



Structural characterization of protein-DNA complexes by hydroxyl radicals



MAREK POLÁK

Laboratory of structural biology and cell signaling |
Institute of Microbiology of the CAS | Prague

Labelling techniques to study higher order structure

HDX

Exchange of amide hydrogens for deuterium in peptide backbone

- Mapping dynamics and solvent accessibility

Chemical labelling

Acetylation of lysine, modification of arginine and tyrosine

- Mapping dynamics and solvent accessibility

Radical labelling

Labelling of protein surface by reactive radicals

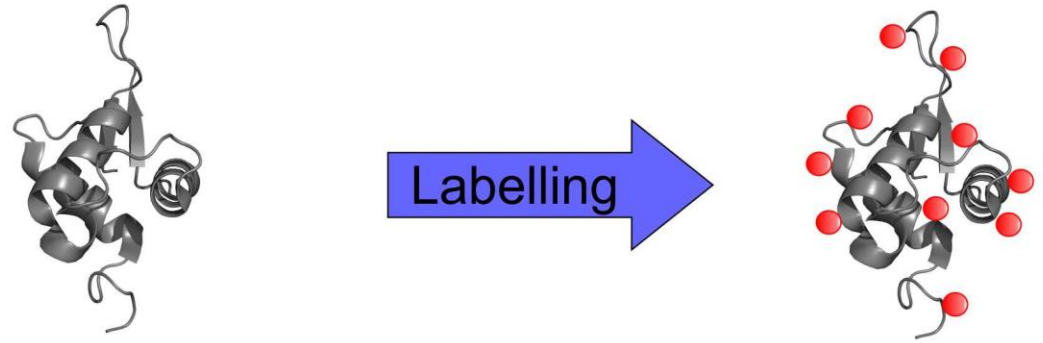
- Mapping dynamics and solvent accessibility

Coupled to high resolution mass spectrometry

Footprinting (Radical)

Labelling of residues:

- **Labelled ones – reactive and exposed to solvent**
- **Unlabeled ones – nonreactive to probe, or consequence of solvent inaccessibility due to structure/interactions**
- **examination of**
 - **higher order structure of biomolecules**
 - **interaction of biomolecules**



Radical labelling

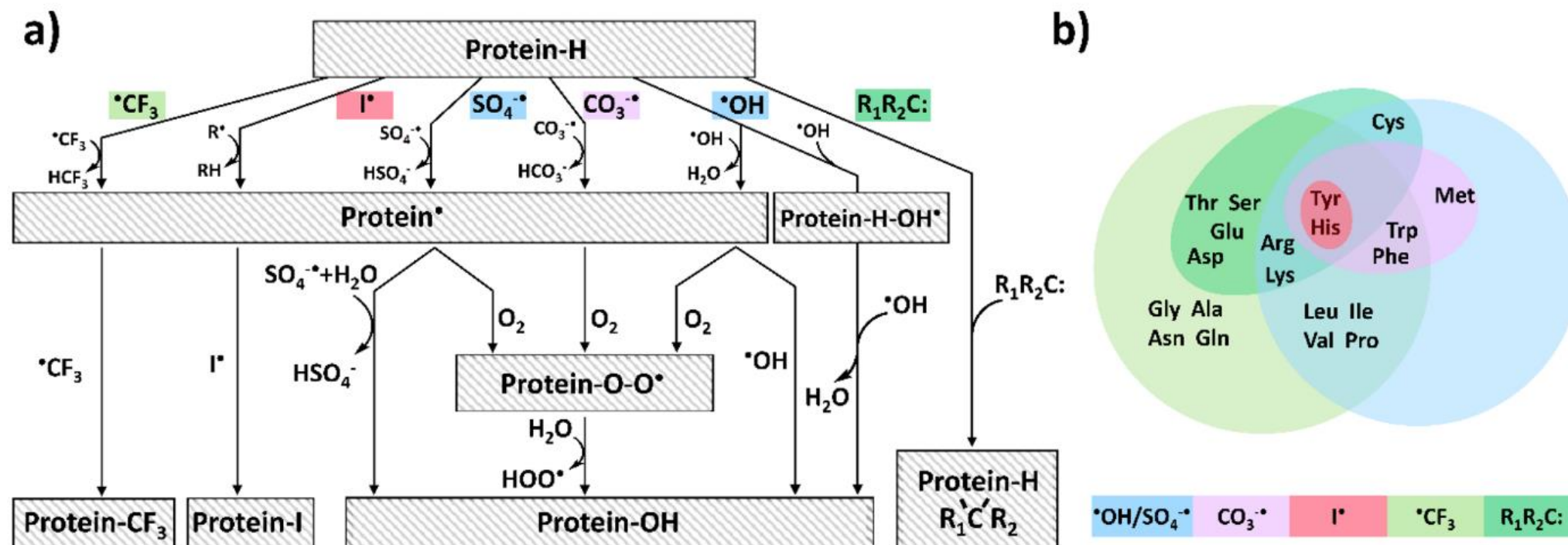


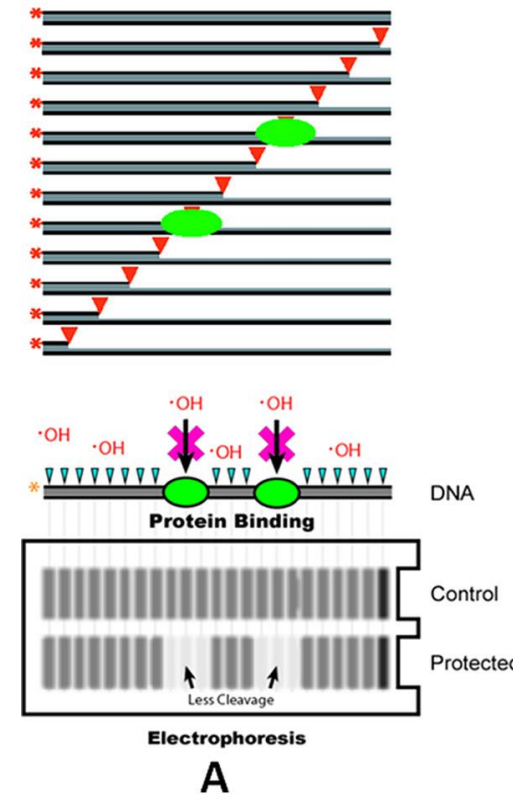
Figure: Summary of radical-based footprinting reagents of (a) proposed pathways and (b) residue specificity.

X. R. Liu, M. M. Zhang, M. L. Gross, *Chem. Rev.* **2020**, *120*, 4355–4454.

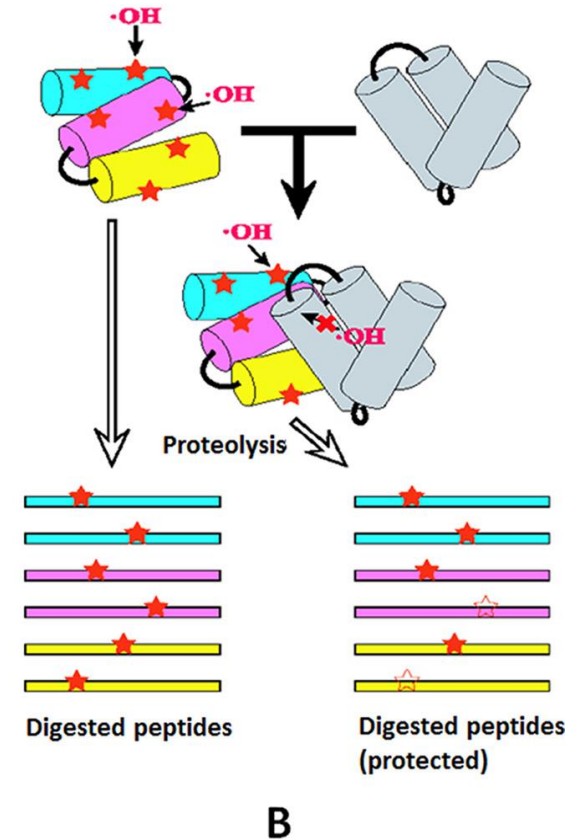
History of footprinting techniques

1. **Limited proteolysis** – proteolysis of exposed protein residues
2. **DNAse footprinting**
1st footprinting method – enzymatic footprinting
3. **Hydroxyl radical footprinting of DNA (Fenton reaction)**
$$\text{Fe(II)} + \text{H}_2\text{O}_2 \rightarrow \text{Fe(III)} + \text{OH}^- + \cdot\text{OH}$$
4. **Hydroxyl radical footprinting of proteins (Fenton reaction)**
Oxidation of protein residues by Fenton reaction – Protein interactions, binding interfaces
5. **Radiolysis of H₂O by synchrotron or electron beam**
Expensive and normally not available
6. **Fast Photochemical oxidation of proteins**

DNA- protein interaction



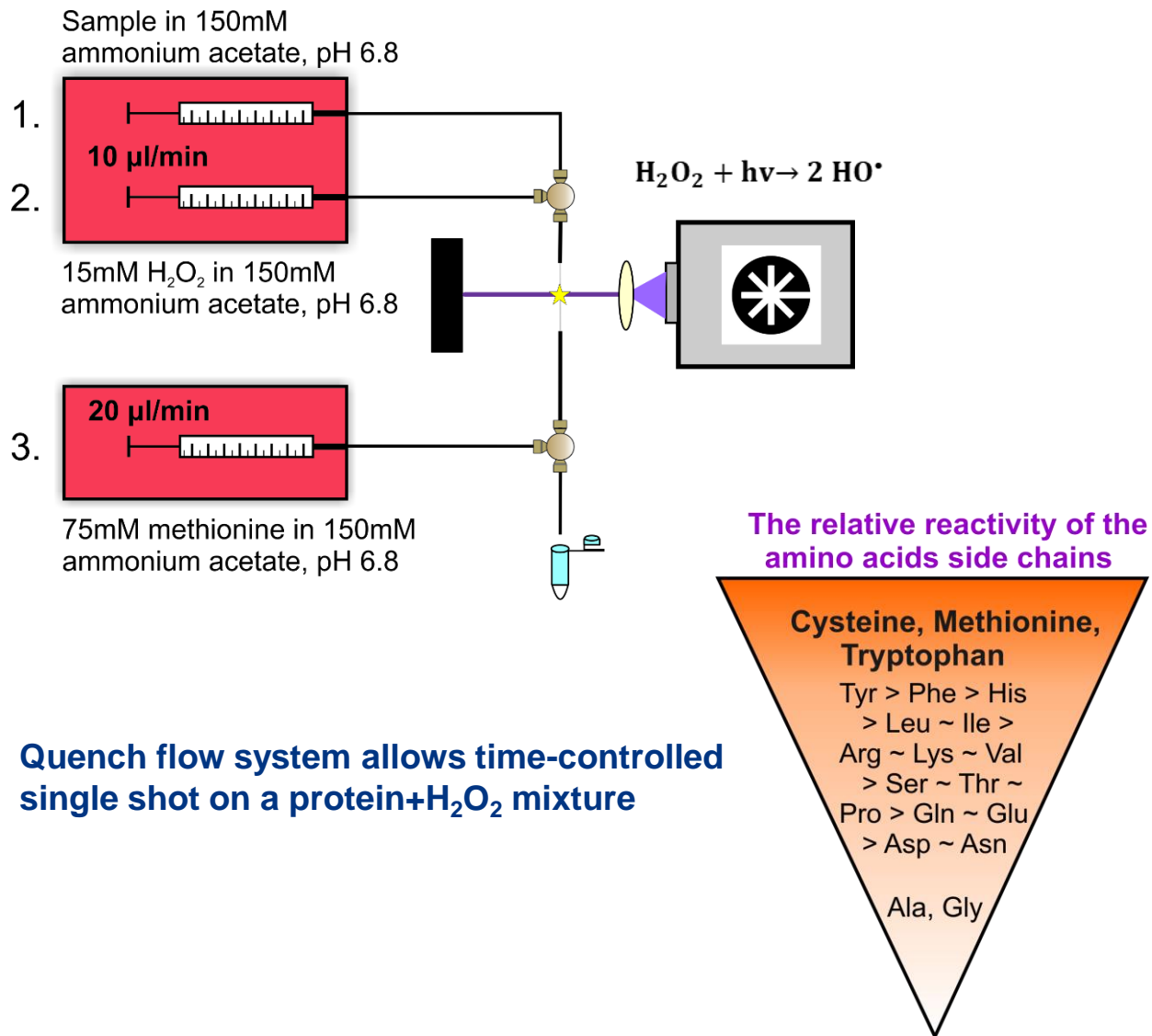
Protein- protein interaction



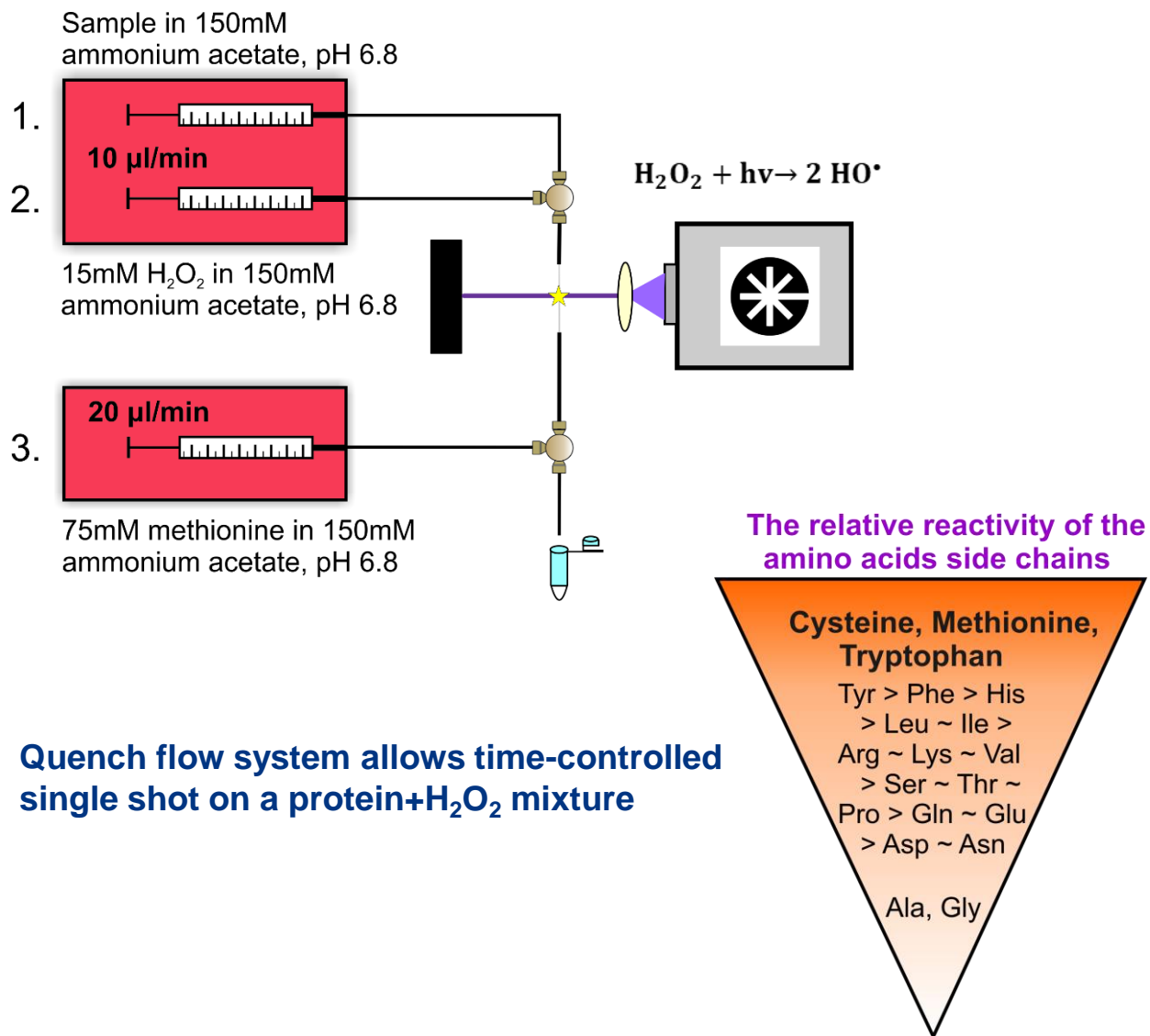
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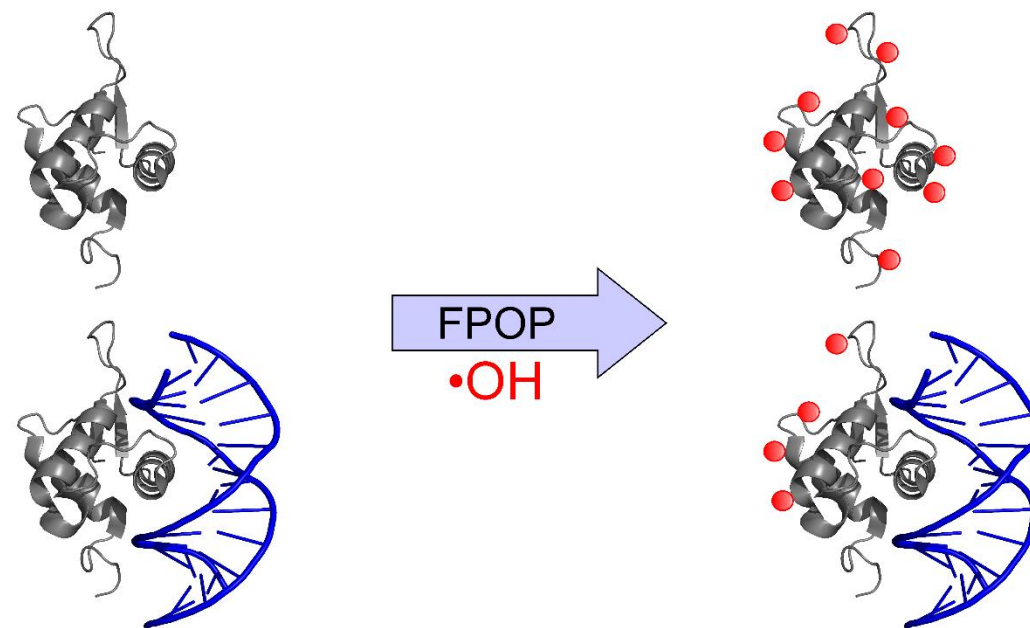
Fast Photochemical Oxidation of Proteins (FPOP)



Fast Photochemical Oxidation of Protein-DNA complex



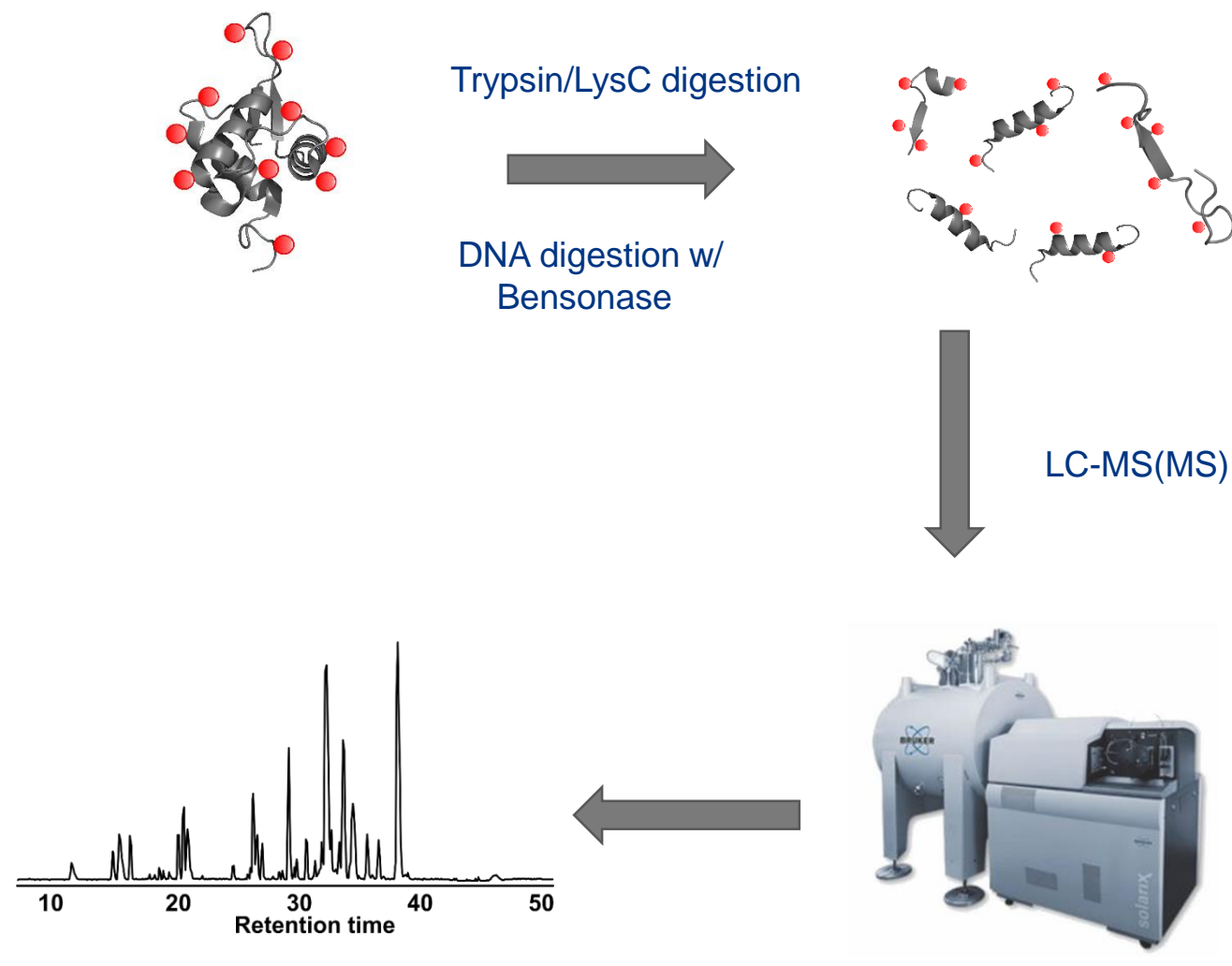
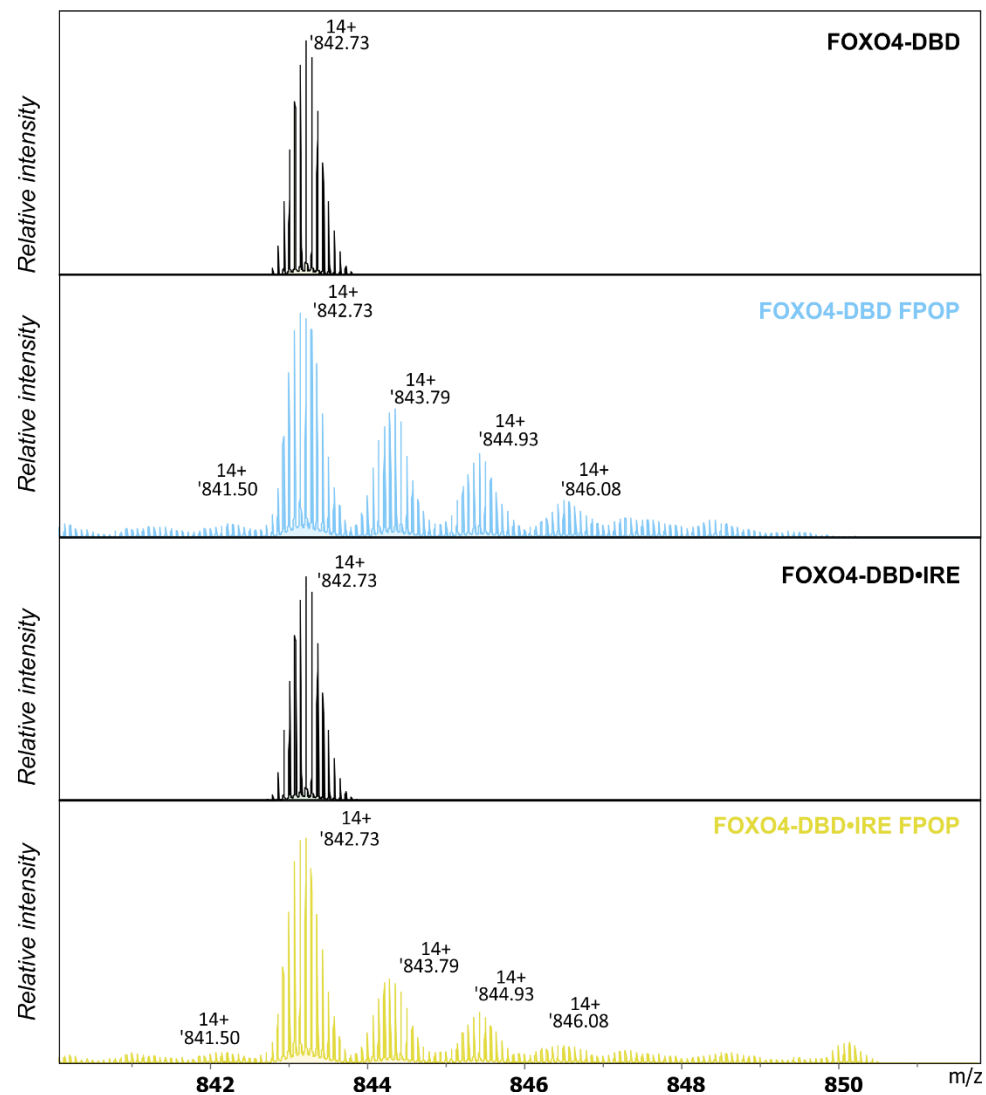
Aim of study: FPOP of protein-DNA complex:



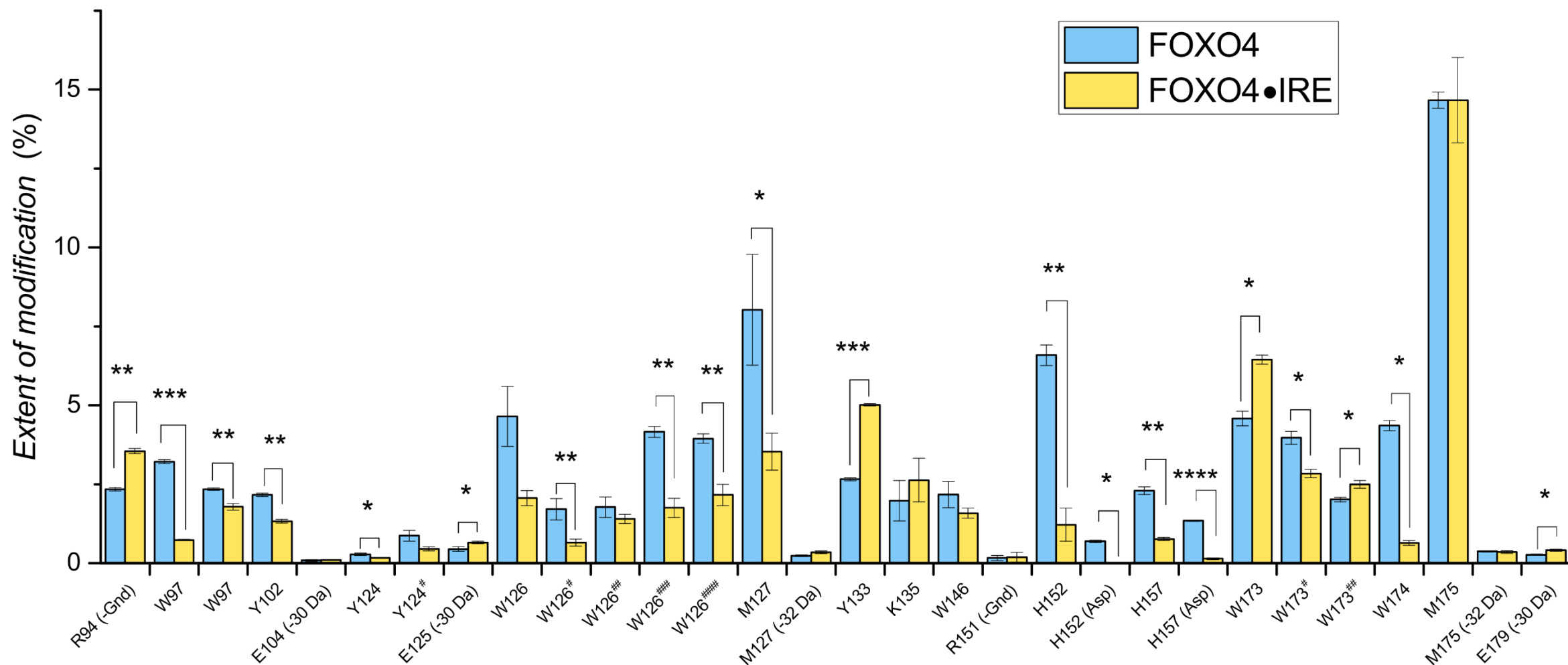
Protein: DNA Binding domain of FOXO4

dsDNA: Insulin Response Element (IRE):
5'-GAC TAT CAA AAC AAC GC-3'

FPOP of FOXO4-IRE complex

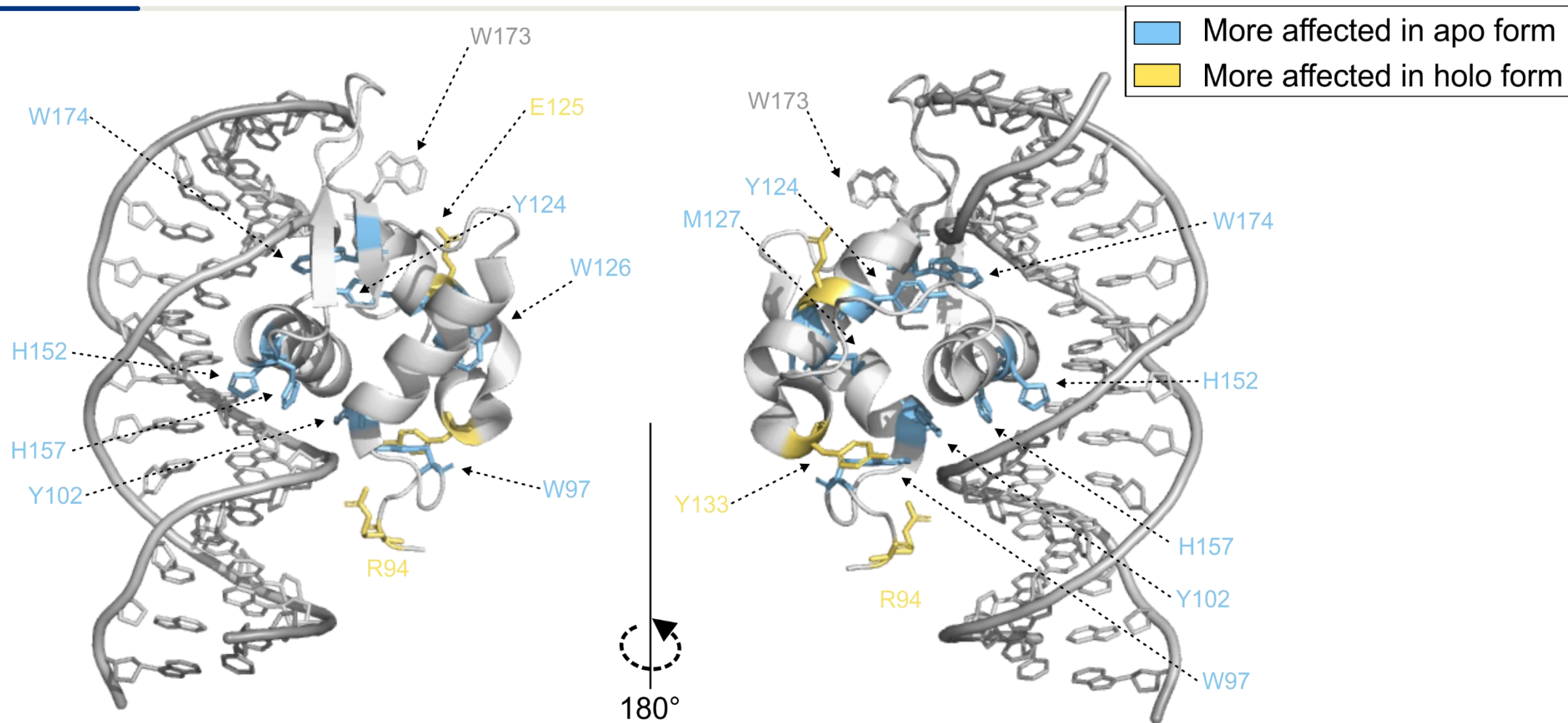


Extent of modification of modified residues



T-test leged: *($P \leq 0.05$), **($P \leq 0.01$), ***($P \leq 0.001$), ****($P \leq 0.0001$)

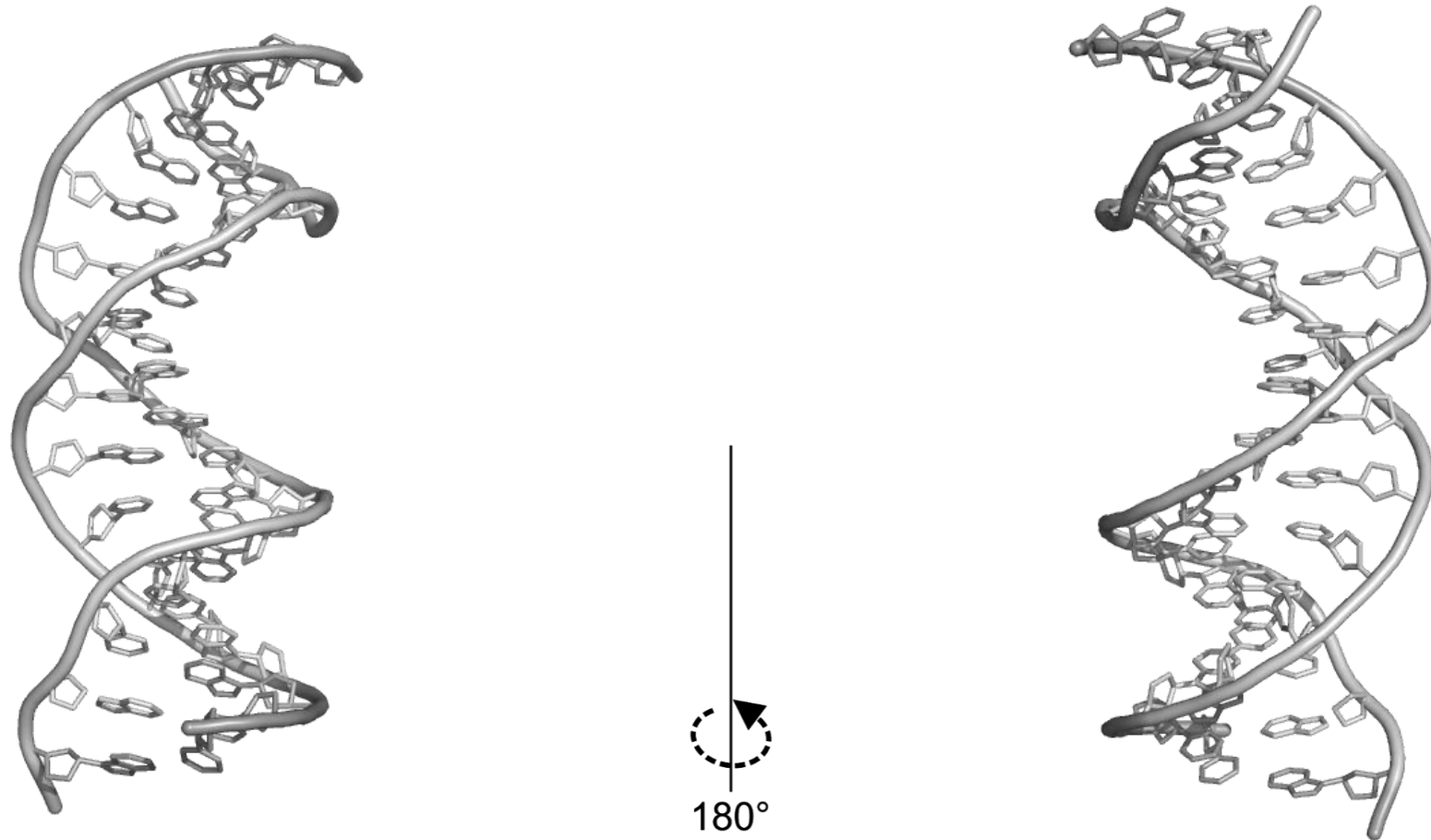
Plotting residues onto a crystal structure



Model of FOXO4-IRE built based on an available structural model in Pymol (entry: 3l2c)

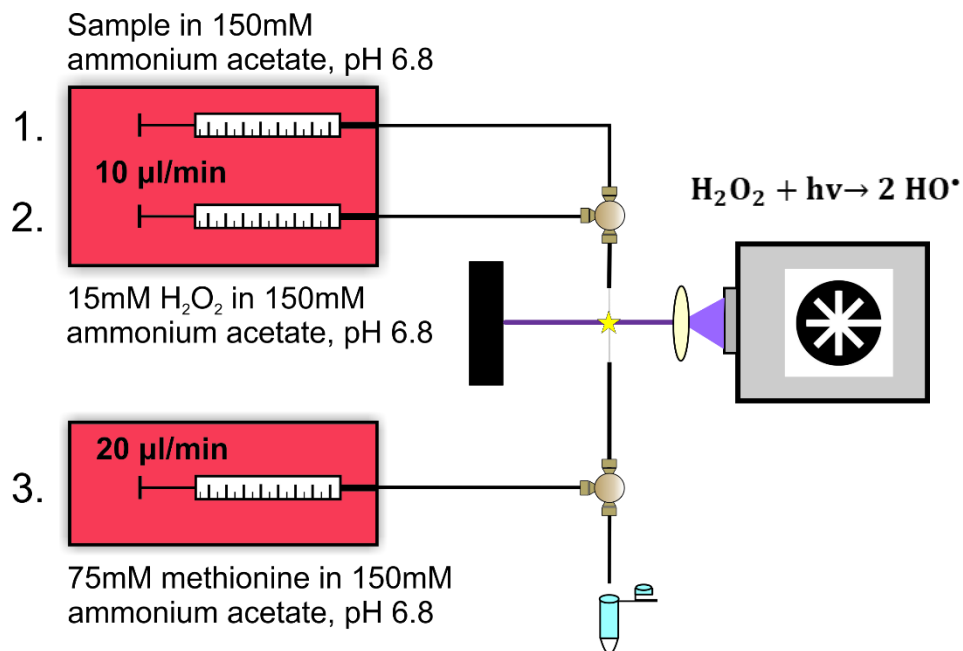
Boura E., *Acta Crystallogr D Biol Crystallogr*, 2010

What information can be obtained from the DNA site?

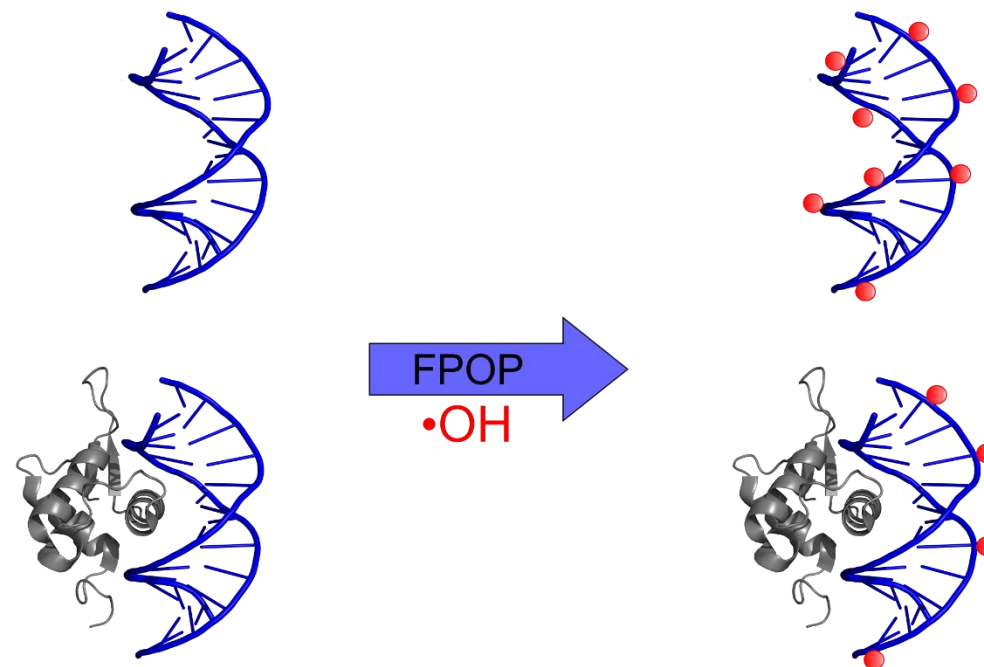


Boura E., *Acta Crystallogr D Biol Crystallogr*, 2010

Fast Photochemical Oxidation of Protein-DNA complex



Aim of study: Oxidation of DNA

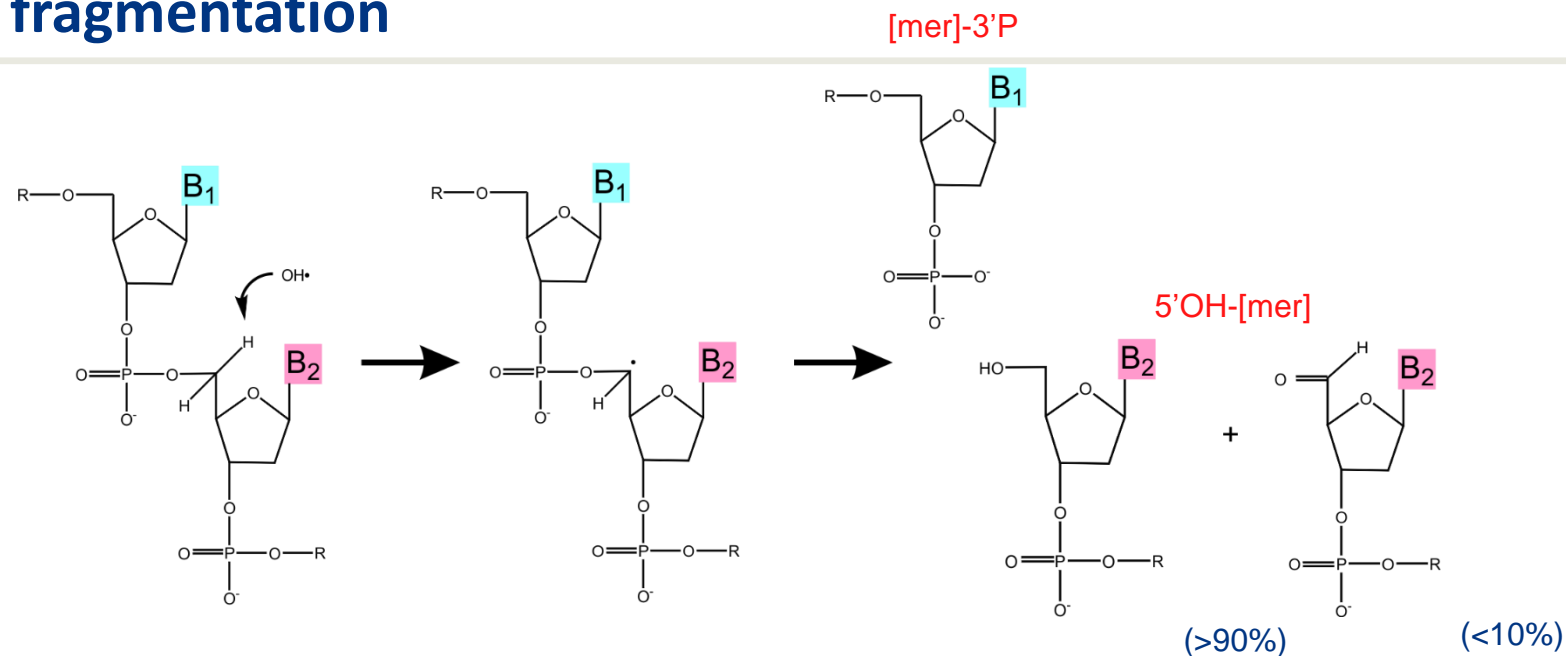


Protein: DNA Binding domain of FOXO4

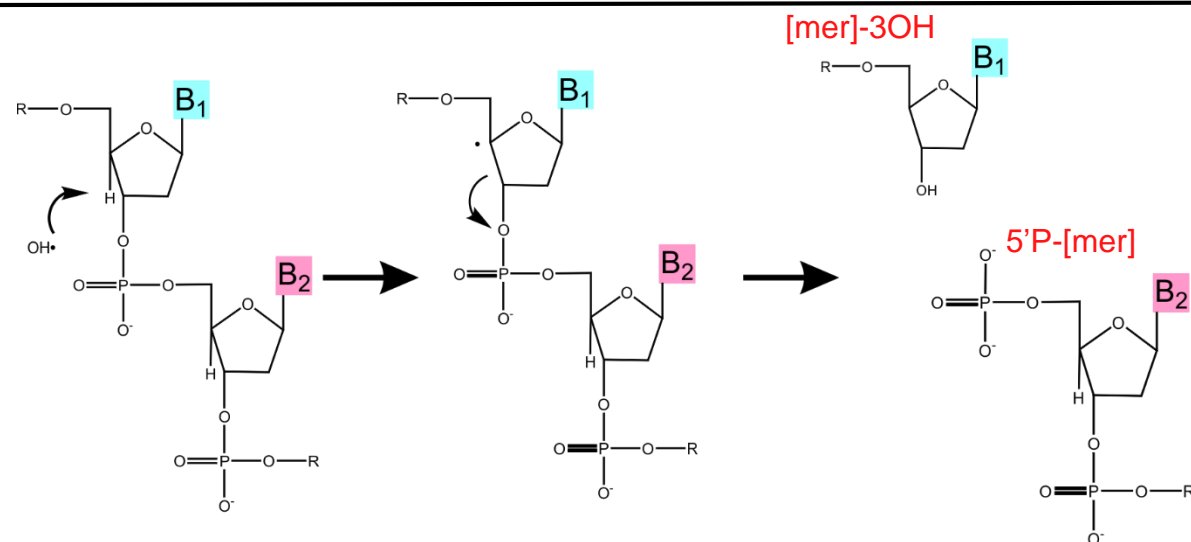
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Mechanism of DNA fragmentation

5'C H-subtraction:

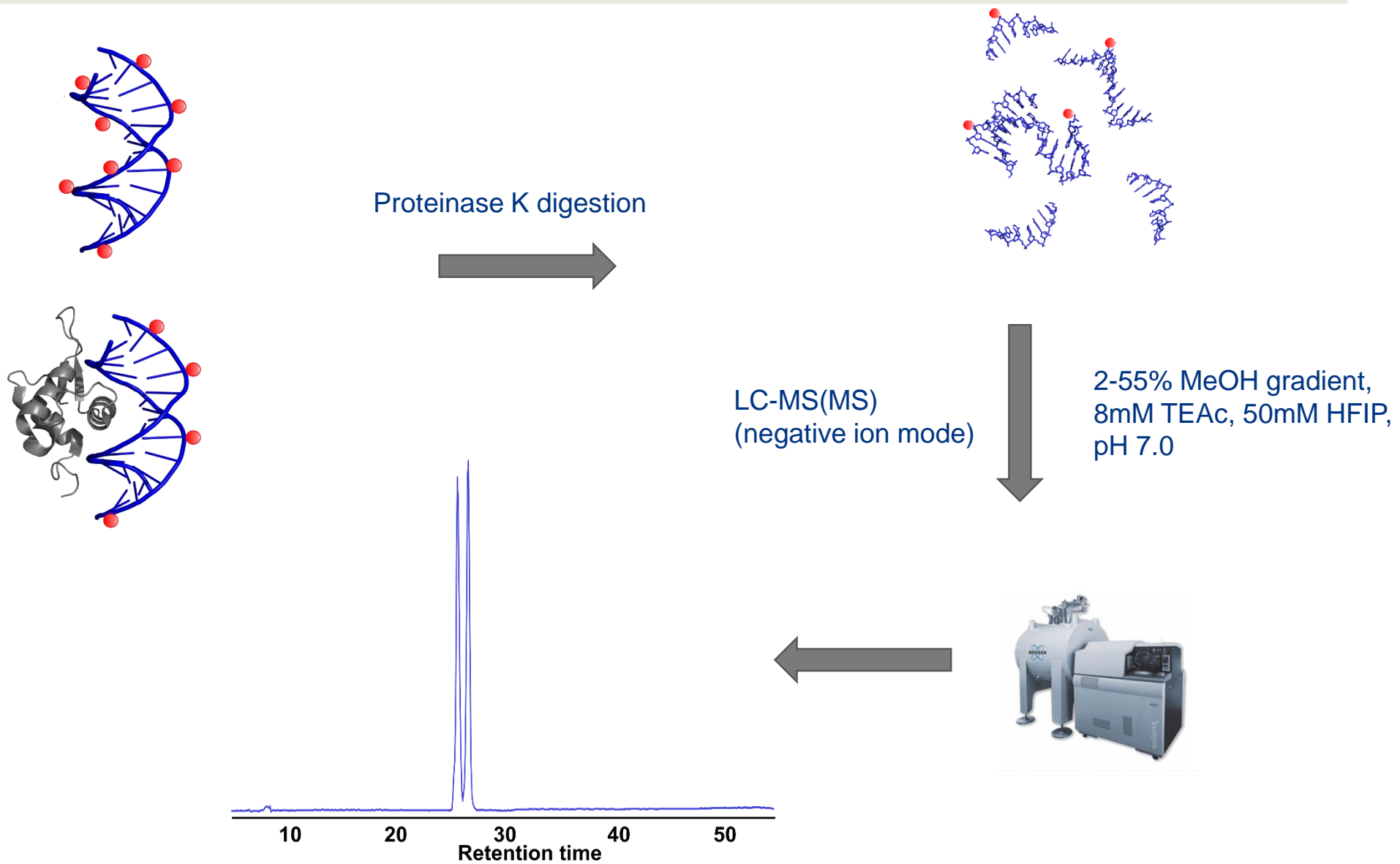


4'C H-substraction:

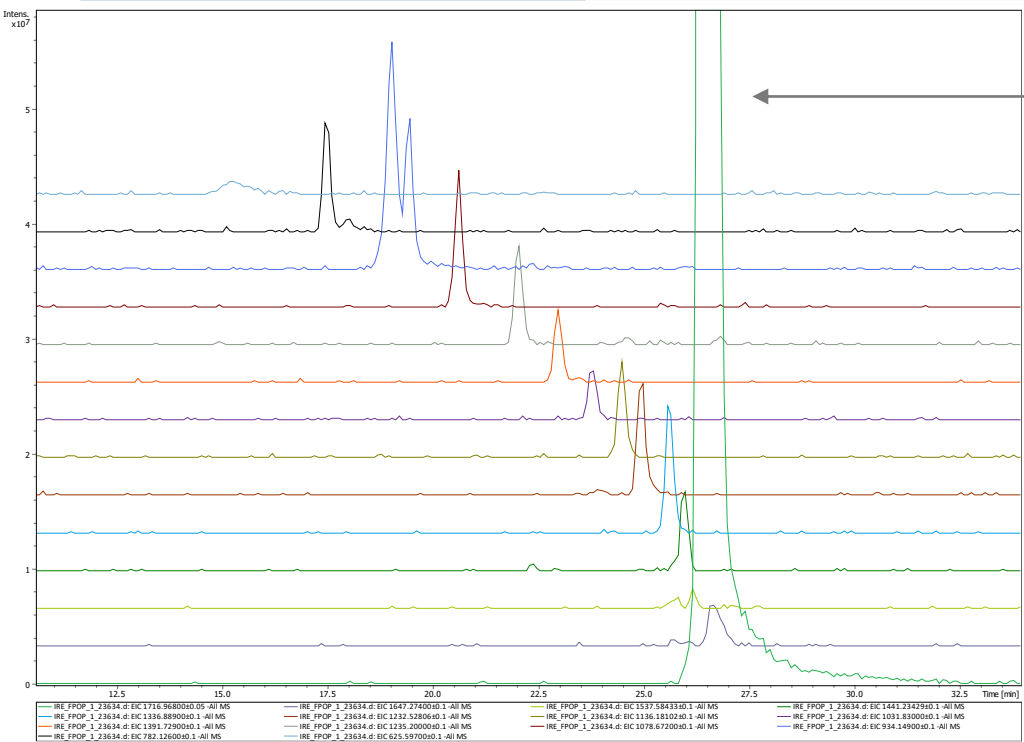


Balasubramanian, B., *PNAS*, **1998**; Jain, S., Tullius, T., *Nature Methods*, **2008**; Dizdaroglu, M., Jaruga, P., *Free Radical Research*, **2012**; Dumont, E., Monari, A., *Frontiers in Chemistry*, **2015**

FPOP of dsIRE

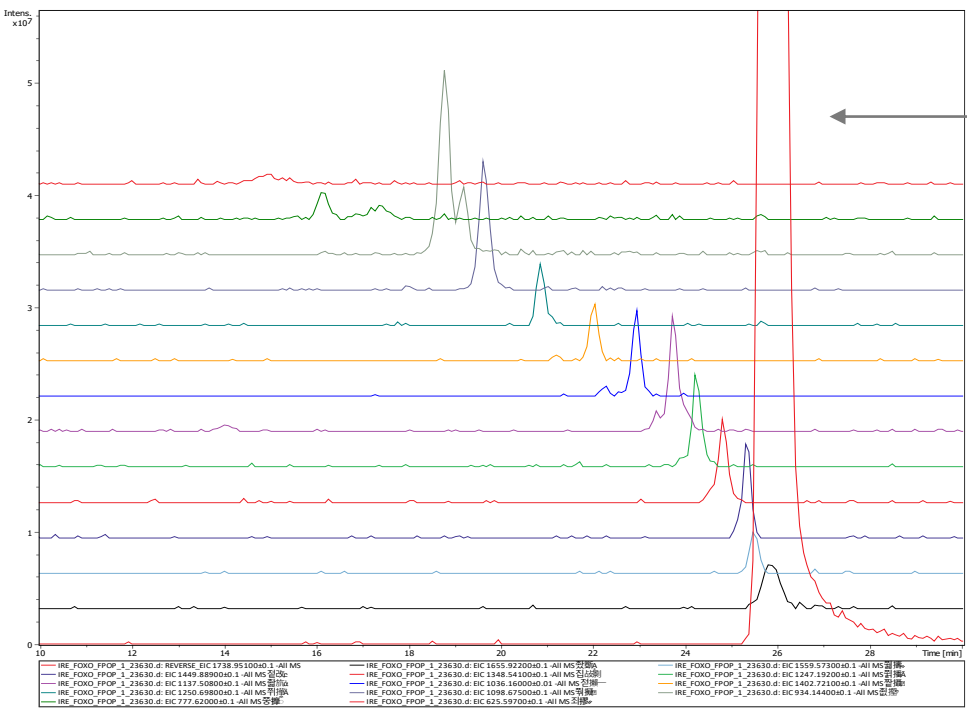
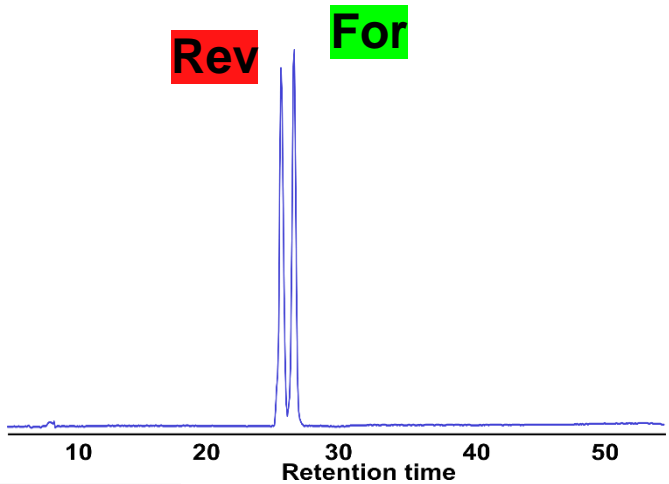


Extracted ion chromatograms of DNA fragments



Forward strand [17mer]

5'OH-[mers]-3'P ← X

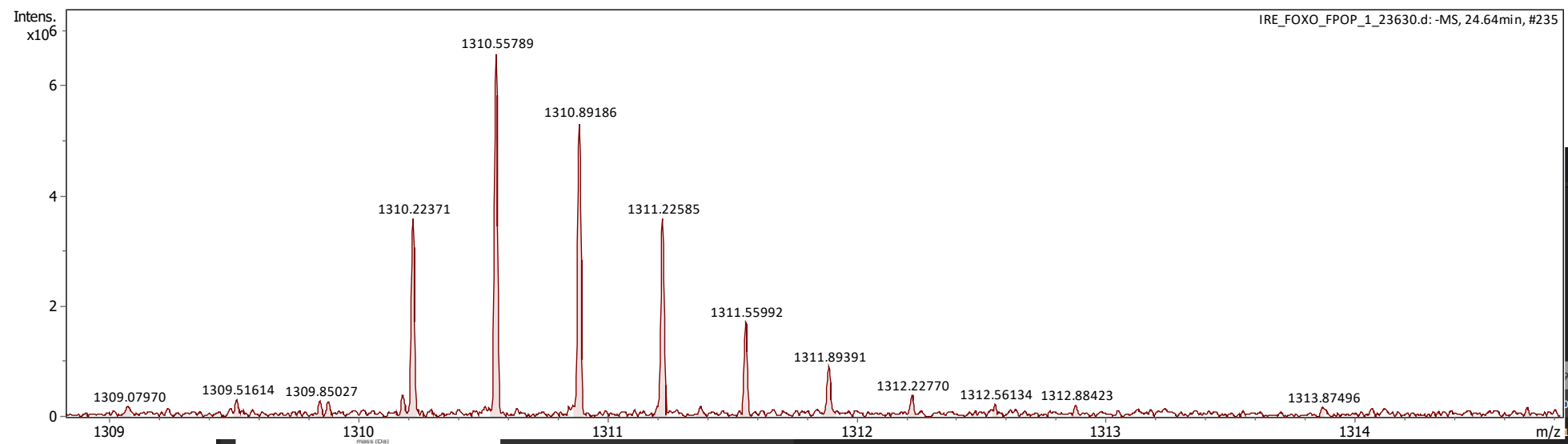


Reverse strand [17mer]

X → 5'P-[mers]-3'OH

Verifying DNA fragments – Isotopic envelope

5'OH-[13mer]-3'OH



Isotopic cluster modelling

Select the input:

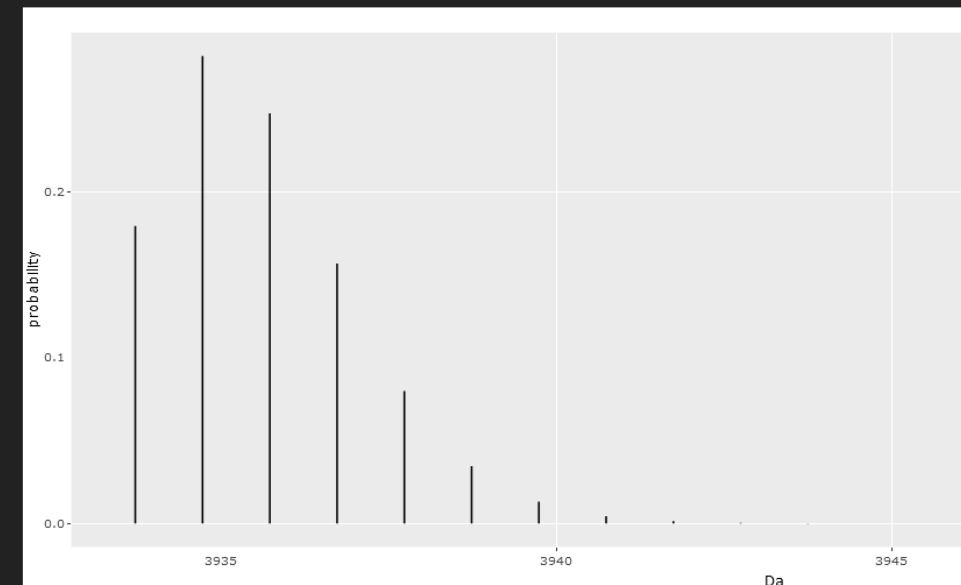
- ☒ Single mass
- ☐ Multiple masses

Select the molecule type:

- ☒ DNA
- ☐ RNA

Please enter the monoisotopic mass:

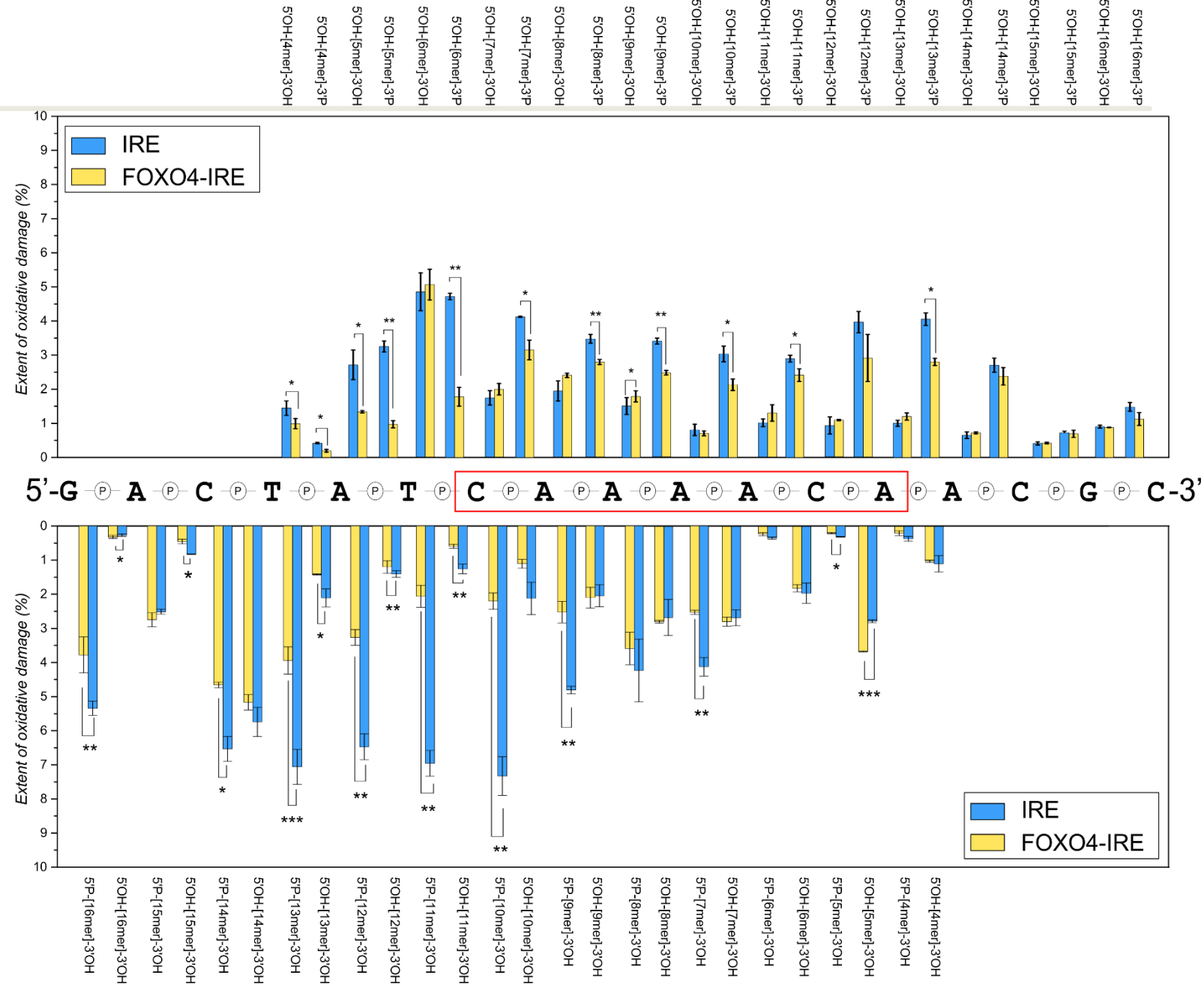
Calculate



Available on: <https://valkenborg-lab.shinyapps.io/pointless4dna/>

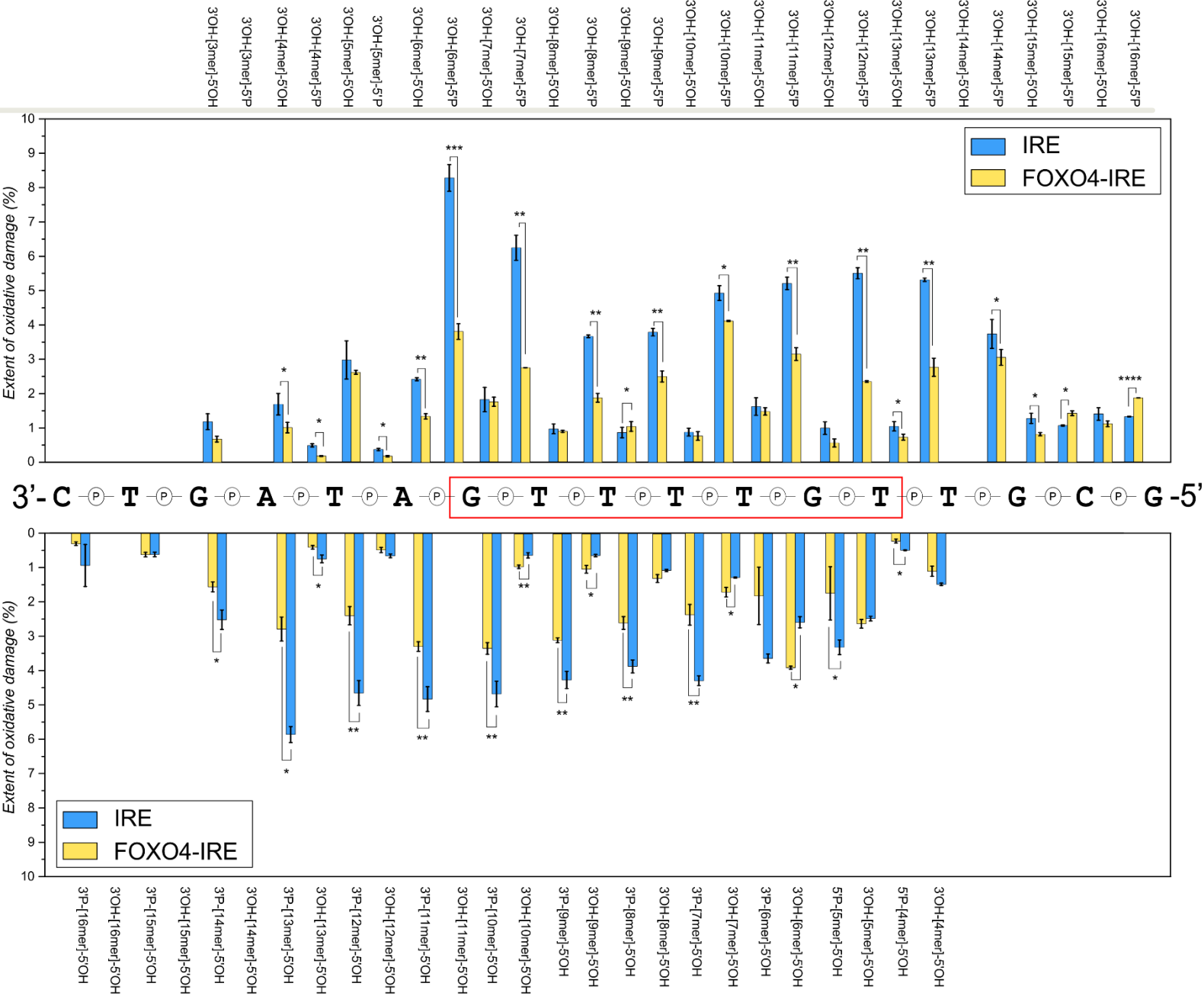
Extent of oxidative damage

DNA damage of Forward strand

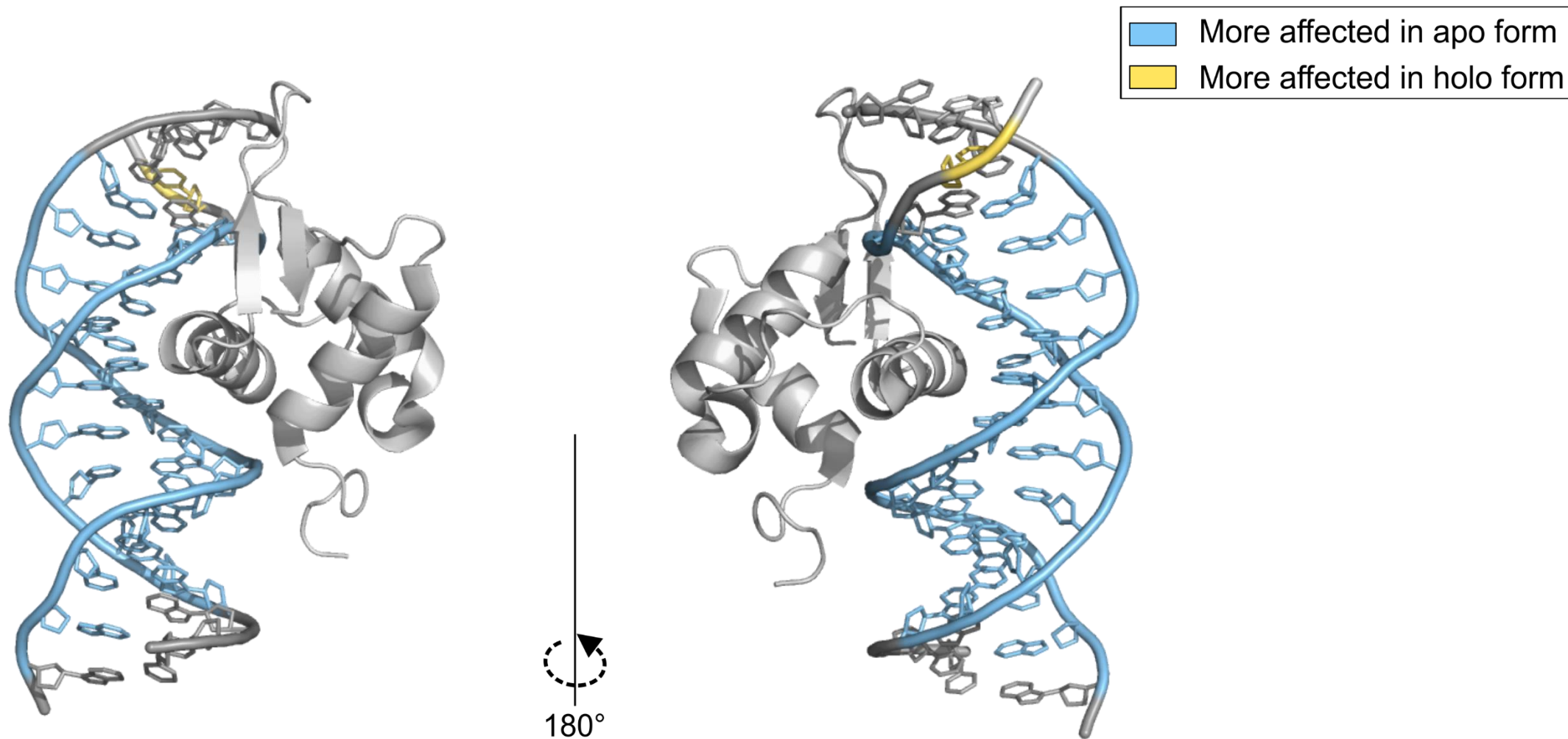


Extent of oxidative damage

DNA damage of Reverse strand



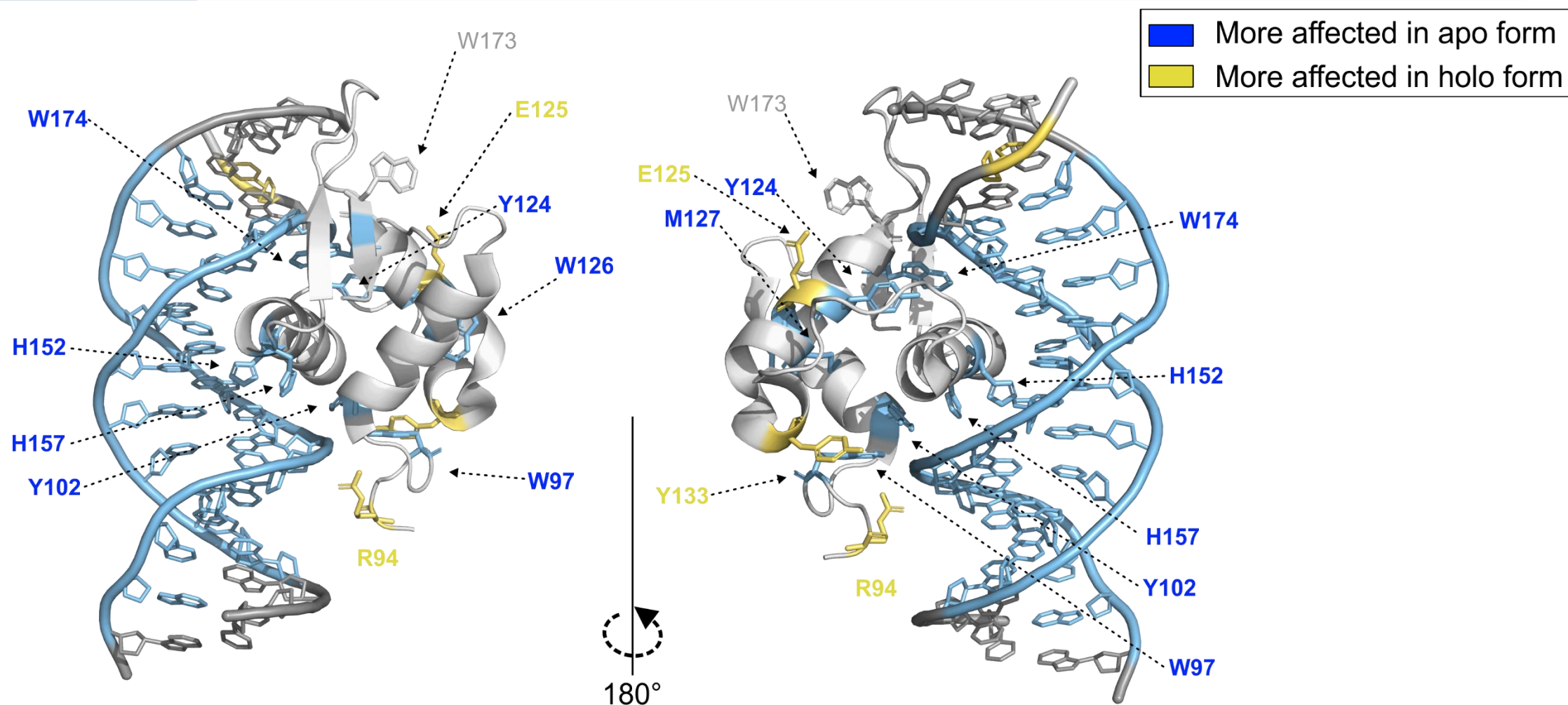
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Conclusion



Acknowledgements

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grant agreement ID: 731077),
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Charles University

