tip can primarily boost their Raman intensity

- A **peculiar superficial structuring** of Type A could justify its tendency to **cause neuron dysfunction**



Fluctuations of His and Trp are expected due to the heterocyclic moieties and possible chemical interactions (charge transfer) with the gold tip

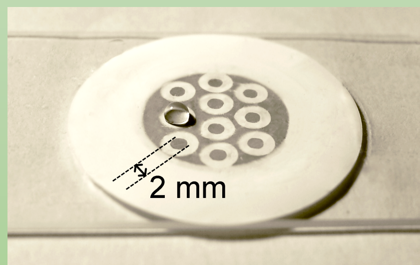


C. D'Andrea et al ***Small*** *14 1800890 (2018)*

Conclusions

SERS & TERS appear effective tools
for neurological disease research

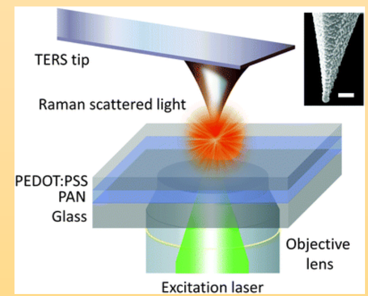
SERS



- **SERS identification of AD biomarkers**
- Simple procedure with minute amounts of liquid sample
- Low-cost SERS substrates are desirable in view of a clinical use

applied research
diagnosis

TERS



- **TERS description of structural characteristics of amyloids that can be connected with particular functional activities (e.g. high toxicity levels)**
- TERS can support pharmacological research in identifying specific molecular targets

fundamental research
therapy

Acknowledgements

People:
NanoBioSpectroscopy group @ IFAC – CNR Florence
Paolo Matteini - 1st Researcher
Marella De Angelis – Researcher
Cristiano D'Andrea - Researcher
Martina Banchelli - Researcher
Chiara Amicucci PhD student
Edoardo Farnesi PhD student

Nanosoft lab @ IPCF – CNR Messina
Pietro G. Gucciardi, Onofrio Maragò
Antonino Foti

Dep. of Biochem., Exp. and Clinical Sciences, Univ. of Florence
Fabrizio Chiti

Projects:

European Commission | Horizon 2020 European Union funding for Research & Innovation

SPEEDY (2019-21) EuroNanoMed3 MIUR

Surface-enhanced Raman scattering with nanophotonic and biomedical amplifying systems for an early diagnosis of Alzheimer's disease pathology - ID221, UE/MIUR, EuroNanoMed3 H2020 (2019-2021)

SENSOGM(2018-21) POR CreO | Unione Europea | Repubblica Italiana | Regione Toscana

Development of biophotonic sensors for the environmental detection of GMOs (Regione Toscana, POR-CREO 2014-2020)

DESWEAT (2019-21) Farnesina – Ministero degli Affari Esteri e della Cooperazione Internazionale

Development of a cost effective wearable metal nanowire-based chip sensor for optical monitoring of metabolites in sweat –MAECI Joint Res Proj Korea-Italy (2019-2021)

PRAMA (2020-23) Regione Toscana

Proteomics, RAdiomics & Machine learning-integrated strategy for precision medicine for Alzheimer's (Regione Toscana, Bando Salute2018)

Thank You For Your Attention

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