Analysis of proteins by microPIXE

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Though many methods, i.e. mass spectrometry or X-ray protein crystallography, for the analysis of proteins are known, it remains sometimes difficult to identify all atoms present in a protein and to quantify their stoichiometry. Especially in the case of metal binding proteins which evade successfully their crystallization, microPIXE becomes a powerful complementary technique for the quantitative analysis of the metal content of a protein.

The LIPSION microprobe facility at the Leipzig University was used with a 2.25 MeV scanning proton micro-beam to determine the metal to protein ratio of protein samples. The sulphur content of proteins, derived from its amino acid sequence (only the amino acids cysteine and methionine residues contain sulphur) serves as an internal standard. We explored the limits of the metal stoichiometry determination of this technique with test samples containing inorganic dissolved copper salts of different concentrations embedded in an organic polymer matrix. For the accurate determination of the metal content the knowledge of the sample thickness is required. We compared scanning transmission ion microscopy (STIM) together with the stopping power tables calculated by the software SRIM (Stopping Range of Ions in Matter) with the determination of the sample thickness by fitting Rutherford Backscattering (RBS). In the case of the proteins samples the STIM method allows a more reliable determination of the thickness of the sample due to sample inhomogeneities. Radiation damage in the form of sulphur evaporation was investigated and no severe effects were detected for low currents. Several results with real proteins are presented. MicroPIXE, combined with STIM proves to be a very sensitive method for metal to protein stoichiometry determinations.