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REFERENCE: Short Term Scientific Mission, COST CM0601
Beneficiary: Dr Aleksandar Milosavljevic, Institute of Physics Belgrade
Host: Alexandre Giuliani, Synchrotron SOLEIL,Gif-sur-Yvette Cedex (FR)
Period: from 06/05/2010 to 20/05/2010 Gif-sur-Yvette Cedex (FR)
Reference code: COST-STSM-CM0601-6005

SCIENTIFIC REPORT

PURPOSE OF VISIT

The purpose of the visit was the joint work on VUV photon interaction with biopolymers in the gas phase, by using a novel, recently developed set-up for synchrotron radiation tandem mass spectrometry. The objective was to investigate, for the first time, the molecular physical properties of large biological molecules isolated *in vacuo*, by means of coupling the commercial linear ion trap mass spectrometer (LTQ XL from Thermo SCIENTIFIC) with VUV synchrotron beam line. Part of the time was also dedicated to investigate a possibility of performing an experiment on electron interaction with large polyions stored in a linear ion trap.

The characterization of large bio-polymers, in particular their ionization thresholds and fragmentation patterns, as well as possibility to fully characterize electron interaction with such complex systems under well defined gas-phase conditions, are of interest for the research on mechanisms to improve the control of the electron induced surface chemistry.

DESCRIPTION OF THE WORK CARRIED OUT DURING THE VISIT

The experimental setup for spectroscopy of electrosprayed ions was coupled with the DESIRS beamline at the SOLEIL synchrotron radiation facility (Fig. 1). The DESIRS undulator emission allowed measurements in the 4-20 eV photon energy range. The wavelength is selected by a normal incidence monochromator, using a grating of 200 gr mm^{-1} which provides a high photon flux (10^{12} - 10^{13} photons/s) with a photon resolution of typically 12 meV at 10 eV photon energy with a 200 µm exit slit. The photon beam can be filtered out for high harmonics using a gas filter.

The experimental system was based upon a commercial linear quadrupole ion trap (“Thermo scientific LTQ XL”), equipped with the ESI probe. The synchrotron beam is introduced into the trap through the back lens of the spectrometer. A special frame has been constructed to allow fine-tuning of the position of the spectrometer, i.e. of the trapping region, with respect to the light beam. The vacuum manifold with a turbo pumping stage has been designed to accommodate pressure difference between the beamline (10^{-8} mbar) and LTQ (10^{-5} mbar). The manifold also includes elements for additional filtering of high order radiation, photon flux measurements and photon beam shutter. A new design of the shutter, with an electro-motor (“KUHNKE”) inside the vacuum attached to a cupper heat-sink, allowed achieving short (~1 ms) and reproducible chopping time under high-vacuum conditions, as well as long exploitation. An acquisition system was made to synchronise the beamline energy scanning with the trapped ions activation, thus allowing an efficient

collection of large amount of data (a number of tandem mass spectra averaged over desired time interval for a desired photon energy).

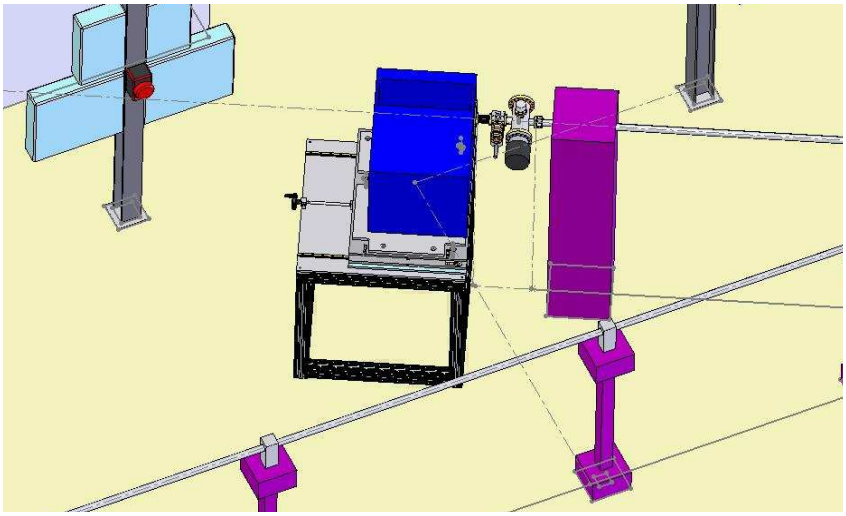

Figure 1. Coupling of the DESIRS bramline at SOLEIL synchrotron facility with the LTQ XL mass spectrometer (Thermo Scientific). A special frame has been constructed to allow fine-tuning of the position of the spectrometer with respect to the light beam.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

The results obtained during the present STSM visit complete-up the most recent results obtained in December 2009 during the first scheduled beam time for the project. They include, for the first time on biopolymers, the photodetachment spectroscopy using a first-order synchrotron light of peptide and protein negative ions and the first ionization spectroscopy of polycations. Some of the preliminary results have been most recently reported on ASMS annual conference (May 2010, Salt Lake City, USA) [1].

Electron photodetachment spectra have been measured for various charge states of insulin and cytochrome c protein. Present results on insulin, obtained with a high energy resolution using the first-order light, fit very nicely to the recent data obtained with zero order light [2] and allow a profound investigation of the mechanism of the electron photodetachment in polypeptides. The recently proposed mechanism [3] of the photon induced photodetachment of negative polypeptides implies electronic excitation of a chromophore state, followed by its crossing with an autoionizing state. An exemplary tandem mass spectrum upon VUV photon activation of a selected, six-fold deprotonated negative precursor of cytochrome c protein (≈ 100 amino acids, ≈ 12.4 kDa) is presented in Fig. 2. The peaks corresponding to single (5-) and double (4-) electron photodetachment are clearly seen in the spectrum. The intensive signal and high signal-to-nose ratio allow a very accurate determination of the photodetachment spectra.

In the positive ion mode, measurement of the mass/charge-selected photonionization signal as a function of the wavelength allows obtaining the photoionization spectra and deriving the ionization thresholds for various peptide's or protein's charge states. We found that the photoionization occurs for all samples in the 10 to 13 eV range (124-95 nm), depending of their charge state. Particularly, the photoionization of polyprotonated cytochrome *c* protein represents the first reported result on photoionization of kDa species in the gas phase.

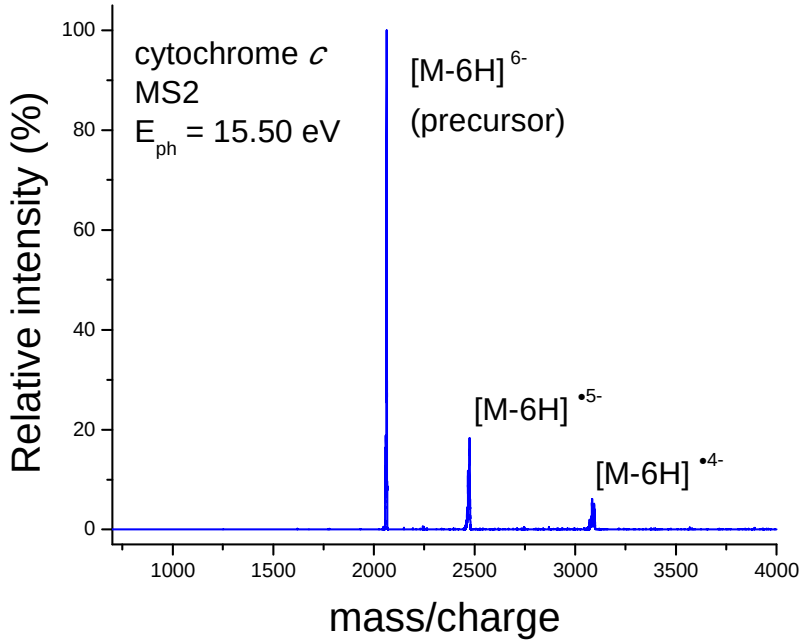

Figure 2. Tandem mass spectrum of cytochrome *c* protein polyanions upon activation by 15.5 eV photons.

References:
[1] A. R. Milosavljević et al., Proceedings of the 58th ASMS Conference, Salt Lake City, May 2010.
[2] A. Giulliani et al., Proceedings of the 57th ASMS Conference, Philadelphia, June 2009.
[3] Joly et al., J. Am. Chem. Soc. 129 (2007) 8428; J. Phys. Chem. A 112 (2008) 898

FUTURE COLLABORATION WITH HOST INSTITUTIONS

The future collaboration is planned as a continuation of the present work. This collaboration will be focused both on new measurements (with the samples including peptides, proteins and nucleic acids) and further development of the experimental system and new techniques allowing accessing physical properties of large biological ions.

PROJECTED PUBLICATIONS/ARTICLES RESULTING OR TO RESULT FROM THE STSM

We expect at least one publication in a leading international journal and several contributions to international conferences in a near future including results obtained during the visit, as well as further important results as a continuation of the started work.

A. Milosavljević

Aleksandar Milosavljević
Institute of Physics, Belgrade

Belgrade, June 1st 2010

CONFIRMATION BY THE HOST INSTITUTE OF THE SUCCESSFUL EXECUTION OF THE MISSION

Herewith I confirm that Aleksandar Milosavljevic, PhD, successfully worked in the SOLEIL synchrotron facility from May 5th until May 20th 2010. The obtained results are of wide interest regarding the investigation of large biomolecules. It was a pleasure to have Aleksandar Milosavljevic working in my group.

Gif sur Yvette, June 1st 2010

Alexandre Giuliani
Division Expérience
Synchrotron SOLEIL
Gif-sur-Yvette