ROBL-CRG	**Experiment title:** Comparative investigation of the uranium complexation by proteins by means of FT-IR and EXAFS spectroscopy	**Experiment number**: 20-01-672
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Names and affiliations of applicants (* indicates experimentalists):

Main applicant: Dr. Johannes Raff*
Applicant: Bo Li*
Applicant: Dr. André Rossberg*
Applicant: Dr. Harald Foerstendorf
Applicant: Dr. Andreas Scheinost

Report:

The aim of the experiment was to determine the functional groups of the S-layer protein interacting with U(VI). In particular, distinguish between carboxylic and phosphate groups in case of U(VI) protein complexation was expected from the results. Thus, polyglutamate and phosvitin were set up as an organic carboxylic and an organic phosphate model compound with the protein backbone to complex with U(VI). An S-layer with a slightly higher phosphate content was also chosen in the experiment.

The infrared spectra of U(VI) polyglutamate complexes show a characteristic frequency for the $\nu_{as}(UO_2^{2+})$ mode representing a U(VI) complexation to carboxylic groups. The EXAFS spectra confirm these findings by observation of a bidentate binding of the U(VI) to carboxylic groups.

Furthermore, in the complex with a U(VI):carboxyl ratio of 1.47:1 two coordinated O-atoms with a shorter U-O distance were detected. The assignment of these O-atoms to distinct functional groups or to the protein backbone has to be verified.

In the phosvitin samples, the U(VI) preferentially binds to phosphate groups, which is demonstrated by the $\nu_{as}(UO_2^{2+})$ peak at 918 cm^{-1} on the IR spectra. With increasing U(VI):phosphate ratio, the binding to carboxylic groups and/or to hydroxyl groups becomes more relevant. This has been demonstrated by a shift of the $\nu_{as}(UO_2^{2+})$ mode to 925 cm^{-1} which is very close to the characteristic frequency of 923 cm^{-1} found for the $\nu_{as}(UO_2^{2+})$ mode after complexation to carboxylic groups of proteins. However, this effect was not observed in the EXAFS spectra. The EXAFS spectra of the phosvitin samples are in good agreement with the spectra of other organic phosphate-U(VI) complexes such as U(VI)–AMP and U(VI)–fructose(1,6)diphosphate complex. In this complexes U(VI) is not only complexed by a phosphate group, but presumably also by a hydroxyl group. Therefore, a formation of a U(VI) complex, which is similar to the so-called "Feldman complex", can be suggested.

In case of the S-layer samples, both IR and EXAFS show that U(VI) mainly binds to carboxylic groups. Particularly, the EXAFS spectra of the S-layer samples are similar to the polyglutamate sample with a U(VI):carboxyl ratio of 1.47:1, where two shorter interatomic U-O distances were found. Furthermore, there are differences between EXAFS spectra of the U(VI)–S-layer complex from this experiment and spectra of the U(VI) complex with an S-layer containing less phosphate groups. Whether the spectral deviations can be assigned to specific U(VI)–phosphate interactions in the S-layer with much lower phosphate content has to be evaluated in future experiments.

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