ABSTRACTS

POSTER PRESENTATION

Identification, characterization and regional localization of extra virgin olive oils from *Olea europaea* via Raman Spectrometry and Discriminant Analysis

Jana Bebek[1,2], Maurizio Musso[1], Beata Sacha-Bielow[2]

[1] Department of Chemistry and Physics of Materials, Jakob-Haringer-Strasse 2a, 5020 Salzburg / Austria,
bebekja@stud.sbg.ac.at
[2] Biospectra Labor GmbH, Griesfeldweg 19a, 5101 Bergheim / Austria

In the Mediterranean region olive oil is one of the most important edible oils. It is extracted from the whole fruit (pulp and stone) of the olive tree (*Olea europeae*). Extra virgin olive oil stands for highest quality and is declared as olive oil produced only by cold mechanical extraction with a maximum temperature of 27 °C for the pressing procedure. This high level of quality can be determined by the following parameters: Free fatty acids (defined as the amount of oleic acid) not exceeding a value of 0,8 %, and peroxide value (an indicator of freshness) having a value below 20 mEq/kg. [1]
Olive oil is produced in countries all over the world, but the main producers are Spain, Italy and Greece. To enable retracing of an oil´s geographical origin, special certificates were introduced, like Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI). These are specific for particular production areas. Because of the existence of such certificates, it is interesting to have a scientific correlation between the product, the olive oil, and its geographical origin. One possibility is to analyze the chemical composition, which is influenced on the one hand by climatic conditions, and on the other hand by geo-botanical variations and other environmental parameters. [2]
Some research approaches have used Electrothermal Atomization Atomic Absorption Spectroscopy to differ between PDO regions within Spain by determining patterns of trace elements [3] or have tried to develop a cluster of physicochemical quality indices and chemical components to describe the typicality of the oils from a single growing region. [4] Furthermore, Raman spectrometry has been used for geographical profiling by analyzing the fatty acid composition. [5]
The aim of our study was to determine origin-specific patterns of the chemical composition of extra virgin olive oils from three different European countries by Raman spectrometry, thus analyzing extra virgin olive oils from Spain, Italy and Greece of one harvest year. Raman spectra were collected with an excitation wavelength of 532 nm. By chemometric treatment (linear discriminant analysis based on a principal component analysis) of the spectral data, country-specific clusters were generated to create a model for prediction of the geographical origin. By this prediction model 89,47 % of olive oils within a training data set could be correctly classified. The excitation wavelength of 532 nm generates Raman spectra containing additional information (additional peaks belonging to carotenoids [6-7]) compared to ones generated by an excitation wavelength of 780 nm, which could have a positive impact on the high success rate.
Sanchéz-Lopéz et al. developed a trustworthy prediction model for the geographical origin of Andalusian extra virgin olive oils by analyzing the fatty acid composition via Raman spectrometry and obtained a success rate of 89 % correct classification. [5] A success rate of 89,47 % correct transnational prediction of extra virgin olive oil, gained during our study, shows the promising potential of Raman spectrometry in transnational geographical fingerprinting of extra virgin olive oils, not only for specification within a single country.

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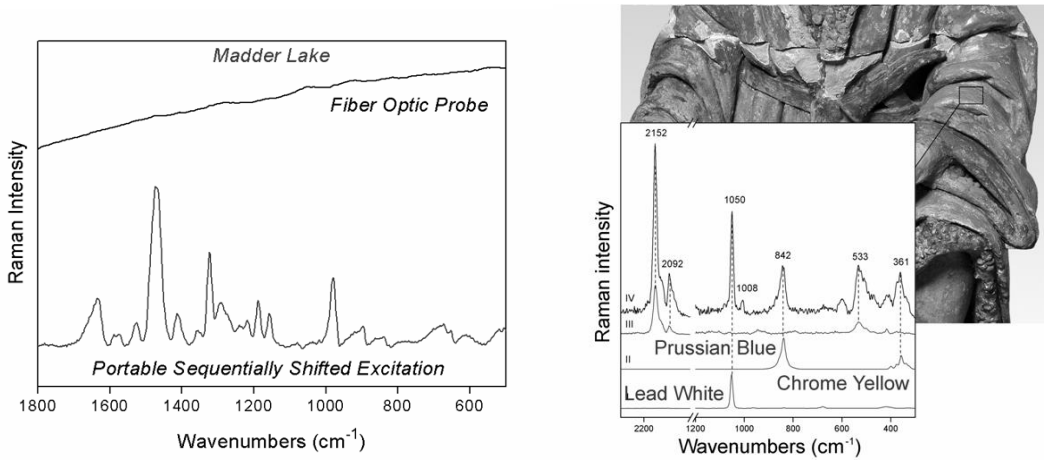
Portable Sequentially Shifted Excitation Raman spectroscopy to reveal painted surfaces in Cultural Heritage

Alessandra Botteon[1], Claudia Conti[1], Moira Bertasa[1,2], Chiara Colombo[1], Marco Realini[1], Diego Sali[3]

[1] Institute for the Conservation and Valorization of Cultural Heritage (ICVBC), National Research Council, Via Cozzi 53, 20125, Milano, Italy, tel: +39 (0)2 66173386
botteon@icvbc.cnr.it
[2] Department of Chemistry, University of Turin, Via Pietro Giuria 7, 10125, Torino, Italy
[3] Bruker Italia S.r.l. Unipersonale, V. le V. Lancetti 43, 20158, Milano, Italy

The non-invasive analysis of painted surfaces is crucially important in Cultural Heritage field to develop a deeper understanding of the artist's technique, artwork history and to supply the background for the suitable conservation procedures. Due to its quick and relatively straightforward molecular recognition of inorganic and organic compounds, portable Raman spectroscopy is one of the advanced analytical techniques currently used for in situ non-invasive analyses, together with portable reflectance FT-IR and handheld XRF spectrometers [1]. However, conventional portable Raman spectrometers suffer from critical limitations in the analysis of art samples, such as low Raman signal sensitivity, short spectral range, interferences from ambient light and, above all, high impact of fluorescence [2,3].
In the present study a new promising generation of portable Raman spectrometers is tested, able to dramatically reduce the fluorescence contribution in Raman spectra. It uses Sequentially Shifted Excitation (SSE) method [4] where each laser (with wavelengths ranging from 700 to 1100 nm) is temperature-shifted over a small wavelength range. The position of Raman intensities in spectral space changes with the excitation wavelength, while unwanted spectral intensities corresponding to fluorescence, stray light, fixed pattern detector noise etc., remain unchanged. This difference allows extracting the Raman spectrum separated from the fluorescence spectrum. Moreover, one of the laser is specifically devoted to the acquisition of the spectrum in the 2000–3200 cm^{-1} range, thus allowing to obtain high sensitive information also in the CH stretching region, usually missing using a conventional portable Raman system equipped with a NIR excitation wavelength. The analytical capability of the spectrometer is critically discussed using laboratory specimens and comparing its performance with other conventional high performance laboratory Raman instruments. The potentiality in Cultural Heritage field has been demonstrated by its application *in situ* to study the complex polychromy of Italian prestigious terracotta sculptures of the 16th century [5].



Comparison between PSSE and conventional Raman on madder lake (left) and PSSE application on painted sculptures (right).

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Study of the processes of unfolding, aggregation and gelation of lysozyme in denaturing conditions through vibrational spectroscopy

Sara Catalini[1], Lucia Comez[2], Paola Sassi[3], Assuntina Morresi[3], Barbara Rossi[1], Cettina Bottari[1], Alessandro Gessini[1], Francesco D'Amico[1], Claudio Masciovecchio[1] and Marco Paolantoni[3]

[1] Elettra-Sincrotrone Trieste, Strada Statale 14 Km 163.5, Area Science 70 Park, 34149 Trieste, Italy
Sara-catalini@libero.it
[2] IOM-CNR c/o Dipartimento di Fisica e Geologia, Università di Perugia, via Pascoli, 06123 Perugia, Italy
[3] Dipartimento di Chimica, Biologia e Biotecnologie, Università di Perugia, via Elce di sotto 8, 060123 Perugia, Italy

The complex structure of a protein is the result of interplay among different types of interactions that are strongly affected by the solvent; the balance between intramolecular and protein-solvent attractions determines the equilibrium between folded and unfolded state of the macromolecule [1, 2]. Protein conformation is strictly connected to its activity and, at the same time, a degree of structural flexibility is also requested for the protein in order to be active, the solvent playing a crucial role in this biological asset. This action, usually investigated under physiologic conditions, can be explored in more depth by studying the solvating properties of proteins as a function of pH, ionic strength and solvent composition [3].
In general, protein systems rearrange to minimize the interaction between hydrophobic residues and the polar solvent [4]. Depending on solvating conditions, the stabilization driving force can favor the folding of the protein system toward its native structure or can lead to misfolding and/or aggregation of protein molecules [5]. Such an aggregation process is characterized by different steps in which the protein undergoes conformational rearrangements and intermolecular association to form stable structures of increasing complexity, going from small clusters to amyloid fibrils, possibly passing through intermediate species. These species are involved in many neurodegenerative diseases [6, 7]. The formation of ordered aggregates is also interesting in biomaterial science since they can originate biocompatible hydrogels whose proprieties can be modulated by changing the aggregation condition [8]. Often transparent thermoreverisible gels, suitable for spectroscopic investigations, can be easily produced.
Due to its relative small size and simple structure, hen egg white lysozyme (HEWL) is one of the most suitable models to investigate protein denaturation.
In this work, we have investigated the unfolding, aggregation and gelation processes of highly concentrated solution of lysozyme in denaturing conditions at different temperatures. The selected concentrated conditions have the double benefit of favoring the gelation process and mimicking the situation of crowding of the cytoplasm in living cells. Infrared (IR) and UV Resonant Raman (UVRR) spectroscopy have been used to probe structural properties of both protein and aqueous environment, in concentrated samples during the phase transformations. This study has allowed to develop a methodology for the preparation and characterization of transparent protein hydrogels with different physical characteristics.

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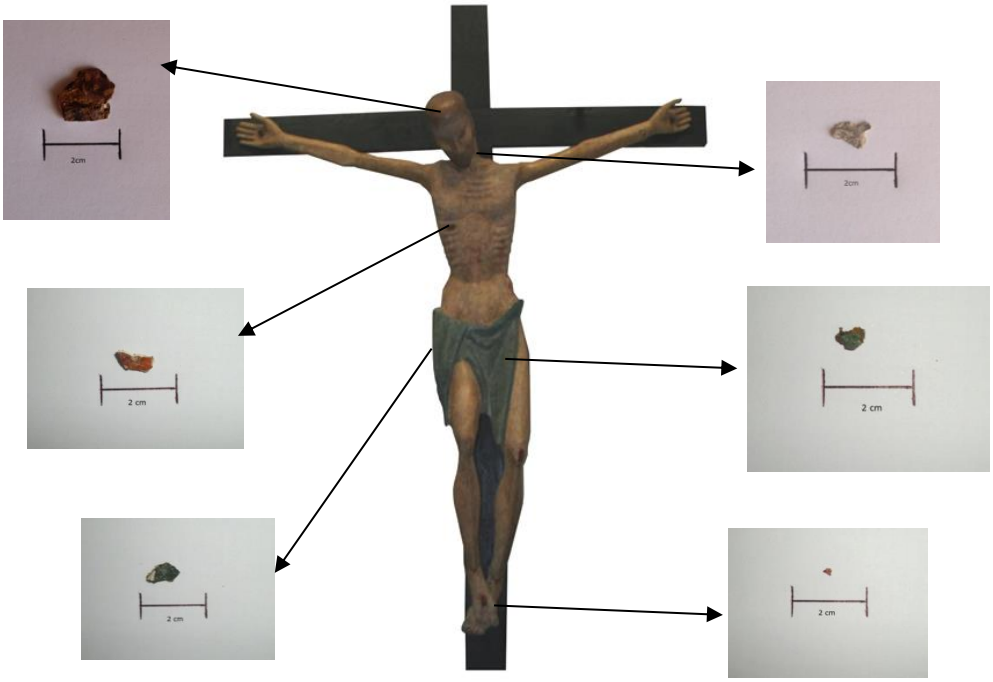
Micro-Raman analysis on the pigments of an ancient cross in Calabria.

Enzo Cazzanelli[1], Danilo Grande[1], Angela Fasanella[1] and Marco Castriota[1]

[1] Dipartimento di Fisica, Università della Calabria, Ponte P. Bucci, Cubo 31 C, 87036-Arcavacata di Rende, Cosenza, ITALY

enzo.cazzanelli@fis.unical.it

This study was carried out on a cross located inside the church of St. Mary Major in Acri. The story of this cross is not very clear and its dating is still uncertain as well, however ranging between the XV and the XIV century. The wooden sculpture of crucified body of Jesus was painted and the conservation state of the color appeared good. A detailed analysis of the pigments has been considered very useful to verify the restoration history of this manufact, and to discriminate between original painting and later restoration activities. Very small pigmented fragments have been obtained during the last restoration work, by using para-destructive methods, i.e. extracting them from zones not visible or else from the most damaged of the manufact; six samples were collected in this way from the different parts of the body, as shown in the figure below.Later they have been analyzed by a micro Raman apparatus LabRam (Horiba-Jobin Yvon), by using the 633nm line of the He-Ne laser as excitation; several spectra from different microscopic regions have been collected for every sample. The micro Raman analysis reveals the presence of the classical pigments expected for the estimated age of the manufact, like gypsum, terra di Siena, vermilion, carbon black and others. In addition, other pigments of more recent use have been found, like Prussian blue, titanium white, lithopone, chromium yellow, mostly in the perizoma around Jesus body, which appears green. This is a clear indication of a relevant modification of the artifact in the modern times, when the perizoma was totally painted again; smaller change has been revealed surely by the presence of “modern” pigments in other points of the body.



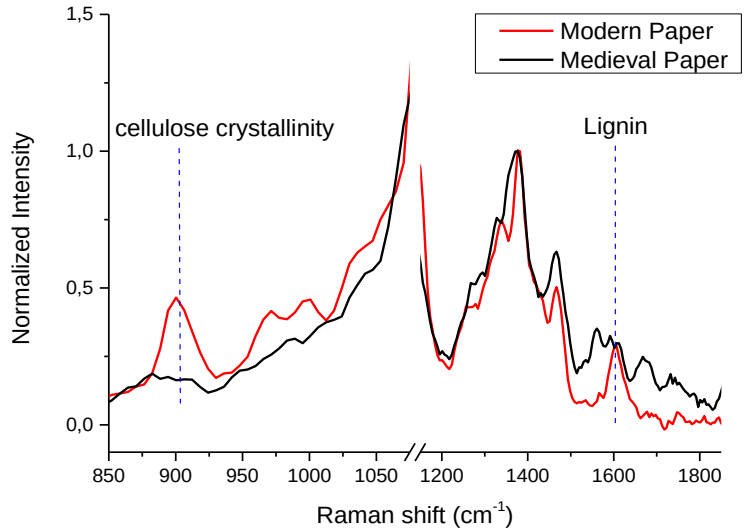
Total image of the cross of St. Mary Major church in Acri, with photographs of the analyzed samples and their origin from the different parts of the sculpture.

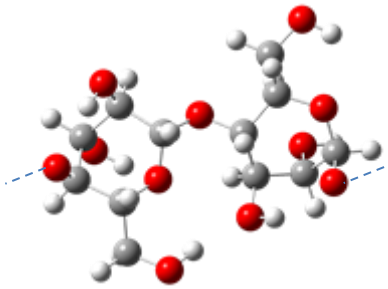
Ancient and Modern Paper: study on degradation process by means of NIR Raman Spectroscopy

Daniele Chiriu[1], Pier Carlo Ricci[1], Giancarlo Cappellini[1], Roberto Cardia[1], and Carlo Maria Carbonaro[1]

[1]Dept. of Physics, University of Cagliari, Cittadella Universitaria di Monserrato, SP n°8 km 0,700, 09042 Monserrato (Italy)
danielechiriu@gmail.com

For many centuries paper has been the main material for recording cultural achievements all over the world. Archives represent the historical memory of our past, many books and documents kept in libraries and archives are at risk because of their fragility [1].
The present study demonstrates the extraordinary power of the non-destructive NIR Micro Raman Spectroscopy, recently applied in the field of the Cultural Heritage [2] [3], to gain new and different information on paper and inks analysed in a set of ancient and modern documents. Micro-Raman scattering measurements have been carried out within back-scattering geometry in air at room temperature neither moving the ancient documents from their seat nor exposing them to possible damages. With the help of Raman spectra, determined after computer simulations, we determined the conservation state of the samples by studying the structure and the grade of crystallinity of the paper.
The Figure shows the raman spectrum of modern paper compared with a medieval paper.





Paper composition is ascribed to mostly treated and bonded cellulose fibres constituting by linear polymers of glucose (β-D-glucopyranose) monomers linked by β-1,4-glycosidics bonds. Molecular chains are held together by strong intermolecular hydrogen bonds that have an important role in the aggregation of single chains into highly oriented structure. The aggregates are ordered up to even 80% (“crystalline” forms). The rest, considered disordered, is called “amorphous” form. The presence of the 900 cm-1 band is very sensitive to the amount of crystalline versus amorphous structure of cellulose. This band is assigned to the angle around the β-glycosidic linkages and hydrogen bond rearrangement and then it depends on the deformation of COC, CCO, CCH and stretching vibrations of C5 and C6 atoms [4] .
Prior to the XIX century, paper was made entirely from rags, using hemp, flax and cotton fibres. After this period, due to the enormous increase in demand, wood pulp began to be commonly used as raw material, resulting in the rapid degradation of paper. The band centered at 1602 cm-1 is typically assigned to the presence of lignin in the composition of the paper, responsible of the principal degradation processes due to its photo-oxidation properties [1] .

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Vitronectin-derived peptides enhancing cell adhesion: a spectroscopic study

Michele Di Foggia[1], Armida Torreggiani[2], Annj Zamuner[3], Monica Dettin[3], Paola Taddei[1], Anna Tinti[1]

[1] Dip. Scienze biomediche e neuromotorie, Università di Bologna, via Belmeloro 8/2, 40126 Bologna.
michele.difoggia2@unibo.it
[2] Istituto ISOF, Consiglio Nazionale delle Ricerche, via P. Gobetti 101, 40129 Bologna.
[3] Dip. Ingegneria industriale, Università di Padova, via Marzolo 9, 35131 Padova.

In the field of implantable materials, the control of surface properties has a key role in determining the biocompatibility of a prosthetic device. An interesting strategy is to create bioactive surfaces able to control and promote cell-implants interactions by using oligopeptides with an amino acid sequence coming from adhesion proteins (i.e. fibronectin, vitronectin), that demonstrated to promote osteoblasts adhesion on dental and orthopedic
biomaterials [1].
Raman and IR spectroscopies have proved to successfully study the structure adopted by oligopeptides and their interaction mechanisms with implants surfaces [2] even at low concentrations (10-11 mol/cm^2). In the present work, a fragment of Human Vitronectin Precursor (HVP, sequence 351-359: Phe-Arg-His-Arg-Asn-Arg-Lys-Gly-Tyr), that has already demonstrated to enhance osteoblasts-adhesion [3], was characterized as synthesized and after covalent grafting on titanium and porous ceramic surfaces (composition: 30% wollastonite - $CaSiO_3$, 45% diopside - $CaMgSi_2O_6$, 8% akermanite - $Ca_2MgSi_2O_7$). This last material has already shown very promising results in preliminary biocompatibility test. Moreover, the HVP dimeric sequence (2HVP), the retro-inverse sequence (DHVP) and its dimer (2DHVP) have been analyzed: both retro-inverse sequences have appealing properties since they are biologically active but not degraded by serum enzymes.
Spectroscopic results indicated that the presence of D-aminoacids poorly affects the secondary structure adopted by peptide (HVP and DHVP) that is mainly β-turn, as already observed by CD and NMR measurements in solution [1, 3], while the dimeric sequence (2HVP and 2DHVP) showed an increased α-helix/random secondary structure content. The interaction with the covalently modified metal and ceramic surfaces significantly affected both the secondary structure and the interactions between the amino acid lateral residues.

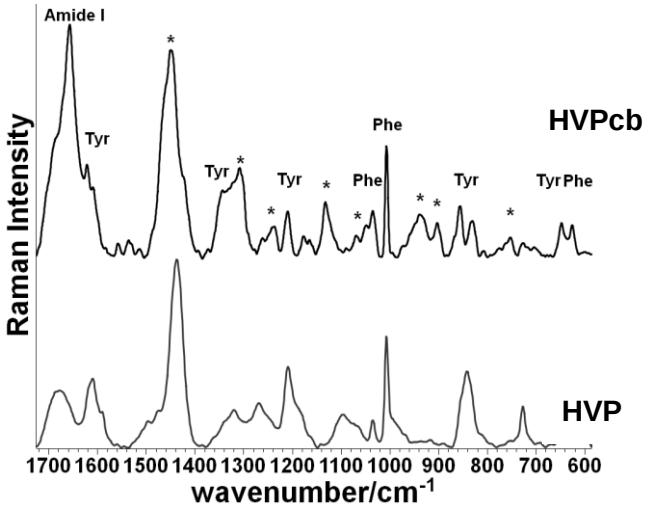

Figure 1: Raman spectra of as-synhtesized HVP compared to HVP covalently bound (HVPcb) to Ti disks.
**: covalent linker bands*

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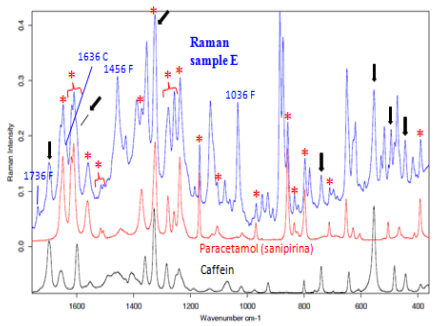
Identification and quali-quantitative determination of cocaine and adulterants by FT-Raman and FTIR/ATR

Michele Di Foggia[1], Armida Torreggiani[2], Maria Augusta Raggi[3], Melania Giasullo[4], Anna Tinti[1]

[1] *Dip. Scienze Biomediche e Neuromotorie, Università di Bologna, Via Belmeloro 8/2, 40126 Bologna, Italy,* micheled@chem.unibo.it
[2] *I.S.O.F., Consiglio Nazionale delle Ricerche, CNR, Via P. Gobetti No. 101, 40129 Bologna, Italy*
[3] *Dip. Farmacia e Biotecnologie, via Belmeloro 6, 40126 Bologna, Italy*

It is well known that cocaine is an alkaloid stimulating the central nervous system. Common forms of cocaine used as drug are freebase powder, hydrochloride powder, freebase paste and crack rock. Numerous health complications are associated with acute and chronic use of cocaine: stroke can result from overdose, caused by the use of high purity cocaine by consumers unaware of its real concentration. Furthermore, various adulterants, used to increase the volume or enhance the drug effects, can be mixed with cocaine. Discriminating the various forms of cocaine, the drug's real concentration, and the adulterants used, is of great interest for forensic toxicology to conclusively identifying and quantifying drugs in cases of drug trafficking [1–3]. Current methods employed in forensic toxicology are mainly based on gas chromatography techniques, but these methods are destructive, time-consuming, and do not allow reexamination of the evidence. Nondestructive and rapid analysis of drugs of abuse could be accomplished through the use of vibrational spectroscopic techniques [1, 4].

The advantages, after the construction of a reference spectra library, lie in the possibility of a rapid analysis of trace samples (1 mg or less of sample) without any destruction or manipulation of the sample itself. Thus, FT-Raman and FTIR/ATR may become powerful methods in fight against drug trafficking. The main substance classes used to obtain our reference spectra library were: cosmetic adulterants, in particular alkaloid psychotropic agents as atropine and caffeine, anesthetics as lidocaine and procaine, some bench drugs as paracetamol (tachipirina and sanipirina) and acetylsalicylic acid (aspirin); diluent adulterants, in particular inorganics as carbonate and hydrogen carbonate, saccharides, polyalcohols. The spectra of cocaine forms were already reported in the literature [1, 3, 4]. Moreover, to identify bands typical of different adulterants and to obtain direct quantitative determinations in a mixture, caffeine-mannitol mixtures containing 5, 10 and 30% of caffeine were prepared and calibration curves were obtained both by calculating IR and Raman ratios between selected bands of the two components. Caffeine was chosen since it is a psychotropic alkaloid and its presence in a mixture can increase the cocaine effect.Two different samples of sequestered cocaine were examined by FT-Raman and FTIR/ATR; as an example, the Raman spectrum of one of these is reported in the figure. The first cocaine sample shows all the bands typical of pure cocaine·HCl without any adulterants (not shown); the second one (see figure) shows typical bands of cocaine both in the free base and crack forms, in addition to bands typical of the paracetamol sanipirina drug, used as adulterant. Moreover, an intensity comparison of the cocaine bands of the pure sample (in particular, FTIR/ATR results) suggests the presence of about 30% of cocaine in sample E. These results underline the good performances of vibrational spectroscopy in identifying the quali-quantitative composition of unknown samples. A comparison with chromatographic methods on samples with different compositions should permit to obtain a reliable calibration line.



Comparison of the Raman spectrum of a cocaine sample E (top) and those of its adulterants paracetamol (asterisks) and caffein (arrows); the bands typical of cocaine freebase (F) and crack (C) are also indicated.

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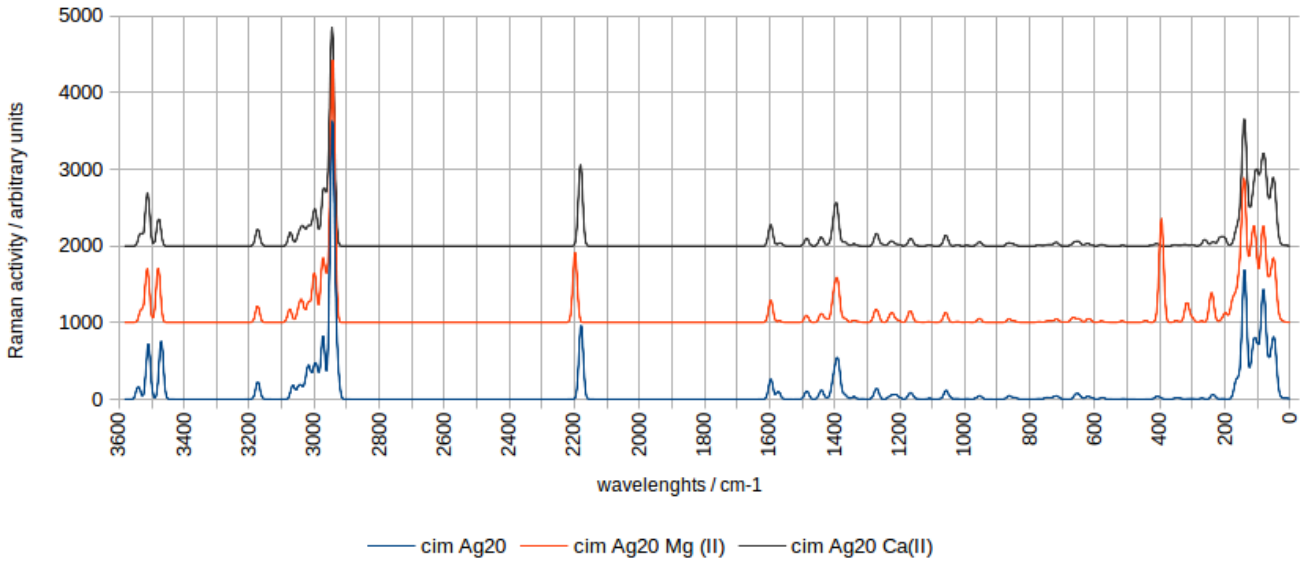
Spectroscopic (IR, Raman, UV and SERS) and computational study on cimetidine complexes with Ca^{2+} and Mg^{2+} ions.

Michele Di Foggia[1], Assimo Maris[2],Vitaliano Tugnoli[1], Enrico Benassi[3]

[1] Dipartimento di Scienze Biomediche e Neuromotorie, Università di Bologna, via Belmeloro 8/2, 40126 Bologna Italy.
michele.difoggia2@unibo.it
[2] Dipartimento di Chimica "G.Ciamician", Università di Bologna, via Selmi 2, 40126 Bologna Italy.
[3] Scuola Normale Superiore di Pisa, Piazza Cavalieri 7, 56126, Pisa, Italy

Different vibrational and electronic spectroscopy tools and DFT calculations were used to investigate cimetidine, one of the most potent histamine H_2-receptor antagonist for inhibiting excessive acid secretion caused by histamine, and its metal complexes with alkaline earth metals (Ca(II) and Mg(II)). The IR, Raman and reflectance UV study on the solid 2:1 cimetidine-Mg(II) and cimetidine-Ca(II) complexes shows that both exhibit the octahedral structure around the central metal, that appears slightly distorted in the presence of Mg(II). SERS spectra and computational simulations suggest that in water diluted solution at ppm level, the formation of stable 2:1 cimetidine-Mg(II) and cimetidine-Ca(II) complexes is excluded, while data are consistent with the formation of 1:1 adduct, as already reported for other divalent metallic cations (Cu, Ni, Co, Zn) [1], in which the metals reach their total coordination shell by complexation of water molecules. Theoretical study exhibits that the stronger interaction site of pure cimetidine with Ag_{20} cluster is the imidazole nitrogen atom, followed by the cyanide nitrogen atom. In presence of divalent metallic ions, the bond between silver and the imidazole nitrogen remains almost unchanged while the Mg(II) and Ca(II) ions bind to the cyanide group. Experimental SERS spectra confirmed that Ca(II) induced a highest distance between silver and the cyanide group, showing that the presence of these ions can modulate the binding mode of the drug to a biological substrate and, consequently its biological function.



Simulated SERS spectra of: ***cim-****Ag_{20},* ***cim-****Mg(II)-Ag_{20}, and* ***cim-****Ca(II)-Ag_{20}*

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Potential use of MCR-ALS for the analysis of hyperspectral Raman maps from pediatric intestinal biopsies

Stefano Fornasaro[1], Luigina De Leo[2], Alois Bonifacio[3], Tarcisio Not[4] and Valter Sergo[5]

[1] Department of Engineering and Architecture, University of Trieste, Trieste, Italy
sfornasaro@units.it
[2] Institute for Maternal and Child Health - IRCCS Burlo Garofolo, Trieste, Italy
[3] Department of Engineering and Architecture, University of Trieste, Trieste
[4] Institute for Maternal and Child Health - IRCCS Burlo Garofolo, Trieste, Italy
[5] Department of Engineering and Architecture, University of Trieste, Trieste

Raman hyperspectral imaging is an emerging tool in biological and biomedical research for label free analysis of tissues and cells [1]. Using this method, both spatial distribution and spectral information of analyzed samples can be obtained. However, there are several limitations related to the high complexity of the data obtained from clinical samples and to the big size of the data sets generated by this technique: behind each pixel of the Raman map stands a whole spectrum consisting of many, typically thousands, wavelengths. Therefore, the importance of chemometric approaches in the data analysis process is unquestionable. Among the different chemometric techniques, multivariate curve resolution alternating least squares (MCR-ALS) is often used to analyze hyperspectral images due to the ability to resolve (unmix) the raw hyperspectral image into the spectral signatures of the major biological components of the sample contributing to the measured spectral signal, and to report their distribution maps on the tissue surface [2].
In this work, a MCR-ALS workflow is applied to images of multiple samples of duodenal biopsy sections from pediatric patients with autoimmune diseases characterized by chronic intestinal inflammation (such as Crohn's disease and celiac disease) compared to healthy controls, to assess whether this approach is able to detect the alterations in the biochemical composition of intestinal tissues associated with these conditions. The image segmentation starting from the MCR-ALS output is also discussed.

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CERIC-ERIC, the multi-technique research infrastructure for materials research in Central-Eastern Europe

Matthias Girod[1], Ornela de Giacomo[1], Jana Kolar[1]

[1] CERIC-ERIC S.S. 14 - km 163,5 in AREA Science Park 34149 - Basovizza, Trieste – ITALY,
Matthias.girod@ceric-eric.eu

Material science and nanotechnology are two of the main research fields to take up future challenges of Europe such as alternative energy sources and energy storage or biomedical and pharmaceutical materials. The scientific problems coming up in this fields have become more and more complex in the recent years and require an ever increasing number of instrumental and analytical techniques and disciplines. Such complexity requires the availability of expertise as well as open access to a wide range of probing techniques and many different complementary instruments. The CERIC-ERIC research infrastructure was developed to face this challenge and to make a wide variety of instruments available through open access.

CERIC stands for Central European Research Infrastructure Consortium and is a distributed research infrastructure unifying several national institutions, under one roof [1]. This multinational facility was set up as a European Research Infrastructure Consortium (ERIC) [2]. It brings together research facilities from Austria, Croatia, Czech Republic, Hungary, Italy, Poland, Romania and Slovenia (Fig 1). Statutory seat is in Trieste, Italy. All partners offer a set of complementary, cutting-edge instrumentation from national institutes for free and open access to excellent researchers all over the world.

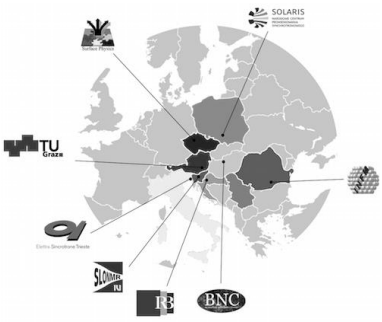

Figure 1. Locations and partners of CERIC-ERIC

CERIC-ERIC comprises synchrotron radiation, neutron radiation, microscopic techniques, ion-beam analysis methods and NMR. All instruments are available for open access through one single entry point. The selection of proposals and experiment time is done in a peer-review process and based on scientific excellence only. Following the nature of CERIC as a multi-probe facility, the open access operation allows to ask not only one instrument per proposal but to get experiment time granted for several complementary instruments with one proposal.

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SERS SPECTRA OF BIOFLUIDS: HOW PROTEIN CORONA AFFECTS THE DETECTION OF SMALL BIOMOLECULES

Elisa Gurian[1], Alois Bonifacio[1], Valter Sergo[1]

[1] Department of Engineering and Architecture, University of Trieste, Via A. Valerio 6, 34133 Trieste, Italy – elisa.gurian@phd.units.it; abonifacio@units.it; sergo@units.it

Surface Enhanced Raman Spectroscopy (SERS) has proven to be a promising technique for biomedical application, such as in the field of cancer diagnosis, provided its sensitivity to analytes at low concentrations in biofluids [1, 2].

In this frame, it is of uttermost importance to optimize the experimental set-up for these kind of SERS measurements, with a specific focus on human serum, considering the necessity of reproducible data.

As a matter of fact, the presence of proteins in biofluids limits the enhancement of the scattering process by forming a corona around the metal nanoparticles substrates that are required for the signal amplification. The aim of this work is to verify systematically how the presence of human serum albumin, one of the most abundant proteins in serum, can affect the free diffusion of small molecular weight analytes toward the metal nanostructured surface and their adsorption on it, by means of SERS of model solutions. In particular, the SERS substrates are in-house developed paper-based solid substrate, in which filter paper sheets are dip coated with citrate-reduced silver nanoparticles [3].

The adsorption kinetics of known biomolecules on silver solid substrates has been studied by means of simple model systems. They are single or mixed solute solutions of low- (adenine, hypoxanthine, uric acid) and high-molecular weight molecules (human serum albumin, HSA). These molecules are easily detected through SERS analysis. The samples were prepared by dipping the paper-silver substrates in vials containing the analyte solutions at the proper concentration for a fixed incubation time. After drying, SERS spectra were collected on many replicas for each sample with the use of a portable Raman spectrometer.

Data suggested a cooperative adsorption trend for most of the analytes, except for the adenine, which always adsorbs fast on the nanoparticles in a non-cooperative way and its adsorption rate is slightly affected by the presence of HSA. The presence of HSA alters the adsorption kinetics of hypoxanthine and uric acid: the first reaches a plateau up to 8 times less intense than the HSA-free case, without a significant adsorption rate variation. Conversely, the latter adsorption rate is lowered by a factor 1.4 and the cooperativity coefficients is doubled.

The systematic analysis of the adsorption kinetic of these biomolecules has provided information about the binding constants and the adsorption rate on solid SERS substrates. This may be helpful in the peak assignment on SERS spectra of complex biofluids. Moreover, it has been verified the possibility to perform quantitative analysis by means of SERS spectra on the in-house developed paper-based solid substrate.

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Investigation of molecular order using Raman Spectroscopy for self assembled adlayers of CoPc and FePc

Abhishek Kumar[1,2], DenysNaumenko[1,4], Luca Cozzarini[1], Luisa Barba[3], Alberto Cassetta[3], Maddalena Pedio[1]

[1]Istituto Officina dei Materiali, Consiglio Nazionale delle Ricerche, TASC Laboratory, Trieste, Italy,
abhishek@iom.cnr.it
[2] Nanotechnology, University Trieste, Trieste Italy
[3] Istituto CNR- IC, Area Science Park Basovizza, Trieste, 34149, ITALY
[4]Elettra Sincrotrone Trieste, Area Science Park Basovizza, Trieste, 34149, ITALY

Metal phthalocyanines are a peculiar class of organic molecules showing molecular magnetism and exhibit potential for spintronic devices. Magnetic properties of metal phthalocyanines are strongly influenced by phthalocyanine polymorphism, crystal structure and central metal atom. In addition, nature of substrate significantly influences the magnetic properties of the metal phthalocyanines thin films by controlling the molecular orientation during growth[2]. Magnetic coupling of the molecule with substrate is governed by whether the substrate is ferromagnetic, metal or semiconductor[1]. The relative orientation of the molecules is driven by the fine interplay between molecule–substrate and intermolecular forces governing the molecular and optoelectronic properties of the system [1].

Here, we present a comprehensive study of CoPc and FePc thin films on technologically relevant substrates Si(111),ITO and polycrystalline gold of about 50 nm thickness in order to decipher the substrate induced effects on molecular stacking and crystal structure. Raman analyses show lower intensity for the frequencies corresponding to phthalocyanine macrocycle for the CoPc and FePc thin films grown on ITO as compare to Si(111) and due to the higher order of phthalocyanine molecules on Si(111). We have observed significant displacement of C–N–C bridge bonds of the phthalocyanine macrocycle for CoPc and FePc depicting the influence of unique central metal atom. In addition considerable modifications for the Raman band frequencies are observed for the powder bulk phase as compare to thin films phase demonstrating the influence of unique molecular stacking in these phases. Atomic force microscopy displays a higher grain size for FePc and CoPc thin films on ITO as compare to Si(111) and polycrystalline gold indicating towards the influence of molecule–substrate interactions on the molecular stacking. Moreover morphology of FePc and CoPc thin films is notably unique for these substrates possibly due to different molecule-substrate interactions. Grazing incidence X-ray diffraction(GIXRD) reciprocal space maps reveal that FePc molecules adopts a combination of herringbone and brickstone arrangement on Si(111) substrate which have significant implications on the optoelectronic properties of the film due to unique molecular stacking.

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Raman and x-ray diffraction studies on the phase coexistence between the pressure-induced structures in black phosphorus up to 18 GPa

Boby Joseph[1], Simone Caramazza[2], Francesco Capitani[2], T. Clartè[2], Andrea Lausi[3], Paolo Postorino[2], Paolo Dore[2]

[1]Elettra-Sincrotrone Trieste, Area Science Park, 34149 Basovizza, Trieste, Italy
boby.joseph@gmail.com
[2]Dept. Physics, Sapienza University of Rome, P.le Aldo Moro 2, 00185, Rome, Italy
[3] Elettra-Sincrotrone Trieste, Area Science Park, 34149 Basovizza, Trieste, Italy

Black phosphorus (BP) is one of the important emerging two-dimensional systems. Here we present a high pressure x-ray diffraction study performed on BP up to 12.2 GPa and a high pressure Raman investigation up to 18.2 GPa. Above 10 GPa, a phase transition to a simple cubic structure is evidenced by from the x-ray diffraction data. However, high pressure diffraction data show that the rhombohedral A7 to the simple cubic transition is not complete even at 12.2 GPa. Simple cubic phase of BP is not expected to show any Raman phonon modes, however, Raman components are observed till 18.2 GPa. In the case of Raman measurements on the small crystalline sample, slight deviations from the ideal hydrostatic conditions at high pressures above 10 GPa inside a diamond anvil cell may be leading the simple cubic phase of BP to adopt a locally non cubic structure. Such a scenario can explain the observation of the board two component Raman spectra centered around 250 cm^{-1} in the pressure range 14-18 GPa. This poster will discuss these results in the context of recent literature [1-4].

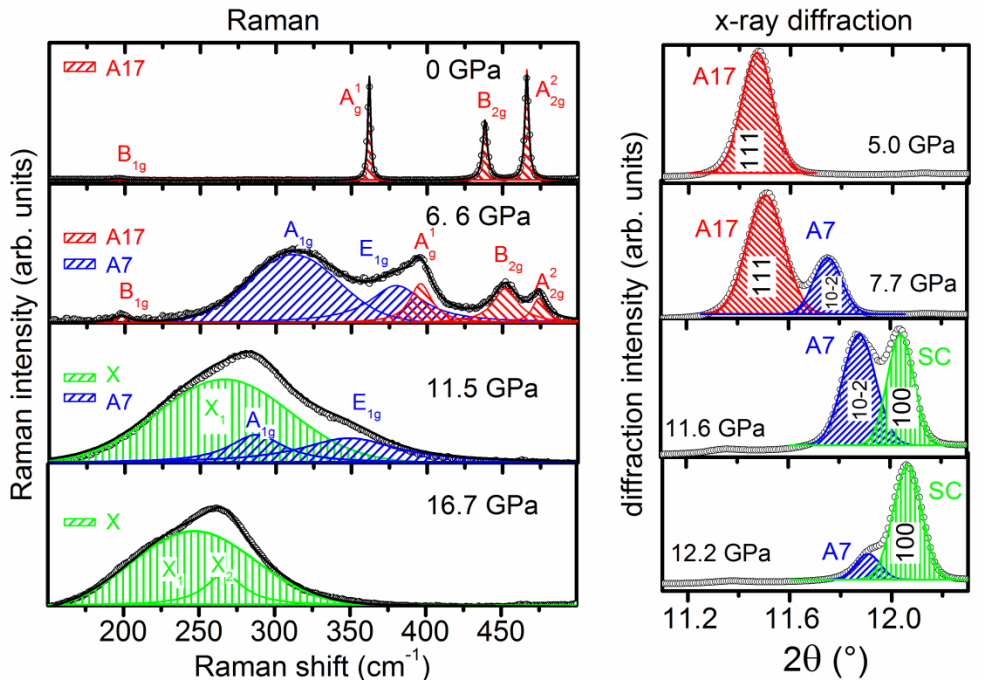

Figure showing the pressure evolution of the Raman (left) and diffraction pattern (right) of black phosphorus at several selected pressures. Three different phases are shown in three different colors. Only a limited 2θ range is shown for the diffraction patterns to clearly evidence the phase coexistence.

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The conversion of coproheme to heme *b* by HemQ followed using resonance Raman spectroscopy

Lisa Milazzo[1], Stefan Hofbauer[2], Barry D. Howes[1], Christian Obinger[2], Giulietta Smulevich[1]

[1] Dipartimento di Chimica 'Ugo Schiff', Università di Firenze, Sesto Fiorentino (FI), Italy, lisa.milazzo@unifi.it
[2] Division of Biochemistry, Department of Chemistry, BOKU–University of Natural Resources and Life Sciences, Vienna, Austria

In 2010, Dailey and coworkers discovered that the Chlorite dismutase-like (Cld-like) proteins of the Gram positive bacteria Firmicutes and Actinobacteria are essential enzymes in heme biosynthesis, and consequently renamed them HemQ [1]. In fact, Firmicutes and Actinobacteria have established an alternative way to the canonical heme biosynthetic pathway, in which the homopentameric coproheme decarboxylase enzyme, HemQ, catalyzes the final step which leads to the transformation of coproheme into heme *b* [2]. Coproheme has four propionate groups located at positions 2, 4, 6, and 7 of the porphyrin ring. Propionates at positions 2 and 4 are decarboxylated by HemQ in a stepwise reaction to form the respective vinyl groups of heme *b*. Since many pathogenic bacteria that have developed antimicrobial resistance, such as *Listeria Monocytogenes* [3], use the HemQ-dependent heme biosynthesis pathway [4] and heme *b* is essential for their survival, HemQ is a very attractive pharmacological target. Therefore, elucidation of both the coproheme/heme *b*-HemQ structures, the structure-function relationships, and the molecular mechanism of conversion of coproheme to heme *b* is crucial. In the present work, the wild type (WT) protein and Met149Ala mutant (in which the conserved Met residue that is H-bonded to the propionate in position 2 [Hofbauer, personal communication] is replaced by the non H-bonding residue Ala), of *Listeria Monocytogenes* HemQ (LmHemQ) were studied via UV-Vis and Resonance Raman (RR) spectroscopies both at room and low temperature (80K). In the RR spectra, the frequencies of the core size marker bands provide information on the oxidation, coordination and spin states of the heme iron [5]. Moreover, complexes with carbon monoxide [6], cyanide [7] and fluoride [8] can be used to gain insight into the interaction of the distal cavity aminoacid residues with the heme moiety, while on the proximal side, information on the Fe-Im bond strength can be obtained by the ν[Fe(II)-Im] stretching mode frequency [9]. The spectra of the ferric WT and M149A coproheme-LmHemQ, obtained with two different excitation wavelengths, 356.4 nm and 406.7 nm, at higher and lower energy, respectively, compared to the electronic absorption maximum of the Soret bands (at 393 and 399 nm), enabled identification of two different 5-coordinated (5c) species for the WT and an additional 6-cordinated low spin (6cLS) form for the mutant. Moreover, in the low frequency region RR spectrum of the WT coproheme-LmHemQ obtained at low temperature, four propionate bands at 375, 382, 389, and 398 cm^{-1} are observed. Due to the lack of the H-bond between M149 and the propionate in position 2 the band at 389 cm^{-1} shifts to 393 cm^{-1} in the RR spectrum of M149A mutant. Therefore, this band is assigned to the propionate 2 substituent. Furthermore, upon addition of H_2O_2 to the coproheme complexes, the conversion of coproheme to heme *b* leads to the disappearance of both the bands at 382 cm^{-1} (propionate 4) and 389/393 cm^{-1} (propionate 2) with the concomitant appearance of a very intense band at 418 cm^{-1}, assigned to the $\delta(CH_2)$ vinyl bending mode. Accordingly, in the high frequency RR spectrum, the bands at 1621 and 1632 cm^{-1} were assigned to the two ν(C=C) vinyl stretching modes of the heme *b* complexes. For all the WT and M149A coproheme-LmHemQ and heme *b*-LmHemQ complexes, the ν(Fe-Im) stretching mode is observed at 214 cm^{-1}, suggesting that the imidazole of the proximal His (H174) is weakly bonded to the heme iron in agreement with the crystal structure of the WT protein [10] and that neither the mutation nor the conversion of the propionates 2 and 4 to vinyl groups has an impact on the proximal heme coordination and architecture.

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Two-dimensional Raman Correlation and THz-Raman Spectroscopic Investigation of the Brill Transition in Nylon 6,6

Maurizio Musso[1], Durval Bertoldo Menezes[1], Andreas Reyer[1]

[1] Department of Chemistry and Physics of Materials, University of Salzburg, Jakob-Haringer-Strasse 2a, 5020 Salzburg / Austria
maurizio.musso@sbg.ac.at

The Brill transition is a well-known and very important phase transition process in polyamides, that has been related with structural changes between the hydrogen bonds of the lateral functional groups (C=O) and (N–H)[1]. In this work, we show the potential of Raman spectroscopy for exploring this phase transition in polyamide PA66 (nylon 6,6). We collected the Raman spectra during a step by step heating and cooling process of the sample from 30°C to 240°C, which allowed us to grasp signatures of the Brill transition from peak parameters (shift, FWHM) of specific vibrational modes, i.e ν(C–C), ν(C–N), ν(C=O) and ν(C–N), and to verify the Brill transition temperature as well as the reversibility of this phase transition. We identified, from two-dimensional Raman correlation spectroscopy, which spectral regions suffered the largest influence during the Brill transition, i.e. around the ν(C–C), and ν(C–N) vibrational modes, as shown e.g. on the left of figure 1 for the Raman shift of ν(C–C). Furthermore, data from Terahertz Stokes and anti-Stokes Raman spectroscopy of a peak around 100 cm^{-1} were also analyzed, delivering complementary information e.g. on the temperature of the sample, as shown on the right side of figure 1.
All our results confirm that Raman spectroscopy can be used as a complementary technique to perform phase transition investigations in polyamides, due to its sensitivity to small intermolecular changes affecting vibrational properties of relevant functional groups [3].

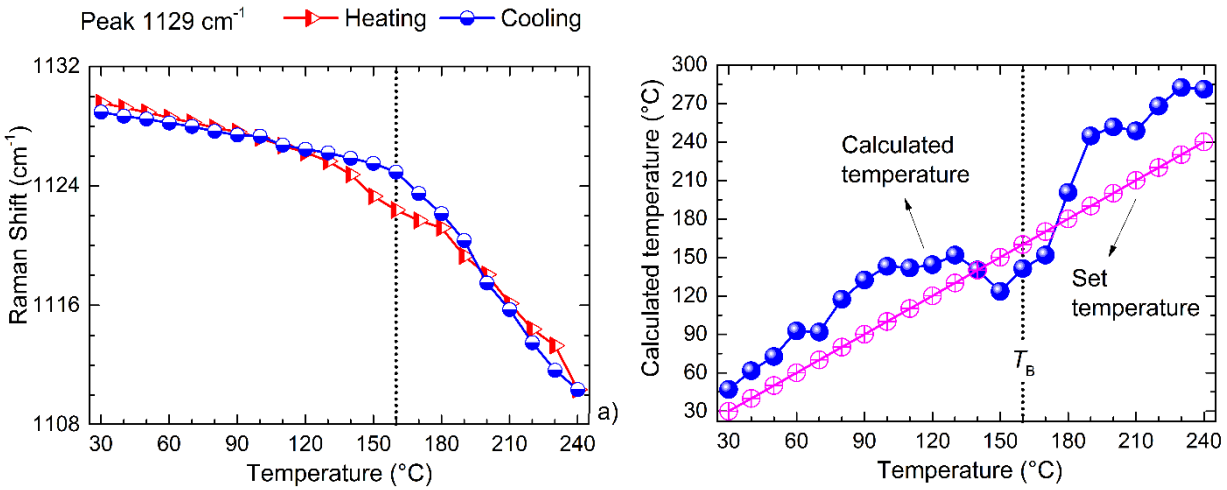

Figure 1. Left side: Raman shift of the peak at around 1129 cm^{-1} associated with the C–C skeletal stretches, in the backbone of nylon 6,6 (N66), as function of a temperature cycle of heating and successive cooling between 30°C and 240°C.
Right side: Temperature set with the help of the heating stage, and temperature calculated from the Anti-Stokes/Stokes ratio of a Raman peak at around 100 cm^{-1}.

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Investigation of SERS Substrates Fabricated via Injection Molding and Surface-Mediated Nanoparticle Formation

Maurizio Musso[1], Andreas Reyer[1], Durval B. Menezes[1], Gilles R. Bourret[1], Stefano Giancristofaro[2], Adrian Prinz[2]

[1] Department of Chemistry and Physics of Materials, University of Salzburg, Jakob-Haringer-Strasse 2a, 5020 Salzburg, Austria, maurizio.musso@sbg.ac.at
[2] STRATEC Consumables GmbH, Sonystrasse 20, 5081 Anif / Salzburg, Austria

Surface-enhanced Raman spectroscopy (SERS) is a very promising characterization technique for biomedical diagnostics. It can be used to overcome current challenges for quick identification of cells, tissue and bacteria with high sensitivity [1], being a non-destructive and rapid method to detect substances even down to trace analysis level without requiring biomarkers or long incubation times that are usually needed for fluorescence spectroscopy and microbiological investigations. For these reasons, creating suitable, reliable SERS substrates usable for specific applications has been the focus of intense research since many years [2].
The aim of our current study is to analyze the SERS performance of newly created, promising SERS substrates and to compare their performance with commercially available SERS substrate [3].
A microstructured polymer slide, produced via an injection molding process, was coated with Ag via a PVD process. This was followed by a chemical post-treatment to produce Ag nanoparticles at the substrate surface and so create large area prototypes of homogenous SERS substrates (see Figure 1). The amount and distribution of the Ag nanoparticles lead to possible couplings, hot spots and arrangements yielding localized surface plasmonic effects, and consequently able to create a surface enhanced Raman signal. In order to check the enhancement factor, two laser excitation wavelengths were used (532 nm and 780 nm) to collect Raman spectra from 1,4-benzenedithiol (1,4-BDT) deposited on our own substrates, and on commercial substrates used as reference. Depending on the laser wavelength used, a Raman resonance effect is also observed [4] for specific molecular vibrations of 1,4-BDT.

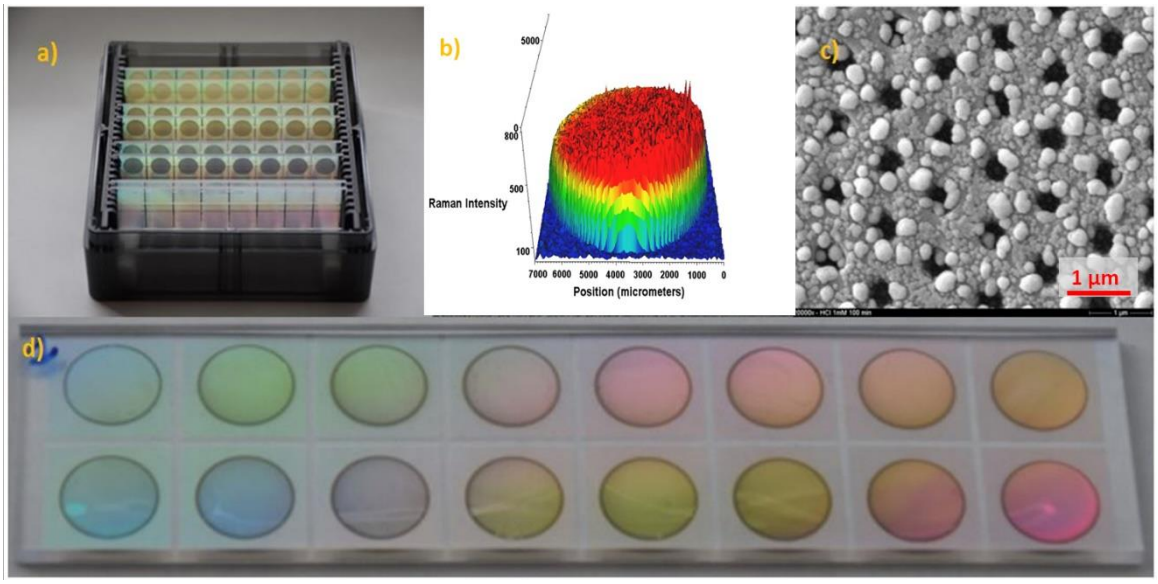

Figure 1. a) *Photograph of the SERS substrates* ***b)*** *3D Raman map of an investigated surface area spot* ***c)*** *SEM picture (20000x magnification) of the Ag-nanoparticles on the microstructured substrate* ***d)*** *Higher magnification photograph of a microsope slide coated with SERS active microstructured discs*

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Composition dependence of $Li[NTf_2]_x/[C_4C_1im][NTf_2]_{(1-x)}$ glasses probed by Raman spectroscopy

Vitor H. Paschoal[1], Mauro C. Costa Ribeiro[1,*]

[1] *Laboratório de Espectroscopia Molecular, Instituto de Química, Universidade de São Paulo, CP 26077, CEP 05513-970 São Paulo, SP, Brazil*
mccribei@iq.usp.br

Room temperature ionic liquids (RTIL) are a class of materials with very attractive properties that can be applied in electrochemical cells and batteries, synthesis of organic compounds and nanoparticles, among others. This compounds also display a rich phase transition phenomenology and good glass forming capabilities[1]. Due to this capabilities, whether by cooling or by compression, glasses can be obtained from ionic liquids with relative ease[1,2]. This enables their applications as model glass formers enabling the study of glass-phase related phenomenology and the glass transition itself. An universal feature of the glasses is the so called boson peak[2], which can be probed with Raman spectroscopy in the low-frequency region (< 200 cm^{-1}). This feature may be related to the intermolecular dynamics of the cations and anions [2] and its features may provide insights on the degree of disorder of the system. The addition of a Li^+ salt in an RTIL, specially those related to the bis(trifluoromethanesulfonyl)imide anion, $[NTf_2]^-$, will result in the increase of the system's viscosity and density correlated to strong interaction between Li^+ and $[NTf_2]^-$. This results in stifier systems what could imply in a increase of the boson peak frequency, as observed for lithium oxide-borate (Li_2O/ B_2O_3) glasses in which the increase in lithium content results in the decrease in boson peak intensity and shifts it to higher frequencies [3]. In this work the effects of addition of $Li[NTf_2]$ to 1-butyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide, ($[C_4C_1im][NTf_2]$) will be investigated. We will show that despite the increase in density caused by the addition of Li^+ (as observed by MD simulations) as for the borate glasses, the RTILs solutions will show a simulatenous decrease of intensity of the boson peak frequency (ω_{BP}) and intensity.

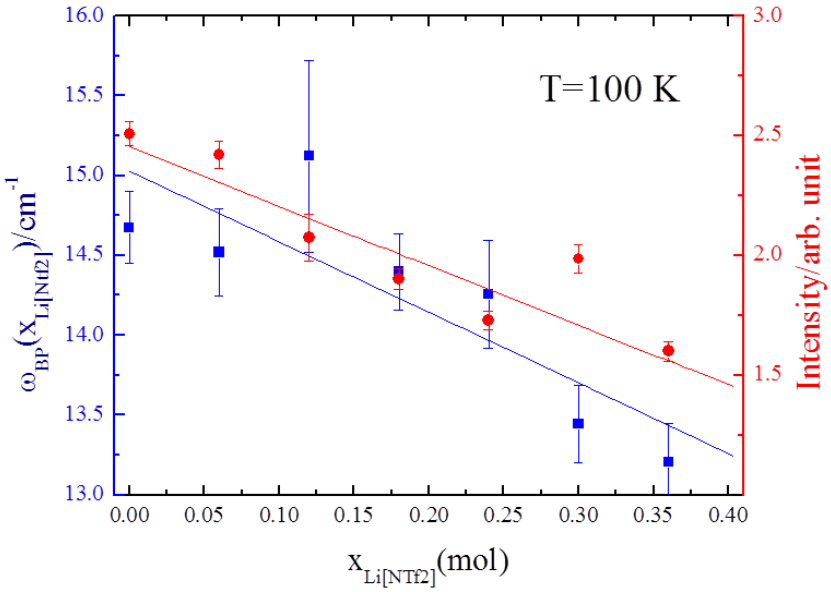

Figure 1: Dependence on boson peak frequency (ω_{BP}) and intensity with $Li[NTf_2]$ molar fraction (x_{LINTF2}) at 100 K

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The lipid bilayer: news from the inside

Paola Sassi[1], Beatrice Gironi[1], Andrea Lapini[2], Marco Paolantoni[1], Assunta Morresi[1], Paolo Foggi[1,2]

[1] Departement of Chemistry Biology and Biotechnology, University of Perugia, Via Elce di Sotto 8, 06123 Perugia, Italy
paola.sassi@unipg.it
[2] LENS, European Laboratory for Nonlinear Spectroscopy, Via N. Carrara 1, Sesto Fiorentino, Firenze, Italy

The solubility of small molecules into a lipid bilayer is central to many biological and biotechnological processes. Lieb and Stein [1] proposed that small molecules dissolve and diffuse by "hopping" between empty voids and molecular dynamics studies have shown that the largest voids are located in the center of the membrane [2], that they increase by increasing temperature and unsaturations in the acyl chains [3], decrease by adding cholesterol [4], and that it depends on lipid composition [5]. Unfortunately, this description can hardly find an experimental confirmation and the diffusion and permeation routes at the atomic level are still not completely understood.
In the present study we use IR spectroscopy, both stationary and time resolved, to characterize the properties of cholesterol loaded POPC liposomes. To this extent we use a vibrational probe, hexacarbonyl tungsten $W(CO)_6$, to follow the solubility of a small hydrophobic molecule inside the membrane at different temperatures and solvent composition (DMSO/water solutions at different DMSO mole fraction), and to follow the dynamics of fat acid hydrophobic tails in the picosecond time domain.

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Label-Free and Site-Specific Probing of Molecular Recognition between Proteins and Multivalent Supramolecular Ligands by Ultraviolet Resonance Raman Spectroscopy

Zakeri Banafshe[1], Gigante Martinez Alba[2], Schmuck Carsten[2], Schlücker Sebastian[1]

[1]Department of Chemistry, Physical Chemistry, University of Duisburg-Essen, Universitätsstr. 5, D-45117 Essen, Germany,
banafshe.zakeri@uni-due.de
[2]Department of Chemistry, Organic Chemistry, University of Duisburg-Essen, Universitätsstr. 5, D-45117 Essen, Germany,

Supramolecular chemistry exploits non-covalent interactions between host and guest molecules. Artificial peptide ligands are versatile molecular model systems for investigating such interactions with peptides/proteins. A class of artificial carboxylate binders/receptors comprising a guanidinium carbonyl pyrrole (GCP) as a carboxylate binding site (Fig. 1 top) has been designed and synthesized by Schmuck and co-workers.
Commonly used techniques for monitoring molecular recognition events require modifications of the system such as external labeling by fluorophores, which are often larger than the system of interest itself, or require the immobilization onto surfaces for surface plasmon resonance, which does not reflect the binding kinetics of the system in solution. Surprisingly, label-free vibrational spectroscopy (IR and Raman) has only rarely been employed for probing molecular recognition processes. In particular UV resonance Raman (UVRR) spectroscopy is ideally suited for site-specific and sensitive probing of binding sites comprising a UV chromophore. In proof-of-concept studies we demonstrated its application to the binding events between monovalent GCP ligands (Fig. 1 top) and small tetrapeptides (Fig. 1 bottom) in water [1, 2].
In this work, we use the site-specificity of UVRR to investigate the binding between a multi-armed GCP ligand (Fig. 2 left) to the alpha-helical protein Leucine Zipper (Fig. 2 right). Making the transition from a tetrapeptide to a protein with more than 40 amino acids, i.e. increasing the size of the molecular system by one order of magnitude, was the major challenge of this dissertation project. We will present the results of a UVRR binding study ("UVRR titration") between a multivalent supramolecular ligand and Leucine zipper.

A 90° scattering geometry with a rotating cell in combination with 266 nm excitation from a cw solid-state laser was employed to selectively enhance the vibrational Raman bands of the GCP subunit in the free and complexed form upon addition of Leucine Zipper. The association constant was determined from a quantitative analysis of the UVRR binding study using non-negative matrix factorization (NMF) [1, 2].

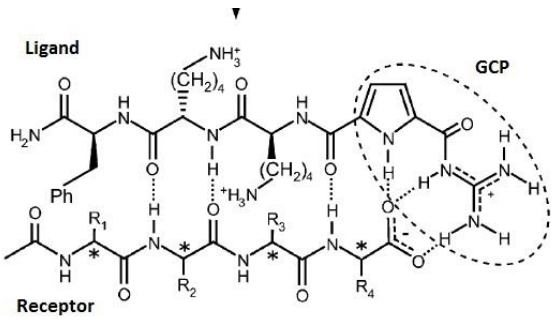

Fig.1

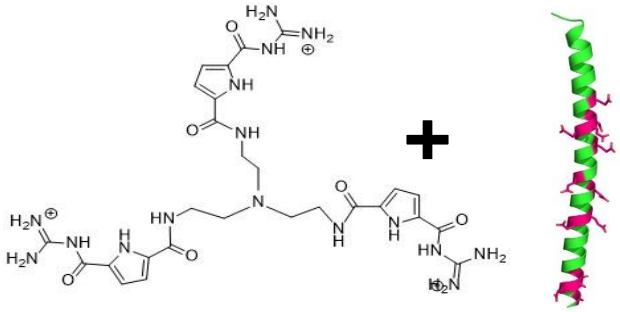

Fig. 2

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Surface modified gold nanorods to provide biofunctionality

Lorenzo Tognaccini[1], Tiziana Placido[2,3], Barry D. Howes[1], Angela Agostiano[2,3], Giulietta Smulevich[1]

[1] Università degli Studi di Firenze, Dipartimento di Chimica "Ugo Schiff", 50019 Sesto Fiorentino (FI), Italy.
lorenzo.tognaccini@unifi.it
[2] Università degli Studi di Bari Aldo Moro, 70121 Bari (BA), Italy.
[3] CNR-IPCF Istituto per i Processi Chimici e Fisici, Sez. Bari, c/o Dip. Chimica Via Orabona 4, 70126 Bari, Italy

In recent years, nanoparticles (NPs) science has progressed extensively and, through refined particle designs, the application of nanosystems has become possible in a broad range of fields. Gold nanorods (AuNRs) are two-dimensional nanoparticles characterized by optical properties, easily tuneable, which depend on their size and aspect ratio (length-to-width ratio) [1]. Moreover, conjugation with biomolecules make AuNRs suitable for many applications, such as imaging of tissues, cells or macromolecules [2], photothermal therapy [3], in vivo delivery [3] and SERS-based biosensing [4]. However, the CTAB bilayers capped on the surface of the AuNRs, derived from the synthetic process [5], limit their application in the fields of biology, not only due to the significant cytotoxicity of CTAB but also since it denatures biomolecules, especially proteins. Therefore, the discovery of new strategies to improve NPs compatibility with biomolecules through surface modification is a challenge of the utmost interest. In this work, we have used horse heart cytochrome c (Cyt c) as a protein probe to test a AuNRs system. Cyt c, a small heme protein localized in the mitochondrial intermembrane space, is one of the best characterized proteins due to its key role in the respiratory chain as an electron carrier and in programmed cell death, apoptosis, as a caspase cascade activator [6]. Consequently, Cyt c has been extensively investigated upon interaction with phospholipids in solution [7], on liposomes [8] and upon immobilization on membranes or appropriate Self-Assembled Monolayers (SAMs)-covered electrodes [9]. However, while preservation of the native structure of Cyt c was achieved on SAMs-covered electrodes [10], bioconjugation [11] and adsorption [12] on colloidal AuNPs has always caused protein denaturation. To our knowledge, retention of the Cyt c native structure has been achieved only upon conjugation to AuNPs decorated with aggregated Ag nanospheres [13].
Herein, "as prepared" CTAB-capped AuNRs have been coated with a negatively charged polyelectrolyte, polyacrylic acid (PAA), and then decorated with the positively charged Cyt c (due to Lys residues) through electrostatic adsorption. This system has been characterized by UV-vis absorption, FTIR and resonance Raman (RR) spectroscopies, transmission electron microscopy and z-potential. UV-vis absorption spectra of Cyt c bound to AuNRs in aqueous solution (AuHCc) show the native protein Soret band at 409 nm and the transverse and longitudinal plasmon bands of the NRs at 523 and 789 nm, respectively. The RR spectra obtained with both 406.7 and 514.5 nm excitation, in resonance with the B and Q transitions of Cyt c, respectively, are characterized by the coexistence of oxidized and reduced Cyt c species. Excitation with the 632.8, 647.1, 785 and 1064 nm laser lines was also attempted, but no SERS effect or Cyt c signals were observed. Nevertheless, the native structure of the protein is fully preserved by adopting this simple coating procedure.

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Resonance Raman spectroscopy of misligated forms of Cytochrome c: insight into the structure-function relationships of the protein

Lorenzo Tognaccini, Lisa Milazzo, Barry D. Howes, Giulietta Smulevich

Università degli Studi di Firenze, Dipartimento di Chimica "Ugo Schiff", 50019 Sesto Fiorentino (FI), Italy.
E-mail: lorenzo.tognaccini@unifi.it

Cytochrome c (Cyt c) is a single-chain hemoprotein whose heme c prosthetic group lies within a crevice and is covalently attached to the polypeptide chain by two thioether bridges formed with the Cys14 and Cys17 residues. Under physiological conditions, His18 and Met80 are the fifth and the sixth axial ligands, respectively, of the heme iron. Cyt c plays important roles in the cell since it acts as an electron carrier in the respiratory chain and as a ROS scavenger [1]. Moreover, direct interaction with cardiolipin, a phospholipid of the mitochondrial membrane, induces acquisition of peroxidase activity and subsequent release of Cyt c into the cytosol, where it acts as an apoptosis initiator [2].

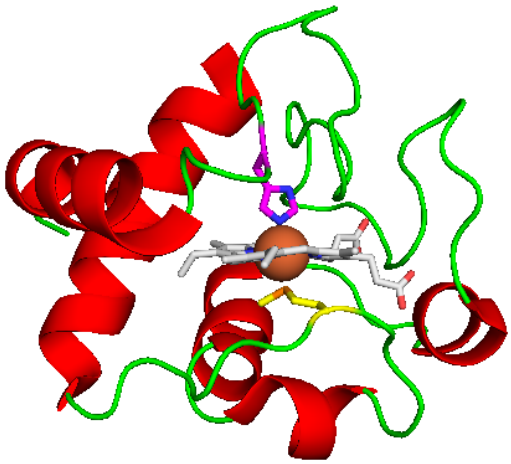

Figure 1: Cyt c structure (PDB entry 1hrc).

Yeast and horse heart Cyt c (YCyt c and HHCyt c) have been studied thoroughly as a model for human Cyt c (HCyt c). However, while the first cannot acquire proapoptotic activity, HHCyt c represents a very good model for the human protein, as its aminoacidic sequence is almost fully conserved. Moreover, Cyt c has also been extensively studied as a model protein to understand the general principles of protein folding. Ligation and misligation of Cyt *c* have been studied since 1941, when Theorell and Åkesson first reported that the oxidized protein populates at least five distinct states between pH 1 and pH 13 [3], where the Met80 sixth ligand is replaced by residues near the heme crevice. In particular, between pH 7 and pH 12, Cyt c undergoes pH-dependent ligand changes: Met80 is replaced by Lys79 or Lys73, with pK_as of 8.7 and 8.9 respectively, and by an OH^- ion at pH > 10.5 [4,5]. Furthermore, studies of the folding mechanism [6,7] highlighted the formation of a His18-Fe-His26 (or His33) species in the presence of urea, GuHCl or SDS denaturing conditions.

The combination of UV-Vis absorption and Resonance Raman (RR) spectroscopy is a precious tool to identify the sixth heme iron ligand in the misligated forms. In particular, in the low frequency RR spectrum, dramatic changes can be observed both in the positions and in the relative intensities of the Raman bands for different heme ligands; in the fingerprint region (300-450 cm^{-1}), specific iron-ligand stretching vibrations can be observed for Lys (Fe-N_{Lys}) [5] and His (Fe-Im_2) [8] misligation and a specific band pattern for His18-Fe-OH^- conformation [5].

In the present work, the combination of site-directed mutagenesis and RR spectroscopy allowed us to unravel the misligated state that is formed upon interaction of HHCyt c with cardiolipin [9], providing new information about the conformational changes that induce proapoptotic activity in Cyt c. Furthermore, RR study of human Cyt c and selected key residues mutants (Y67H, Y67R, M80A and Y67H/M80A) [10,11], allowed us to correlate the structural changes induced by mutations with the role played by the residues in the initiation of apoptosis. Interestingly, in human Cyt c, an interplay between Tyr67 and the Met80 ligand has been found to affect the conformation of the distal side of the heme pocket.

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