# N K-SHELL X-RAY TANDEM MASS SPECTROMETRY OF GAS-PHASE UBIQUITIN PROTEIN

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**Abstract.** We present results on the N K-shell X-ray tandem mass spectrometry of gas-phase, multiply charged ubiquitin protein. The results have been achieved by coupling a linear ion trap mass spectrometer, fitted with an electrosprayed ion source probe, to a soft X-ray synchrotron beamline. The tandem mass spectrum of the 5+ charge state precursor of ubiquitin at the activation energy of 402 eV is presented and discussed. The results show a predominant ionization channel of the protonated protein precursor, but accompanied also by losses of small neutral fragments.

## 1. INTRODUCTION

The controlled study of protein degradation upon exposure to X-rays is of particular importance for the radiation damage research. Additionally, this field has become highly relevant in the last few years, when it was demonstrated that short and intense X-ray pulses from the X-ray free electron lasers (XFEL) can be used for protein 3D structure determination using single-object coherent X-ray diffraction [1]. This is of special interest for membrane proteins, which are difficult to crystallize and study using conventional protein crystallography methods at synchrotrons. There has been a large effort to understand the details of radiation damage of biomaterials at the molecular level, which can also favor important medical applications such as cancer therapy [2]. With this respect, an immense number of publications have been devoted to the electron/ion/photon interaction with DNA and its components [3]; however, protein ionization/degradation and associated secondary effects were less investigated

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although equally important in damage modeling [1]. It is well known that X-ray irradiation induces strong fragmentation in amino acids and peptides [4]. Nevertheless, surprisingly, gas phase proteins appeared to be much less prone to dissociation, whereas dominant channels corresponded to ionization and losses of neutral fragments [5]. Still, it should be noted that intensive production of low-energy secondary electrons, due to direct ionization or to the normal/resonant Auger decay of the core-ionized/-excited states, can also strongly affect the degradation of the biomaterial [6]. Very recently, it has been also predicted that resonant X-ray photoabsorption can effectively produce slow electrons, through the initial inner-shell excitation triggering a resonant-Auger intermolecular Coulombic decay (ICD) cascade [7]. Although the latter study [7] has been performed for a simple ArKr model system, the authors pointed out that the process might have consequences for fundamental and applied radiation biology. With this respect, the inner-shell mass-resolved action spectroscopy of a protein can indeed resolve the dominant relaxation channels upon resonant X-ray photon absorption.

## 2. EXPERIMENTAL SETUP

The experimental setup and coupling of the linear quadrupole ion trap to a synchrotron beamline has been described in previous publications [5,8,9]. Briefly, the setup is based on a commercial linear quadrupole ion trap mass spectrometer (Thermo Finnigan LTQ XL) equipped with an electrospray ionization (ESI) source. The electrosprayed ions are introduced from the front side into the trap, while the soft X-ray photon beam enters the ion trap from the backside. The irradiation time (about 500 ms in the present case) of the mass-selected precursors is regulated by a special photon shutter [10]. The setup includes a differential pumping stage to accommodate the pressure difference between the beamline ($10^{-9}$ mbar) and the LTQ ($10^{-5}$ mbar of He in the main chamber).

The setup is connected to the soft X-ray beamline PLEIADES [11,12] of the SOLEIL storage ring in Saint-Aubin (France). The photon beam is produced by a quasi-periodic APPLE II type of undulator (80 mm period), followed by a modified Petersen plane grating monochromator with varied line spacing and varied groove depth gratings. For the present experiment, a 400 lines $mm^{-1}$ grating was used, which provides high photon flux on the order of $1-2 \times 10^{12}$ photons $s^{-1}$/0.1% bandwidth for the used energy. The energy resolution used (full with at half maximum, FWHM) was about 430−460 meV. The photon energy was calibrated according to the N 1s→π* resonance in molecular nitrogen. Multiply protonated ubiquitin protein (Sigma Aldrich) was generated by the ESI source from a water/acetonitrile (75:25) solution at 10 μM.

## 3. RESULTS AND DISCUSSION

The tandem mass spectrum of the 5+ charge state ubiquitin precursor, upon resonant X-ray photon irradiation during 500 ms, below N 1s ionization

threshold (IT) is presented in Figure 1. The spectrum shows that the inner-shell photo-excitation of a protein dominantly leads to its ionization accompanied by losses of neutral fragments. The ionization of the photo-excited precursor at photon energies below the direct inner-shell IT is the consequence of the resonant Auger decay [7] triggered by the promotion of a core (1s) electron to an unoccupied, bound molecular orbital, forming a highly-excited state. A valence electron fills the core vacancy and the molecule decays by ejecting another (Auger) electron. It is also possible that more than one valence electron be ejected during decay leading to multiple resonant Auger decay [13].

The finding that the ionization process, accompanied by low-mass neutral fragment losses, represents the dominant relaxation channels upon X-ray absorption by ubiquitin protein is very important for the radiation damage research, imposing limitations to the widely applied building blocks approach based on the premise that the properties of complex macromolecules can be elaborated through investigation of their components. Note that the channelling of the absorbed energy into multiple electron ejection and neutral fragment losses, instead of backbone destruction, does not necessarily mean a better protection of the biological material; the secondary electrons can further produce damage [6] and neutral fragment losses from amino acids can induce mutations. However, the present results certainly bring new insights for a more accurate modelling of the complex radiation damage phenomenon. Multiple electron ejection, rather than the formation of small ions and radicals, suggests that the resonant X-ray irradiation of protein-reach regions in the cell would produce a significant number of secondary electrons.

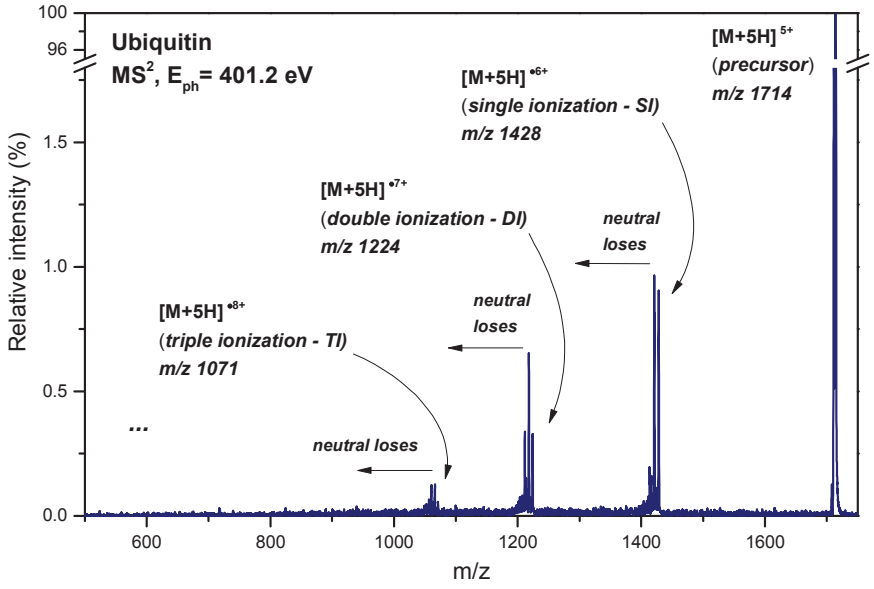


**Figure 1.** Tandem ESI/photoionization mass spectra of the 5+ (m/z 1714) ions of ubiquitin protein, obtained after 500 ms of irradiation at the photon energy of 401.2 eV.

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