HYDROXYL RADICAL FOOTPRINTING OF PROTEIN/DNA COMPLEX COUPLED TO HIGH-RESOLUTION MS ANALYSIS

Marek POLÁK[1,2], Michael Volný[1], Daniel KAVAN[1,2] and Petr NOVÁK[1,2]

1 - *BioCev* - Institute of Microbiology, The Czech Academy of Science, Prague, CZECH REPUBLIC

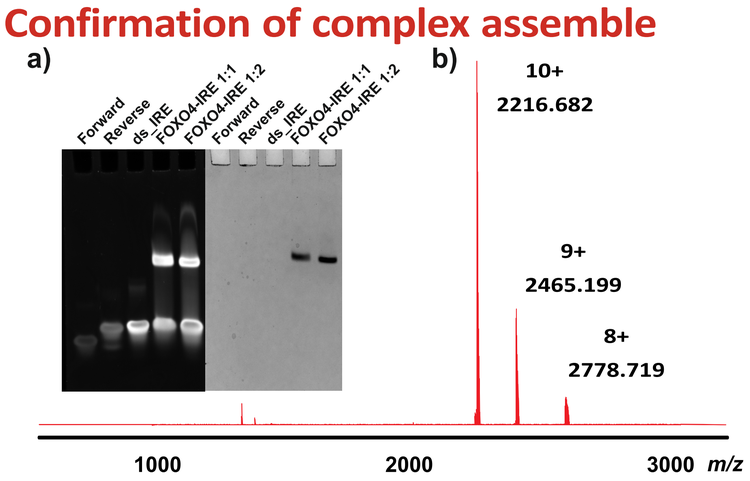
2 - Faculty of Science, Charles University Prague, CZECH REPUBLIC

INTRODUCTION

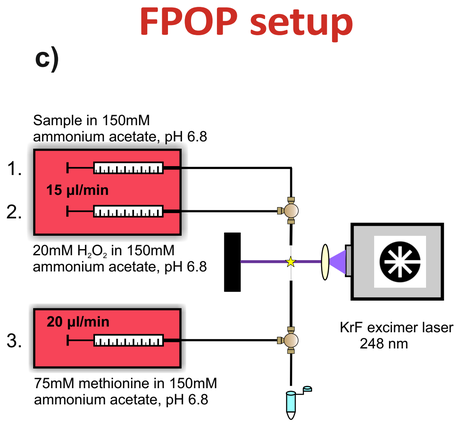
Interaction between transcription factors and nucleic acids plays crucial role in cells. Understanding the mechanism of protein-DNA interaction and its mutual dynamics gives a broad overview of its role in various biological processes. Structural proteomics has undergone a remarkable growth in recent years, which had a huge impact in the field of structural and molecular biology. Structural proteomics methods offer the possibility to answer questions related to structure and dynamics of protein complexes. Beyond well-established MS-based methods, radical covalent labelling has evolved as an effective analytical tool for characterization of such complexes.

RESULTS

Confirmation of complex assemble



FPOP setup



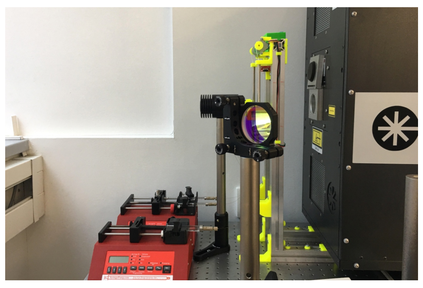


Figure 1: Optimization of the assembly of FOXO4-DBD/IRE complex using native electrophoresis and GelRed® and CBB R-250 staining (**a**). Confirmation of FOXO4-DBD/IRE complex formation by native electrospray experiment (**b**). Experimental FPOP setup (**c**).

Analysis of oxidized samples

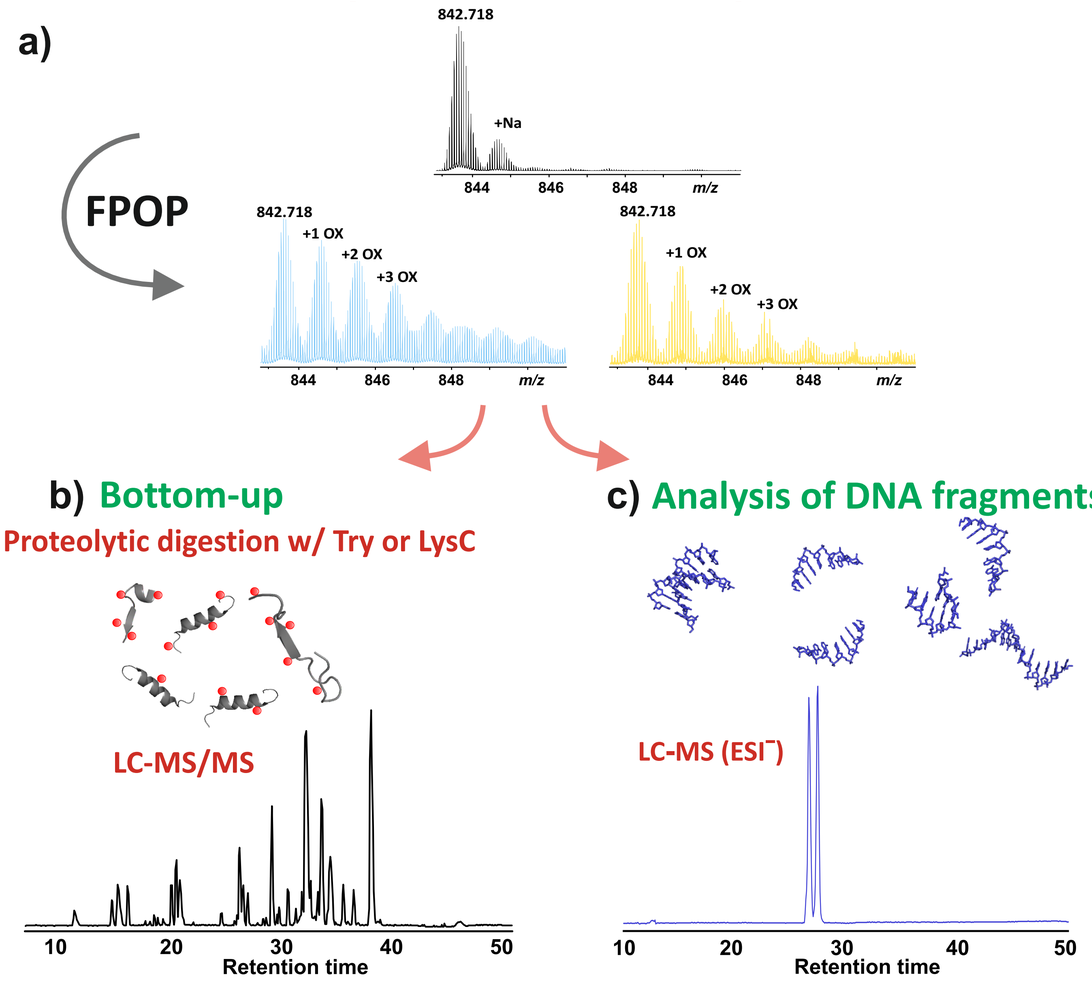
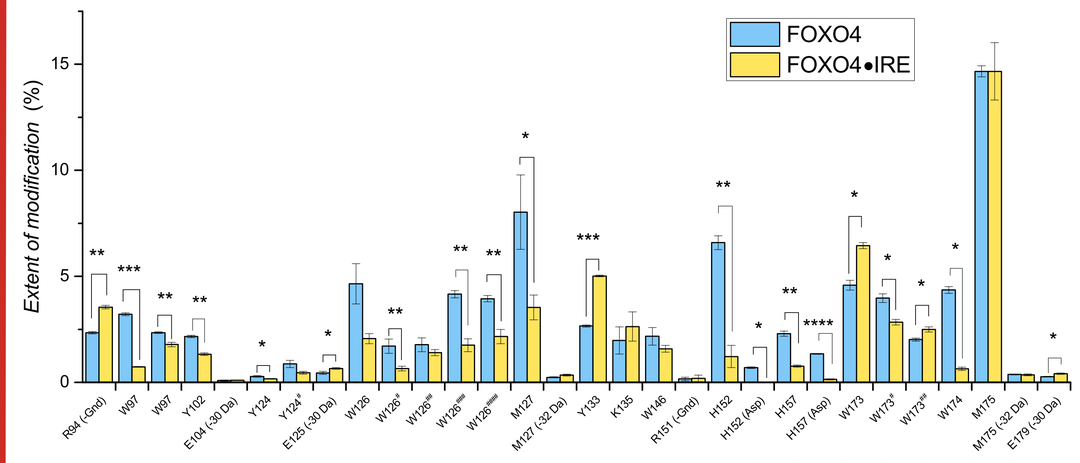


Figure 2: Intact mass spectra of +14 charge state of FOXO4-DBD (**black**) and its respective oxidized apo (blue) and holo (yellow) forms after laser irradiation **(a)**. Bottom-up analysis comprised of sample digestion with Trypsin and LysC followed by identification/quantification by LC-MS/MS **(b)**. Quantitative analysis of DNA fragments was done using LC-MS analysis in DIA mode **(c)**.

METHODS

- **Model protein:** We used a DNA binding model consisted of FOXO4 protein and its cognate Insulin response element, IRE.
- **Complex formation:** To form a complex, FOXO4-DBD and IRE were mixed in molar ratio 1:1. Native electrophoresis (**Figure 1a**) and native nano-electrospray ionization (**Figure 1b**) were used to confirm its formation.
- **FPOP setup:** Both protein and dsDNA were submitted to FPOP alone and in a complex. FPOP was performed in 150 mM ammonium acetate buffer, pH 6.8 . To modify the apo/holo forms (**Figure 1c**), a KrF excimer laser (Coherent Inc., USA) was used to dissociate H_2O_2 (10 mM).
- **Mass spectrometry:** (**Figure 2**) Collected samples were split for bottom-up and top-down analyses. Modified AA residues were identified in DDA mode and extent of modification was calculated from MS trace (timsToF Pro, Bruker Daltonics). The DNA damage initiated by hydroxyl radicals was evaluated by DIA analysis (SolariX XR 15T, Bruker Daltonics).

a) Bottom-up



Analysis of IRE fragments

b)

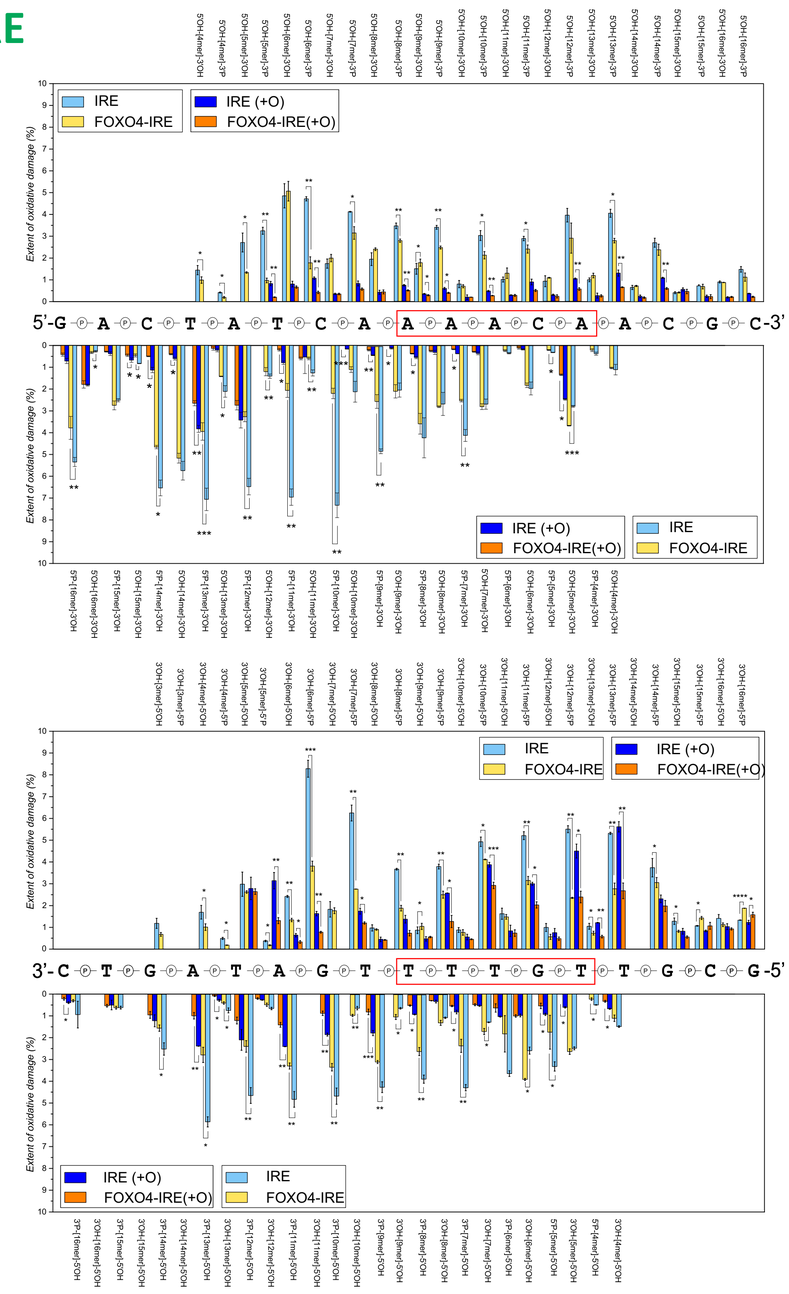


Figure 3: Quantification of the modified residues detected by bottom-up analysis **(a).** Quantified DNA fragments originated from forward strand **(b)**, quantified DNA fragments originated from reverse strand **(c)** for both apo (IRE, blue) and holo (FOXO-IRE, yellow) forms. Extent of oxidative damage determined by quantification of individual DNA fragments separated in LC-MS analysis.

Visualization on a structural model

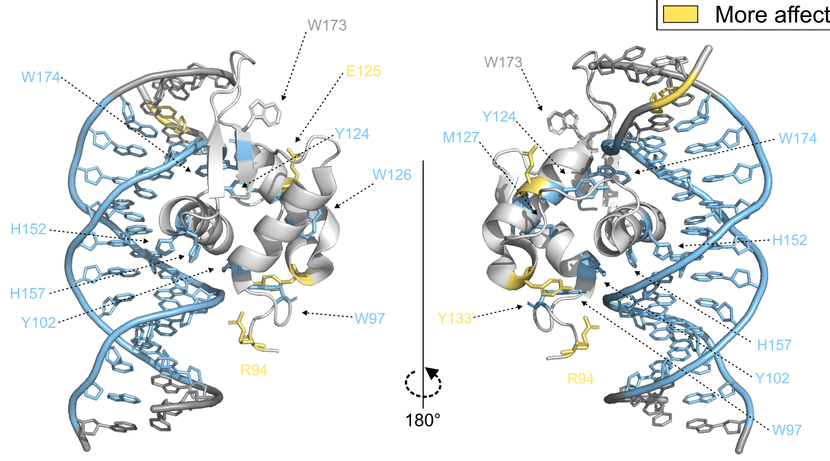


Figure 4: Changes detected on both FOXO4 and IRE are visualized in an X-ray structural model of FOXO4/IRE (based on 3L2C).

CONCLUSION

- Data were resolved at a single amino acid resolution using bottom-up approach (**Figure 3a**). The DNA damage initiated by hydroxyl radical attack was examine to obtain information about the damage of DNA (**Figure 3a, 3b**).
- Results, which were visualized on FOXO4/IRE structural model (based on 3L2C), demonstrate the potential of FPOP as a technique for surface analysis of protein/DNA complexes (**Figure 4**).

LABORATORY OF STRUCTURAL BIOLOGY AND CELL SIGNALING

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