



“What Sherlock Sorely Missed: The EVA Technology for Cultural Heritage Exploration

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Abstract

Capture of proteins and metabolites from Cultural Heritage (paintings, manuscripts, parchment etc.) has been done in the past via surface scraping and erasing a method today strictly forbidden. At present, two techniques stand out: the EVA film technology (here presented) and the rubbing method of Collins (*Proc. Natl. Acad. Sci. USA* 2015). The last technique is based on tribo-electric extraction, i.e. electrostatic molecular harvesting elicited by rubbing the surface of document with a PVC polymer. The EVA (ethyl vinyl acetate) method consists of a plastic polymer in which strong cation and anion resins with C₈ and/or C₁₈ are embedded. These three types of resins are able to efficiently capture from any surface macromolecules and metabolites, including drugs of abuse. The humidified EVA film is placed in contact with different locations (in general along the margins) on the surface to be analysed for up to 1 h. Proteins (or other metabolites) are then extracted in 2% SDS at room temperature. This is followed by SDS-PAGE separation, band excision and trypsin digestion. The peptides, after Zip tip purification, are analysed by nLC MSMS, using an Orbitrap Fusion trihybrid mass spectrometer coupled with a nano-flow UHPL system. The raw data are analysed by Proteome Discoverer software by consulting different protein databases according to the aims of investigation.

We have explored different items stored in public libraries and archives: (a) the original manuscript of the novel *Master y Margarita* by Bulgakov (in Moscow's library); (b) the death registries of the lazaretto in the 1630 Milano plague (in the State Archives in Milano); (c) the shirt worn by A. Chekhov in his death bed (in his museum in Melikovo, Russia); (d) Kepler's script on Plutarch (in St. Petersburg National Archives); (e) the Memoirs of G. Casanova (in Paris National Library). In Bulgakov's case, we could ferret out three biomarkers of his renal pathology as well as abundant traces of morphine that the author consumed to quench the pains of his disease. In the case of Chekhov, the Koch bacillus responsible for his death was spotted. In the 1630 plague bout in Milano, not only 17 proteins specific of the *Y. pestis*



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could be tracked down, but also clear traces of a secondary infection due to anthrax were discovered. In Casanova's case, we searched for the gonorrhoea bacillus, since he confessed several episodes of this disease in his lifetime. In Kepler's case several metals, as identified by X-ray photoelectron spectroscopy (XPS), followed by TOF-SIMS (Time-of-Flight secondary ion mass spectrometry) and by ICP-MS (Inductively coupled plasma mass spectrometry, could be detected, suggesting that he practiced alchemy.

The EVA technique coupled to MS instrumentation could open new, unexpected scenarios belonging to our past history and Cultural Heritage.

The lecture will take place **at 14.30 – Sala Verde – Cà Vignal – Strada Le Grazie, 15**

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For each hour of seminar, 1 CFU (provided for the specific activities of PhD Program in Biotechnology) will be recognized to students attending the event.
