



# Structural characterization of protein-DNA complexes by hydroxyl radicals



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# Labelling techniques to study higher order structure

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## 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

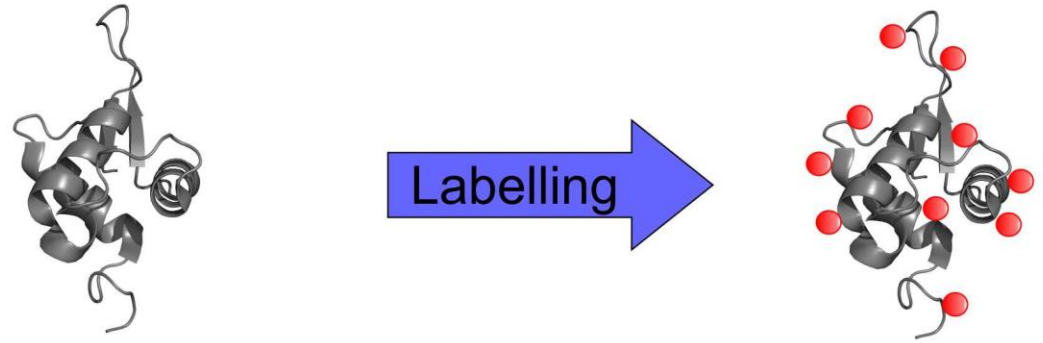
- Mapping dynamics and solvent accessibility

**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

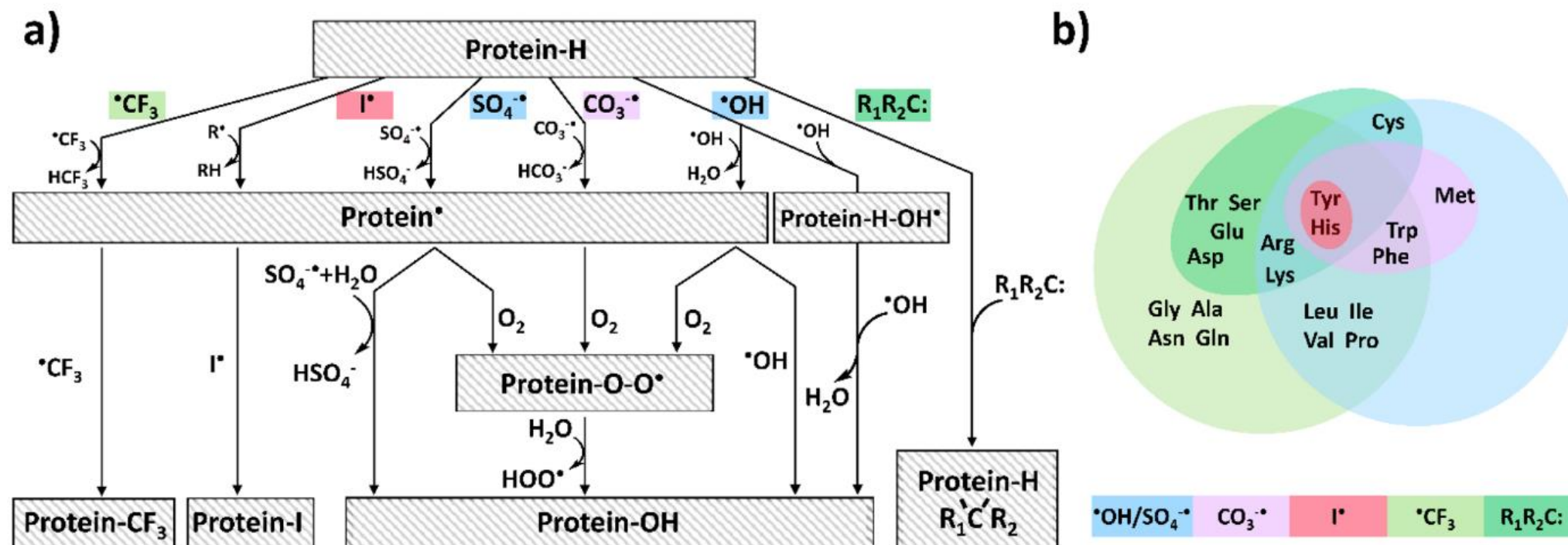


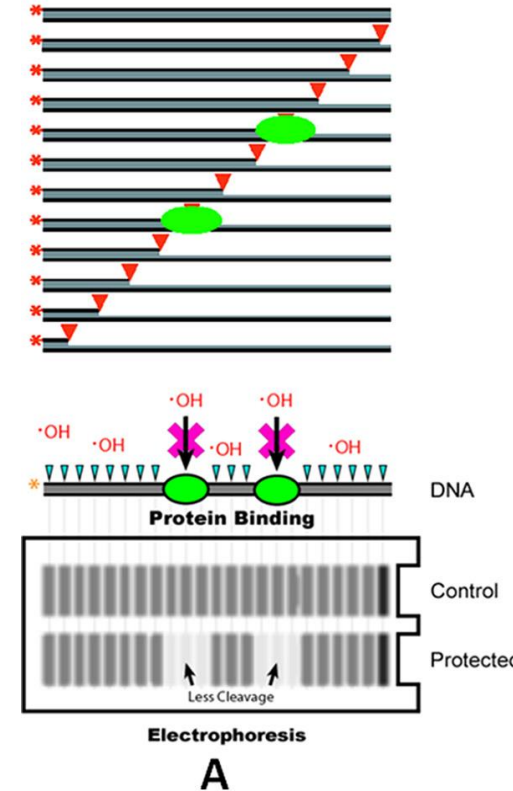
Figure: Summary of radical-based footprinting reagents of (a) proposed pathways and (b) residue specificity.

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

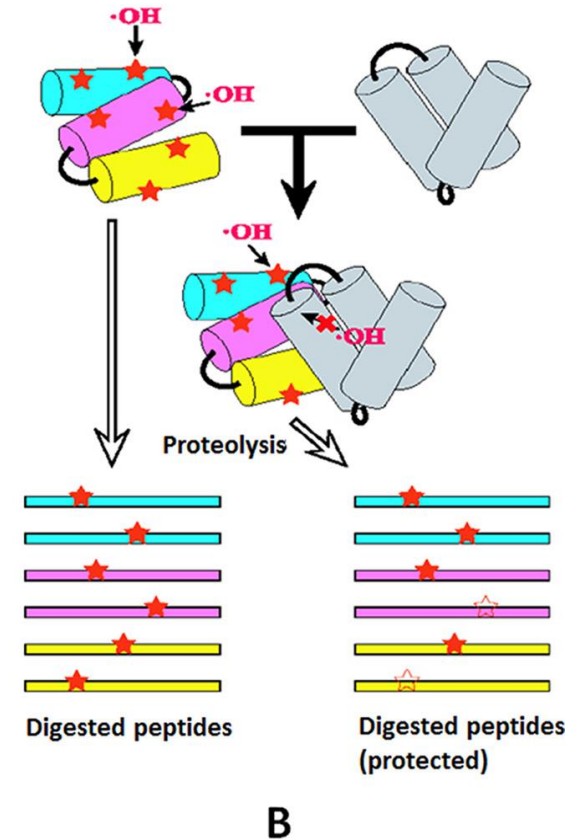
# History of footprinting techniques

1. **Limited proteolysis** – proteolysis of exposed protein residues
2. **DNAse footprinting**  
1<sup>st</sup> 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 H<sub>2</sub>O by synchrotron or electron beam**  
Expensive and normally not available
6. **Fast Photochemical oxidation of proteins**

DNA- protein interaction



Protein- protein interaction

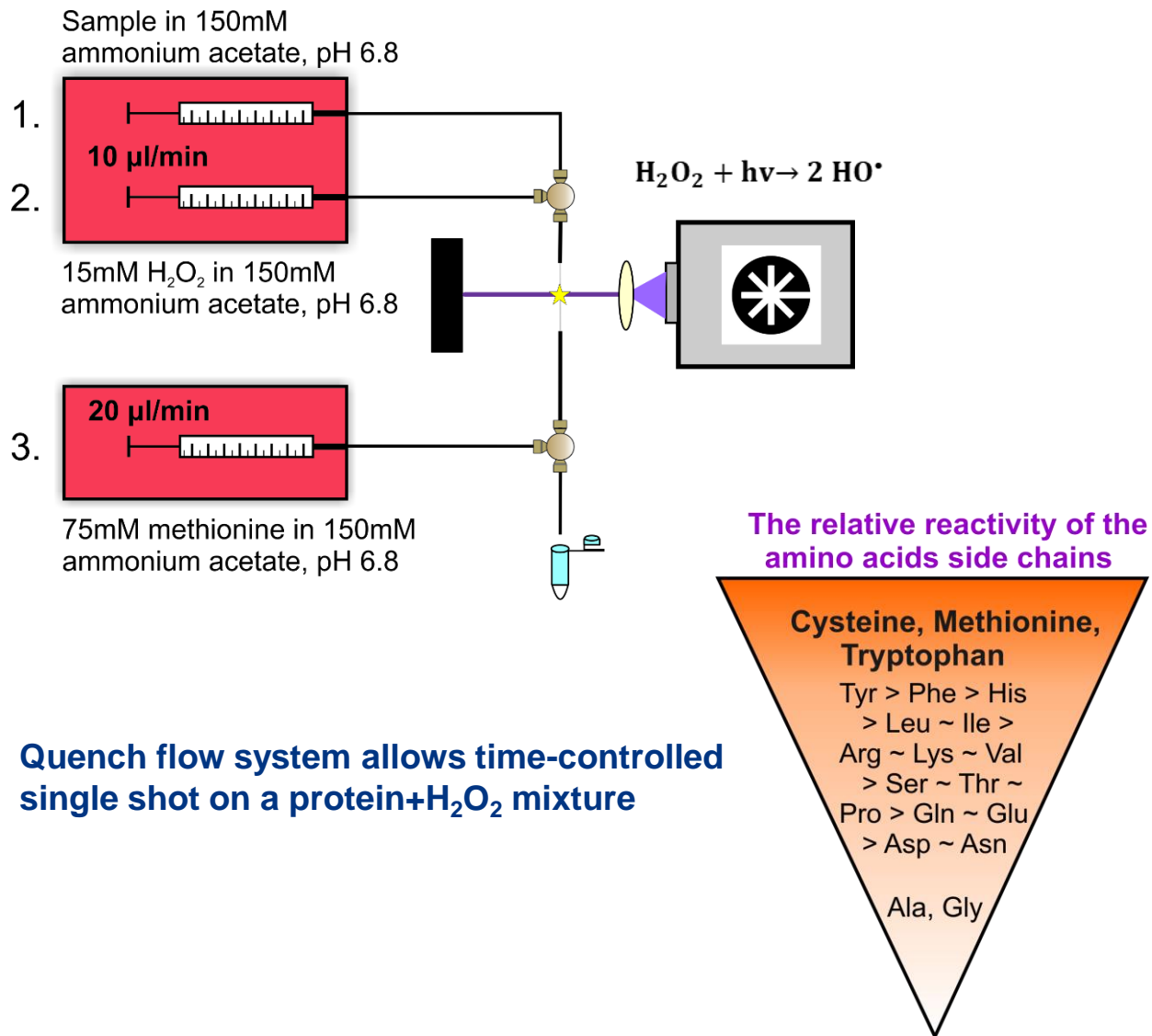


# History of footprinting techniques

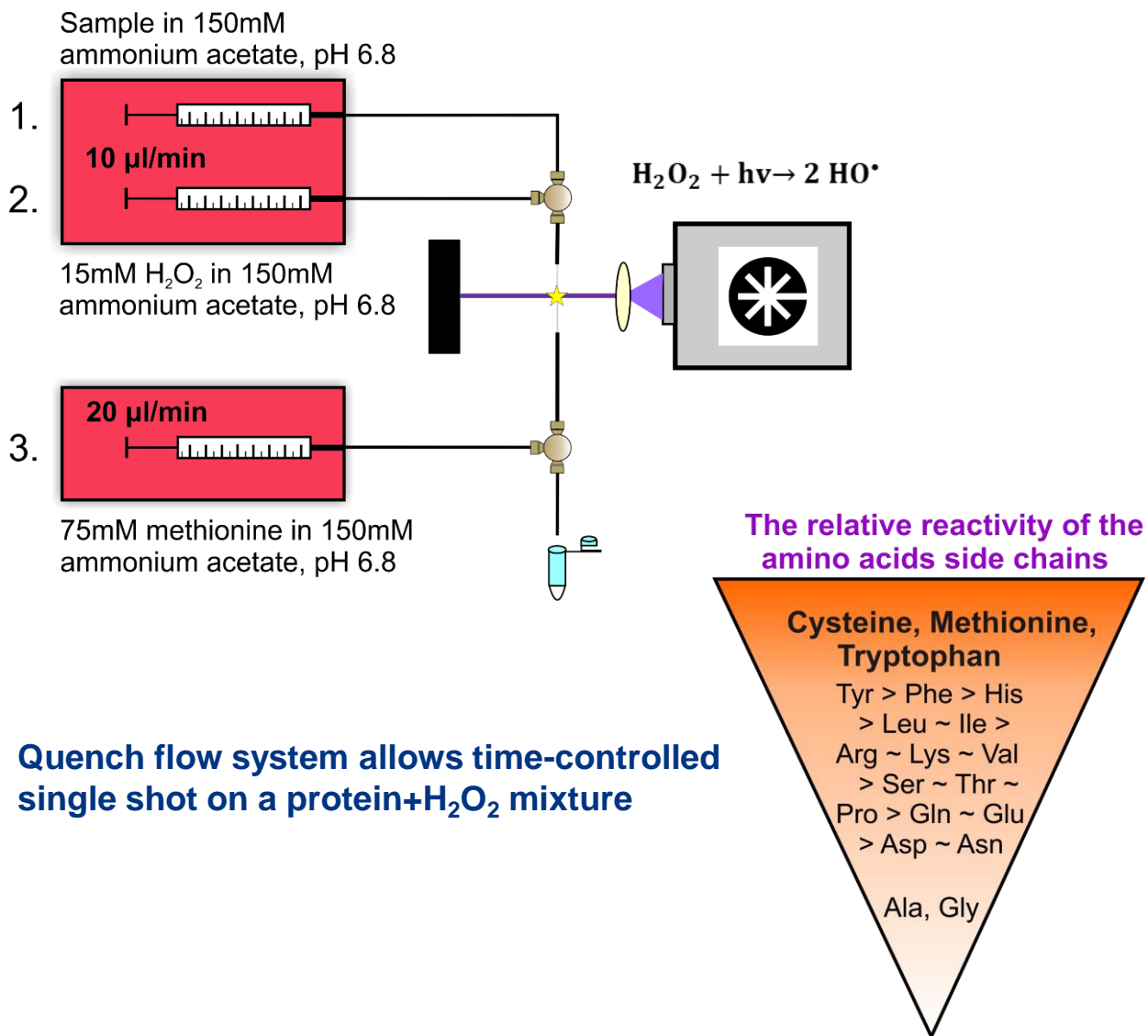
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Expensive and normally not available
6. **Fast Photochemical oxidation of proteins (FPOP)**

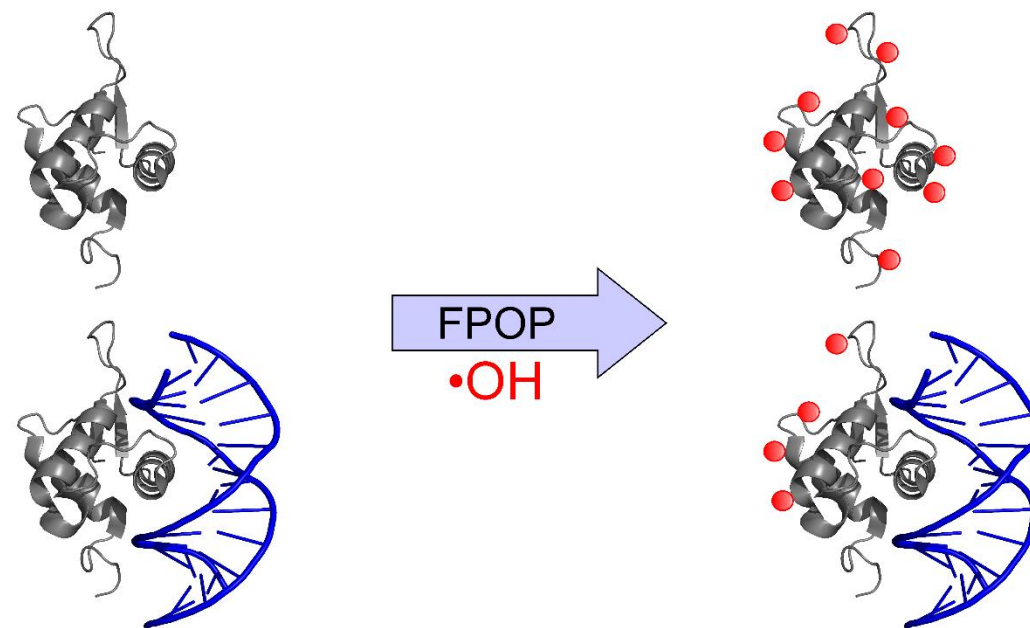
# Fast Photochemical Oxidation of Proteins (FPOP)



# Fast Photochemical Oxidation of Protein-DNA complex



Aim of study: FPOP of protein-DNA complex:

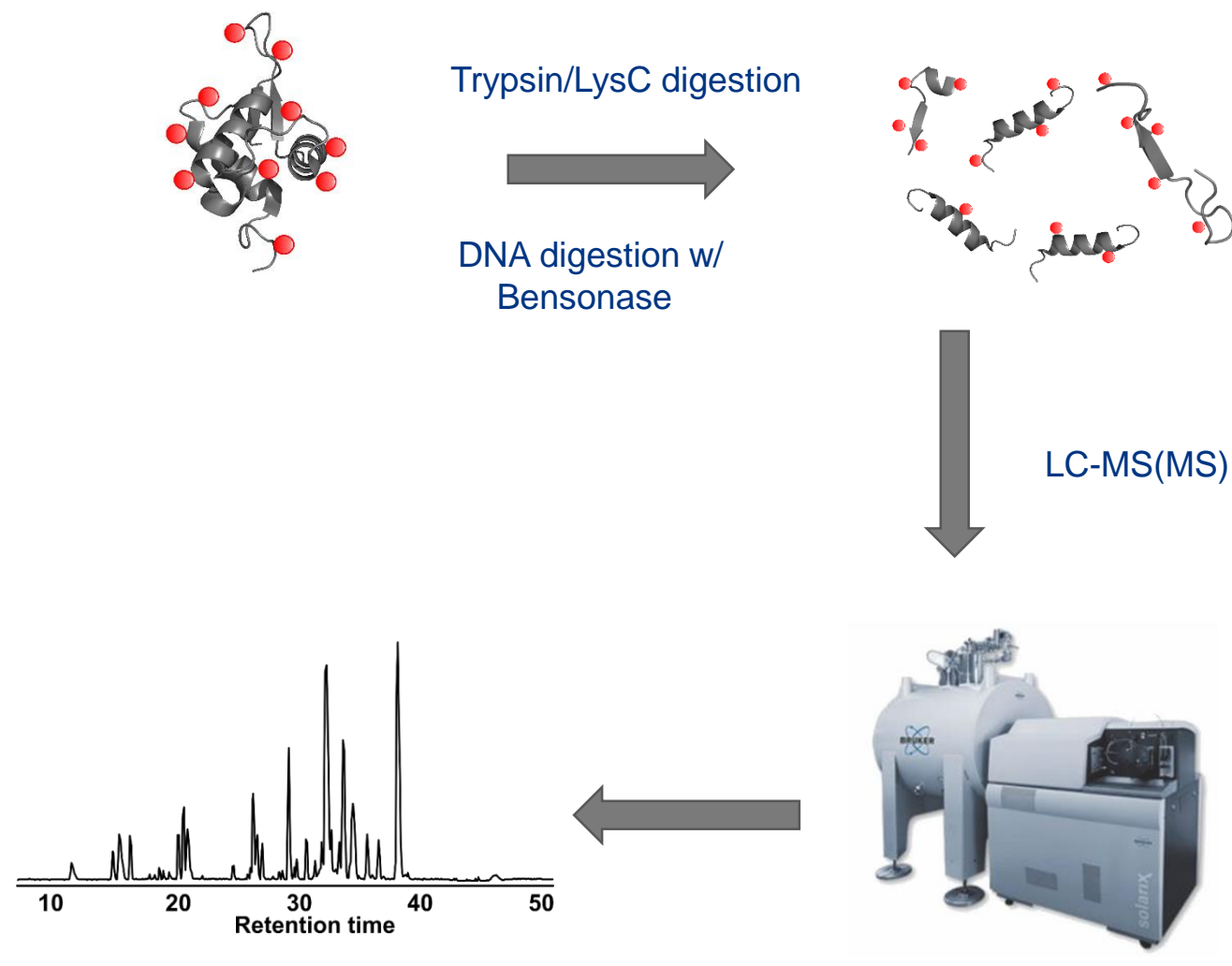
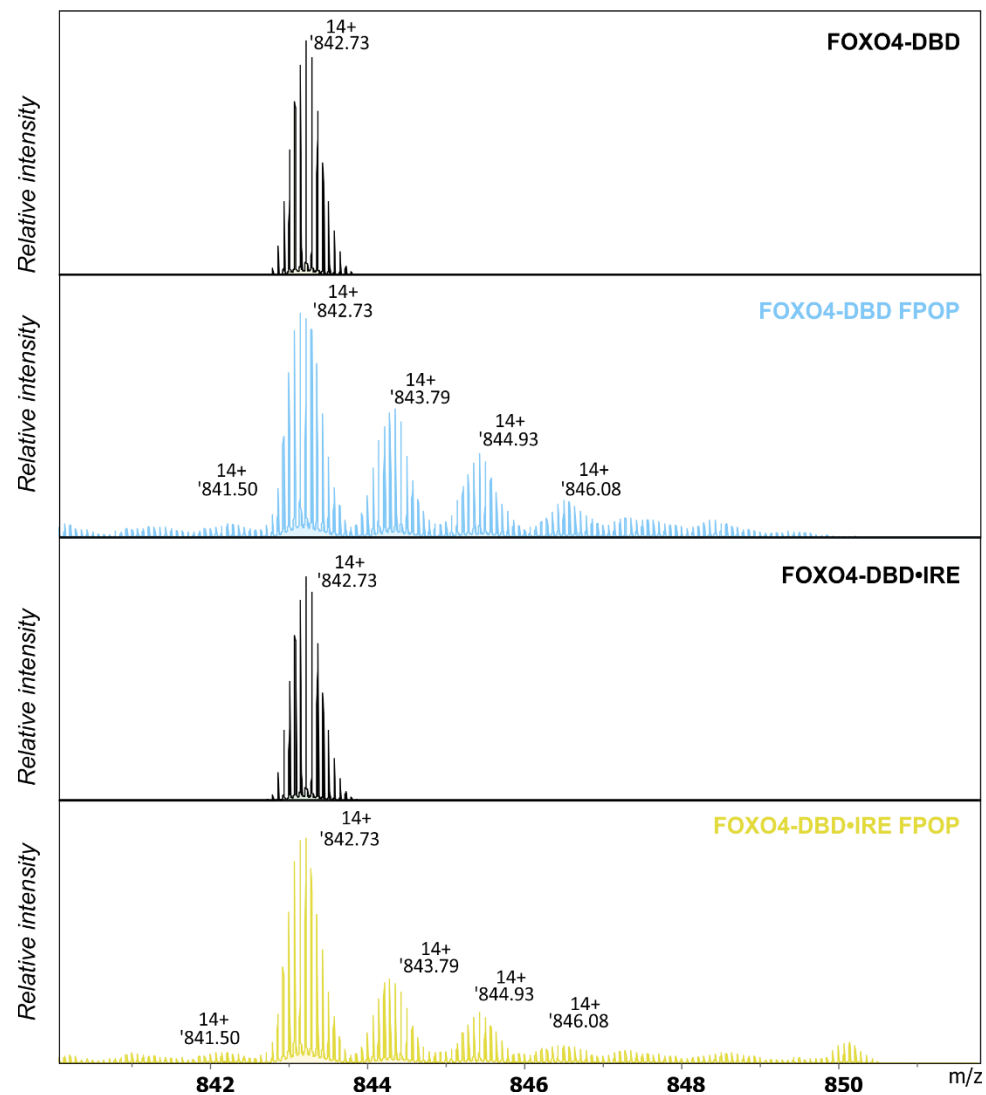


Protein: DNA Binding domain of FOXO4

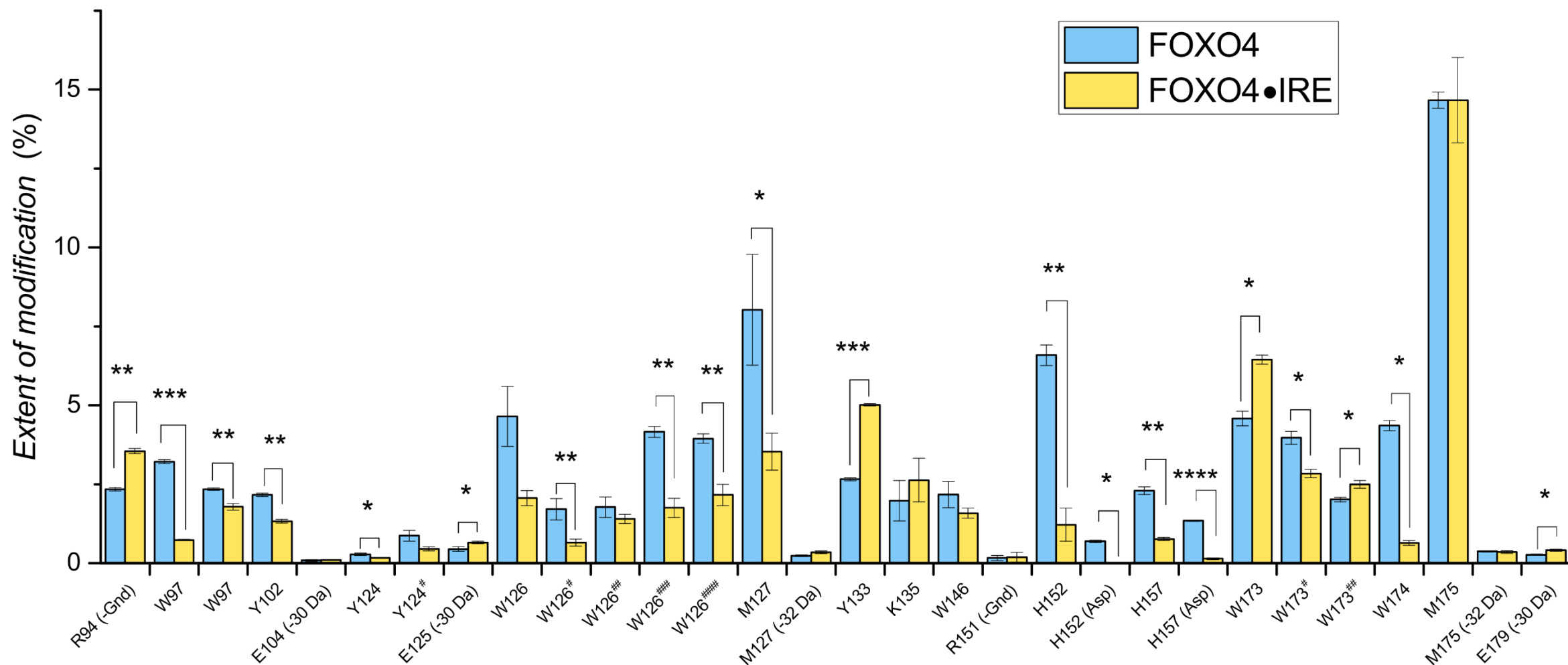
dsDNA: Insulin Response Element (IRE):  
5'-GAC TAT CAA AAC AAC GC-3'



# FPOP of FOXO4-IRE complex

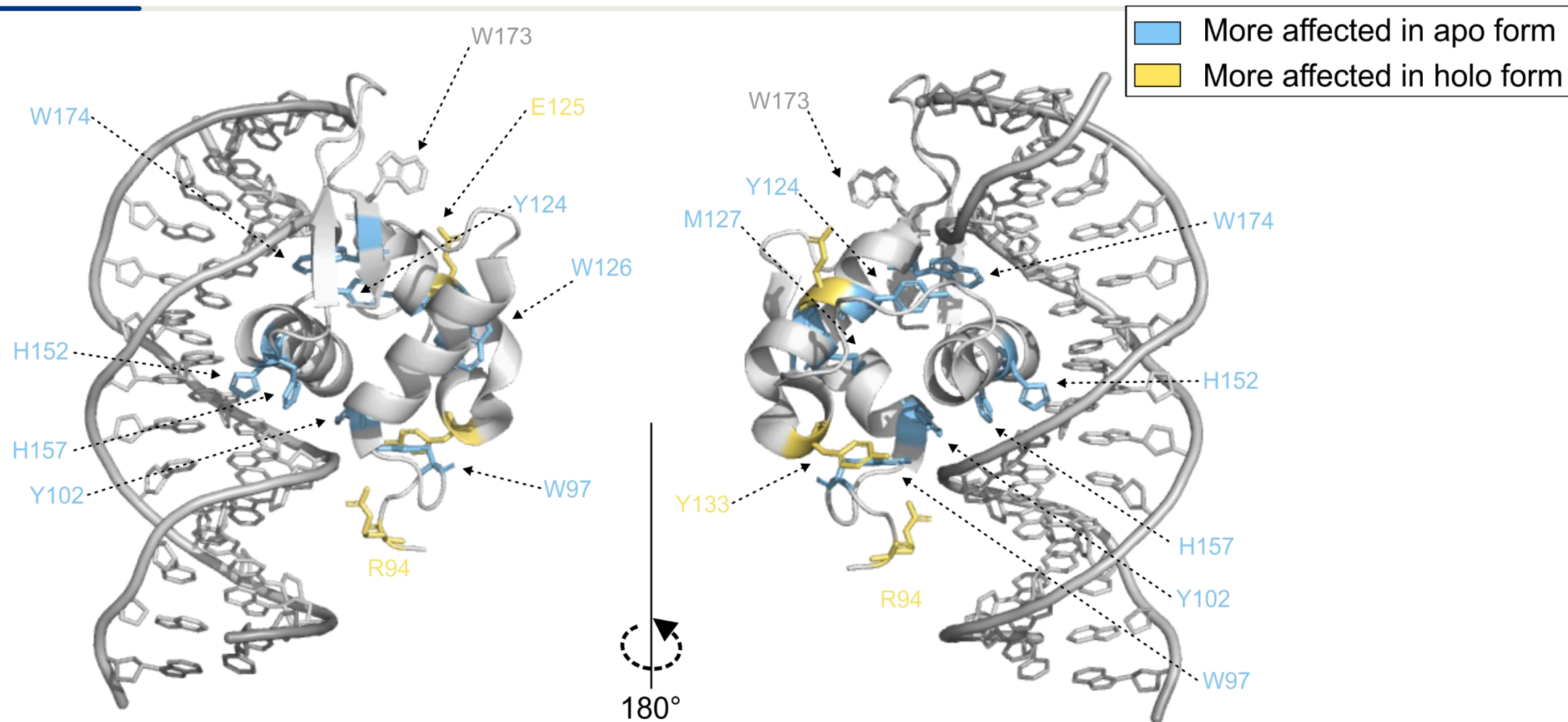


## Extent of modification of modified residues



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

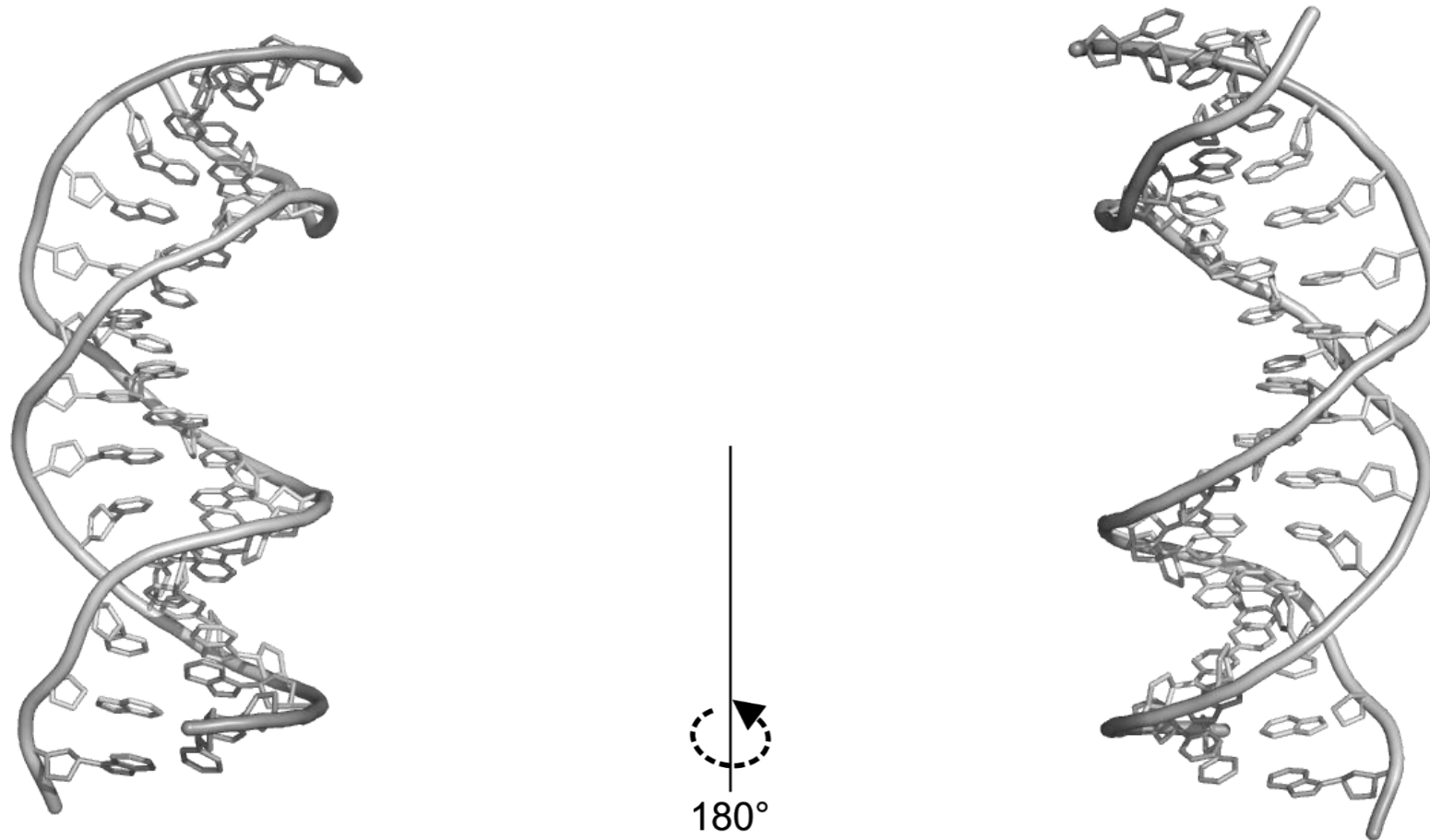
# Plotting residues onto a crystal structure



Model of FOXO4-IRE built based on an available structural model in Pymol (entry: 3I2c)

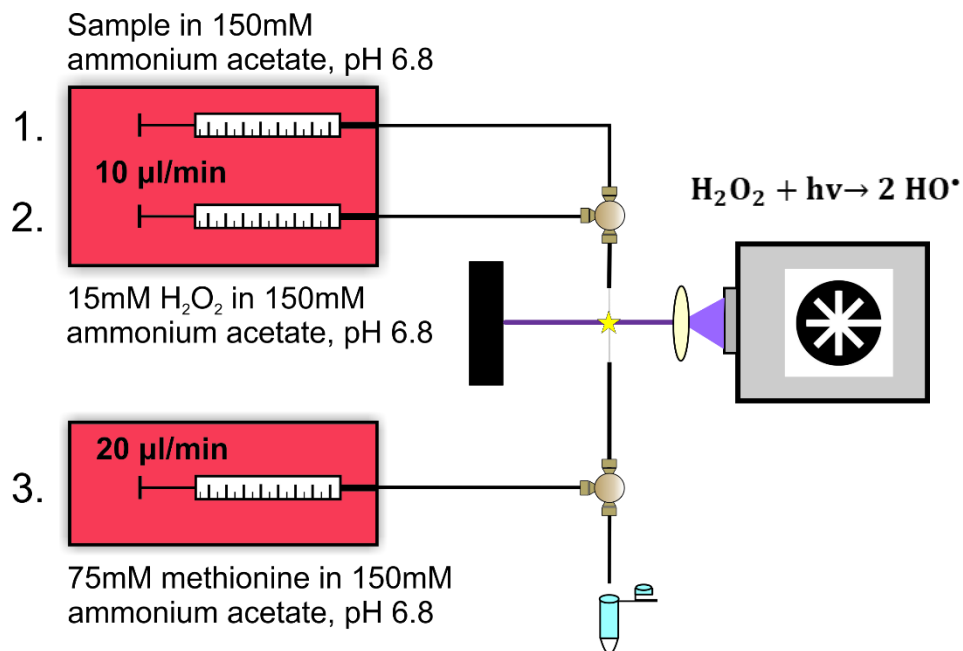
Boura E., *Acta Crystallogr D Biol Crystallogr*, 2010

## What information can be obtained from the DNA site?

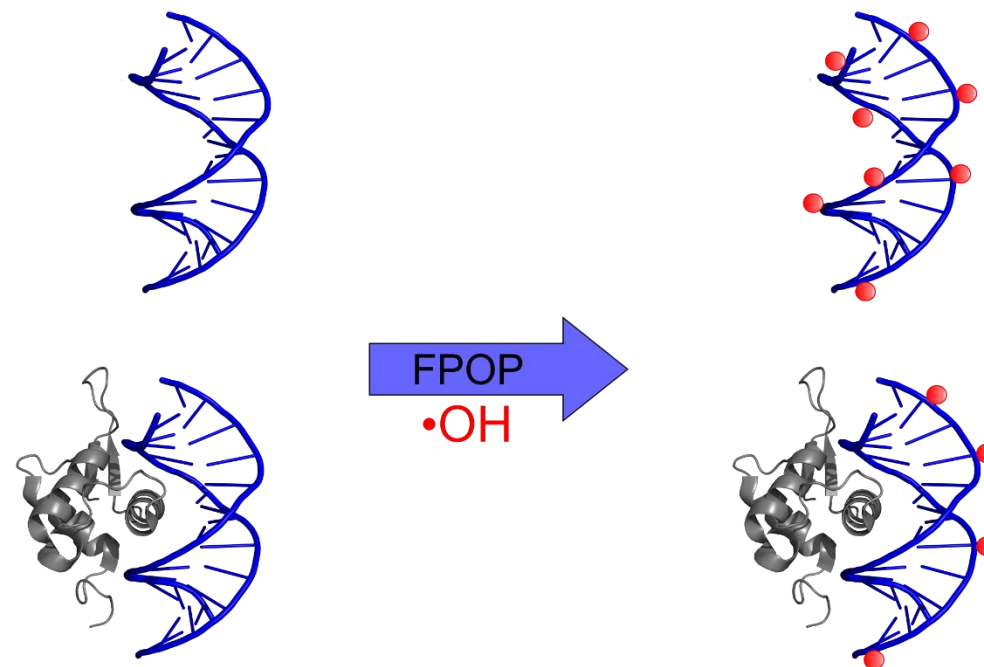


Boura E., *Acta Crystallogr D Biol Crystallogr*, 2010

# Fast Photochemical Oxidation of Protein-DNA complex



## Aim of study: Oxidation of DNA

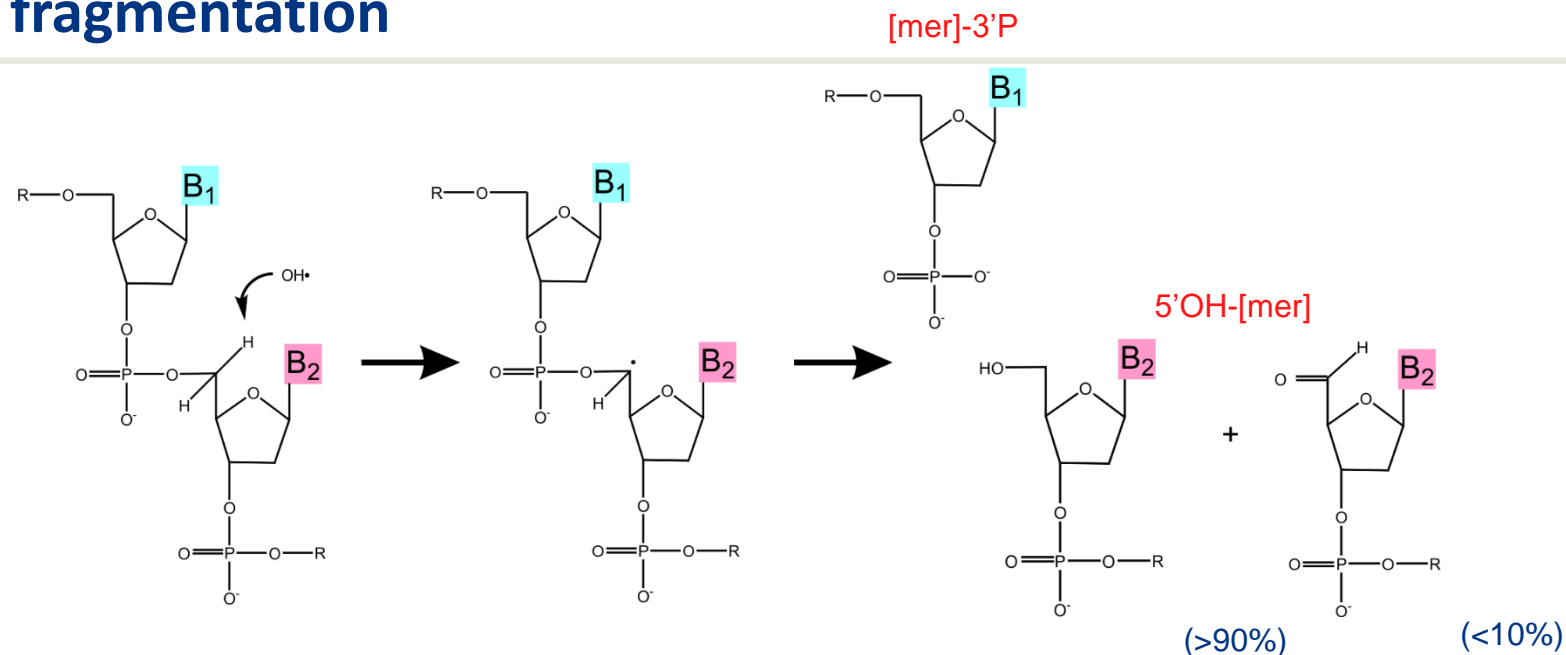


Protein: DNA Binding domain of FOXO4

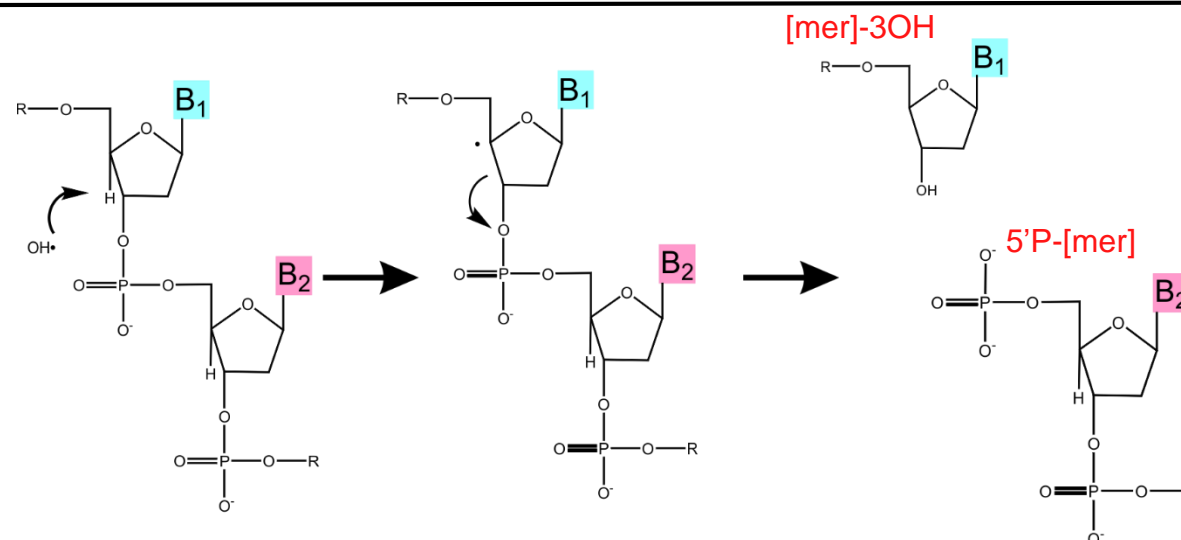
dsDNA: Insulin Response Element (IRE):  
5'-GAC TAT CAA AAC AAC GC-3'

# Mechanism of DNA fragmentation

5'C H-subtraction:

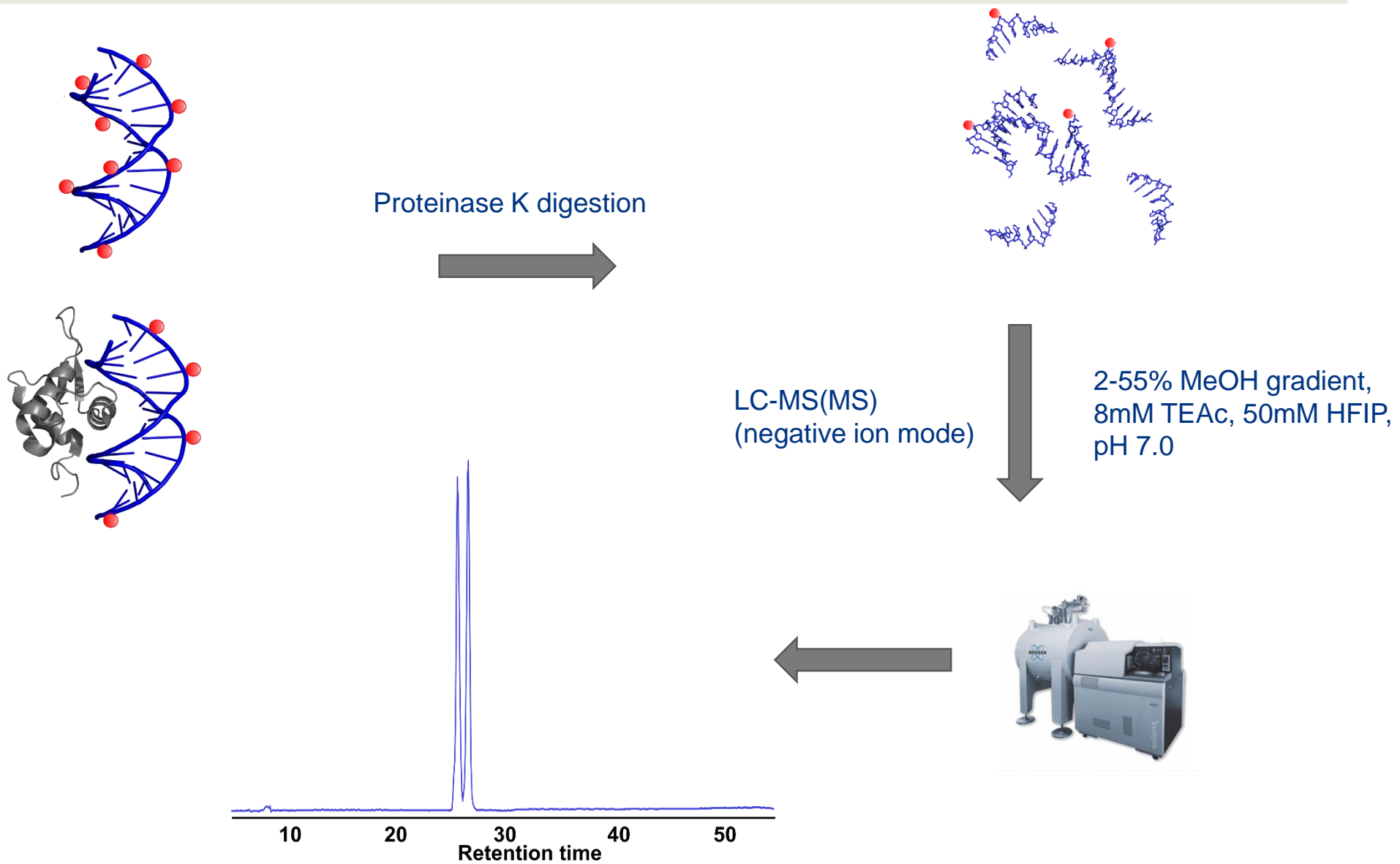


4'C H-substraction:

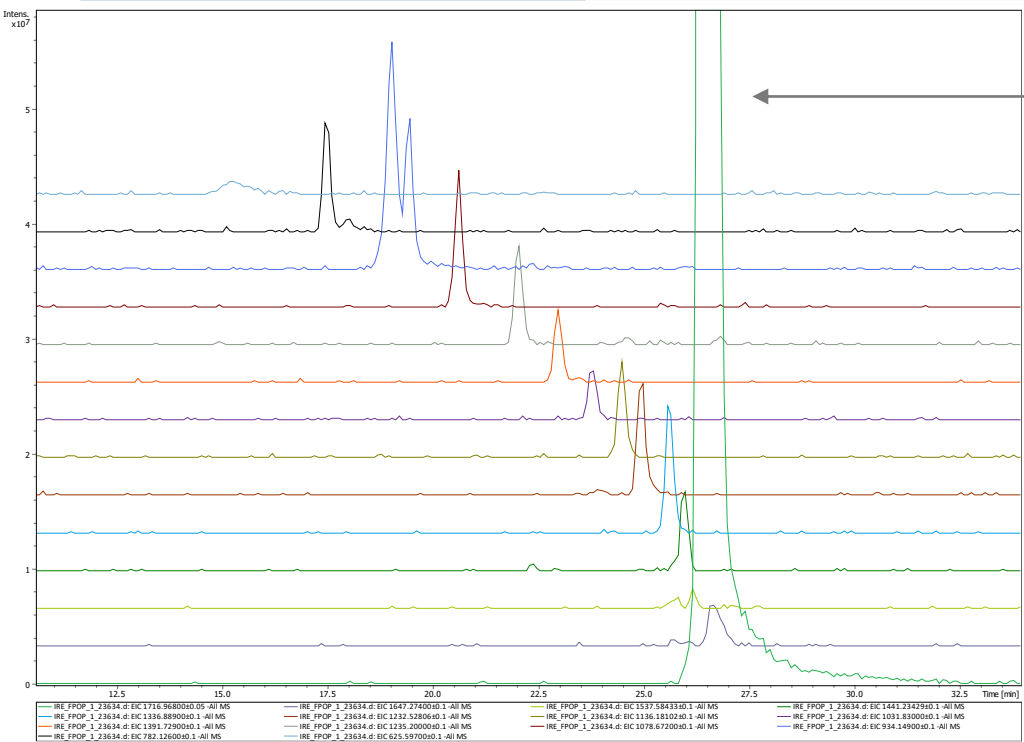


Balasubramanian, B., *PNAS*, **1998**; Jain, S., Tullius, T., *Nature Methods*, **2008**; Dizdaroglu, M., Jaruga, P., *Free Radical Research*, **2012**; Dumont, E., Monari, A., *Frontiers in Chemistry*, **2015**

# FPOP of dsIRE

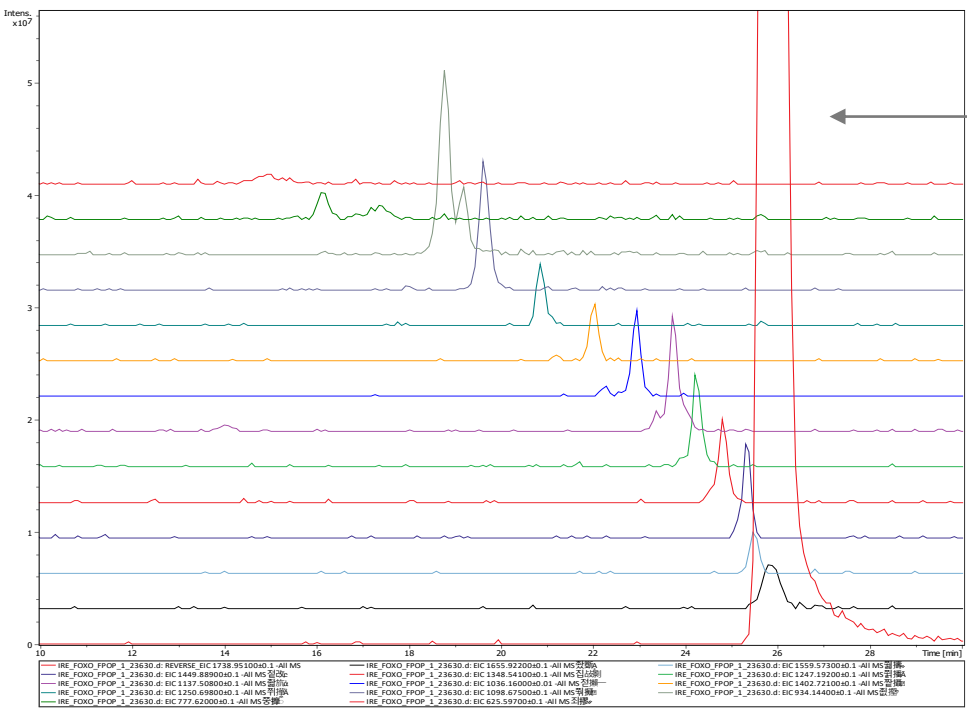
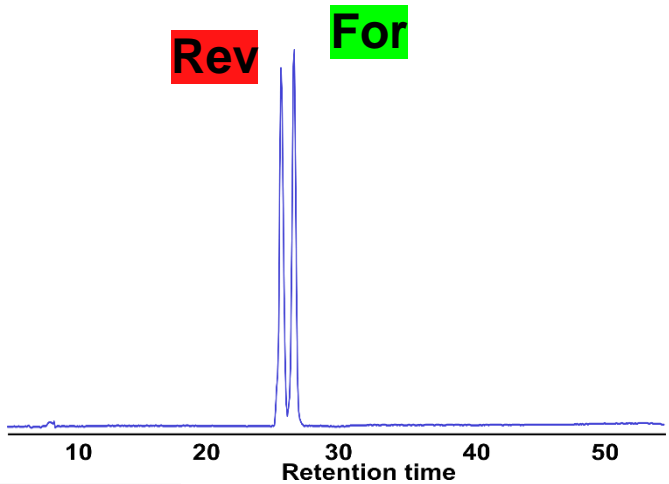


# Extracted ion chromatograms of DNA fragments



Forward strand [17mer]

5'OH-[mers]-3'P  $\leftarrow$  X



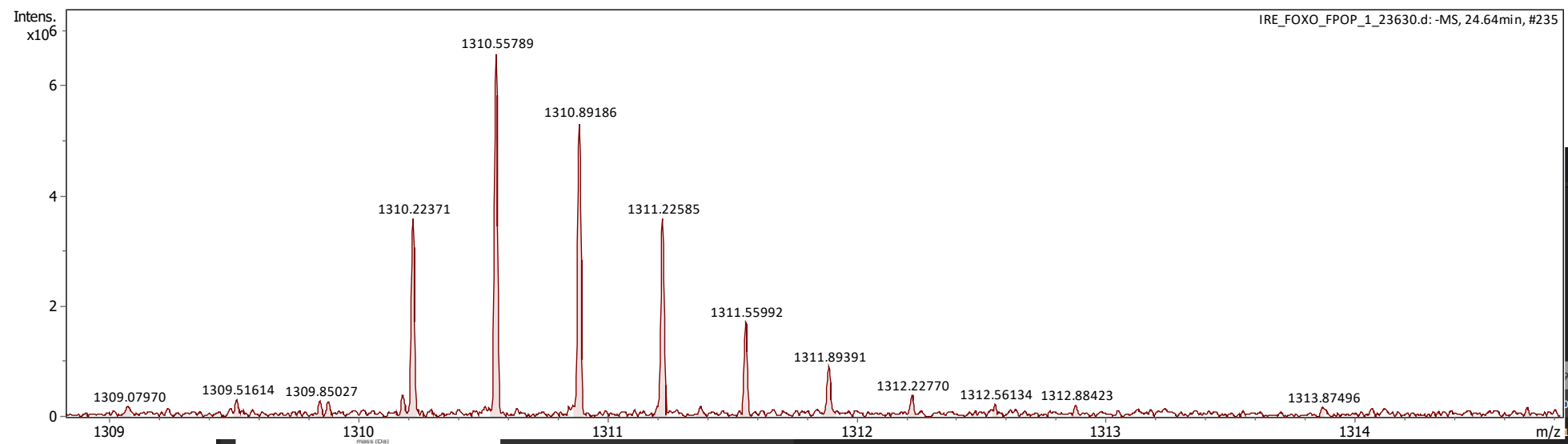
Reverse strand [17mer]

X  $\rightarrow$  5'P-[mers]-3'OH



# Verifying DNA fragments – Isotopic envelope

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



Isotopic cluster modelling

Select the input:

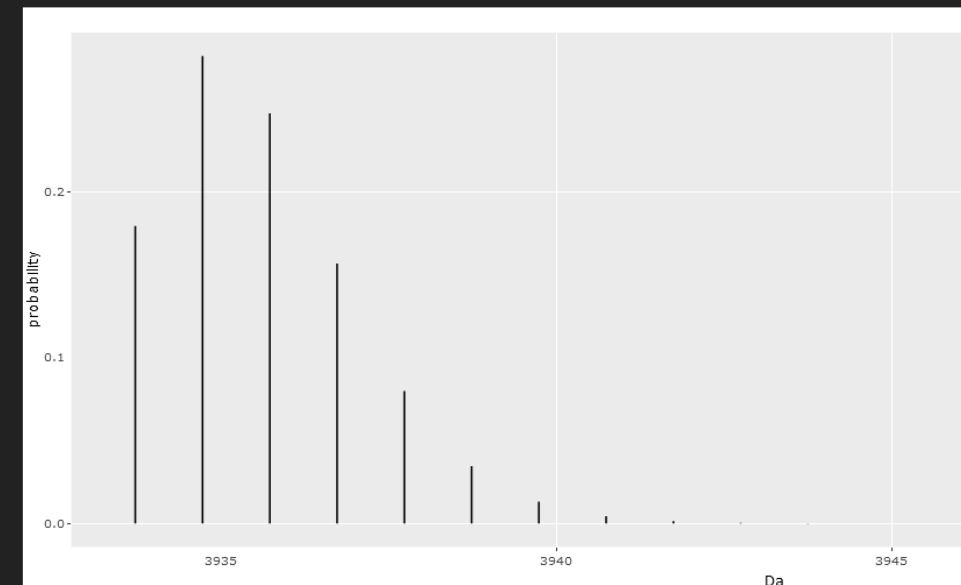
- ☒ Single mass
- ☐ Multiple masses

Select the molecule type:

- ☒ DNA
- ☐ RNA

Please enter the monoisotopic mass:

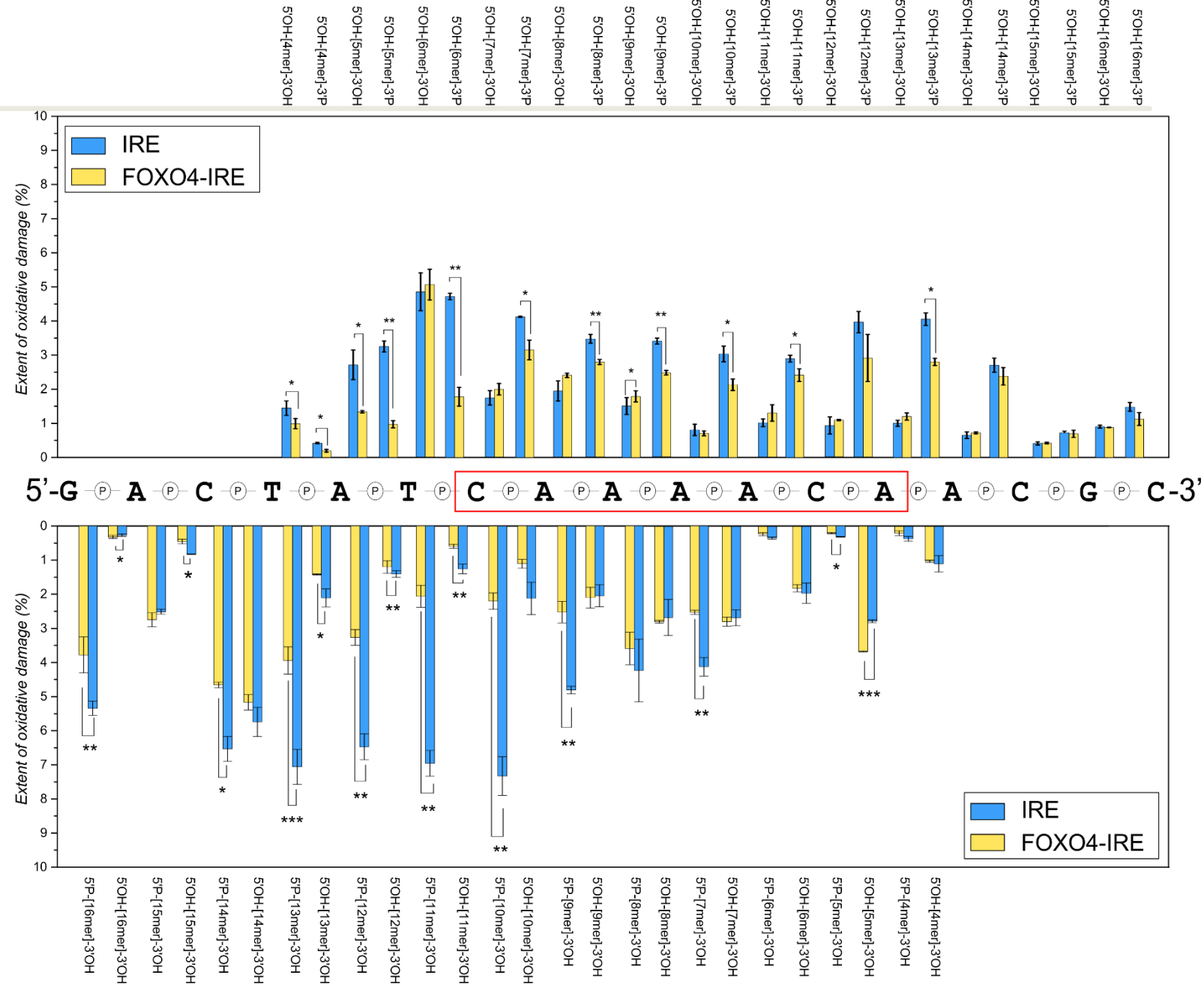
Calculate



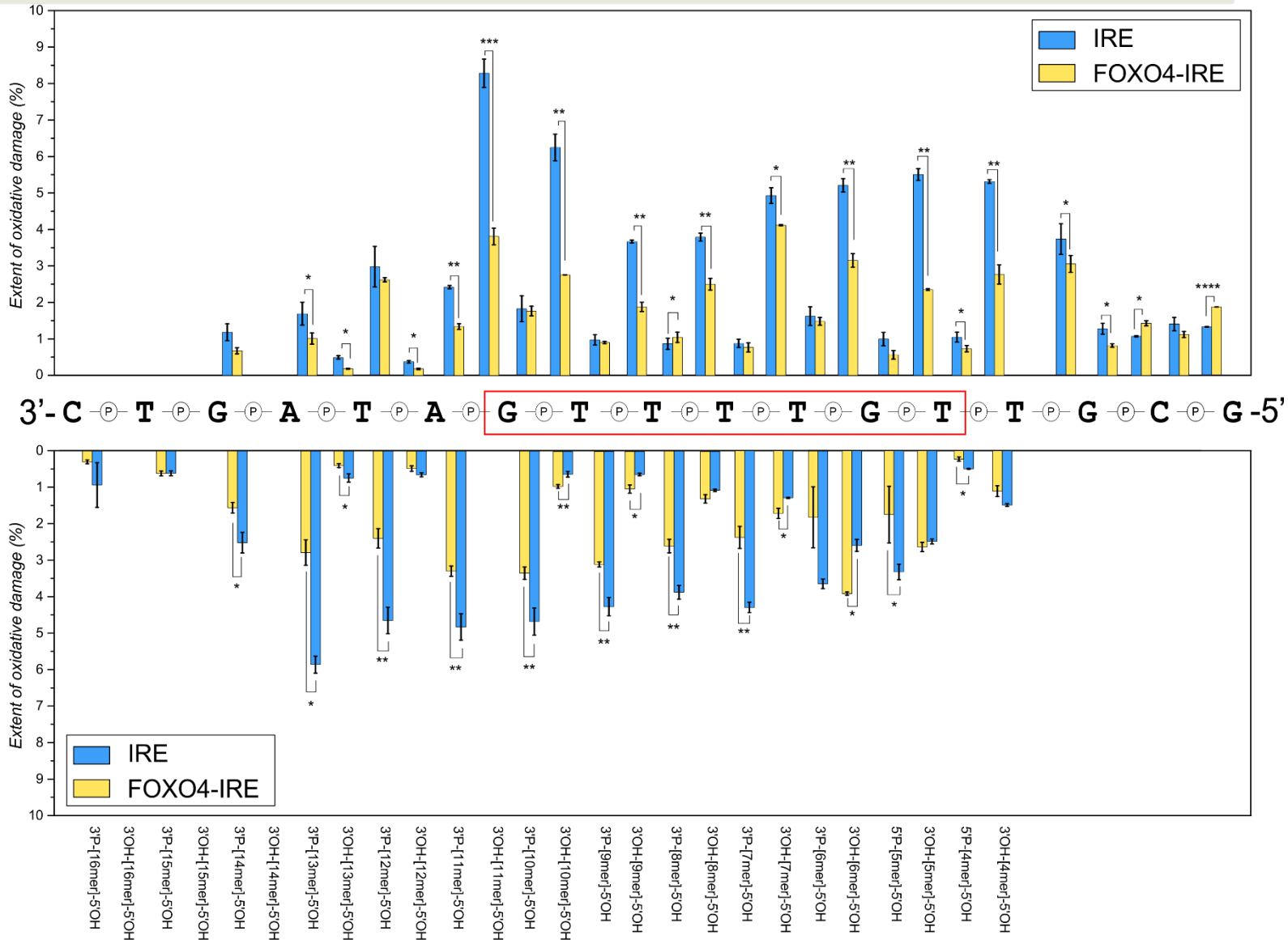
Available on: <https://valkenborg-lab.shinyapps.io/pointless4dna/>

# Extent of oxidative damage

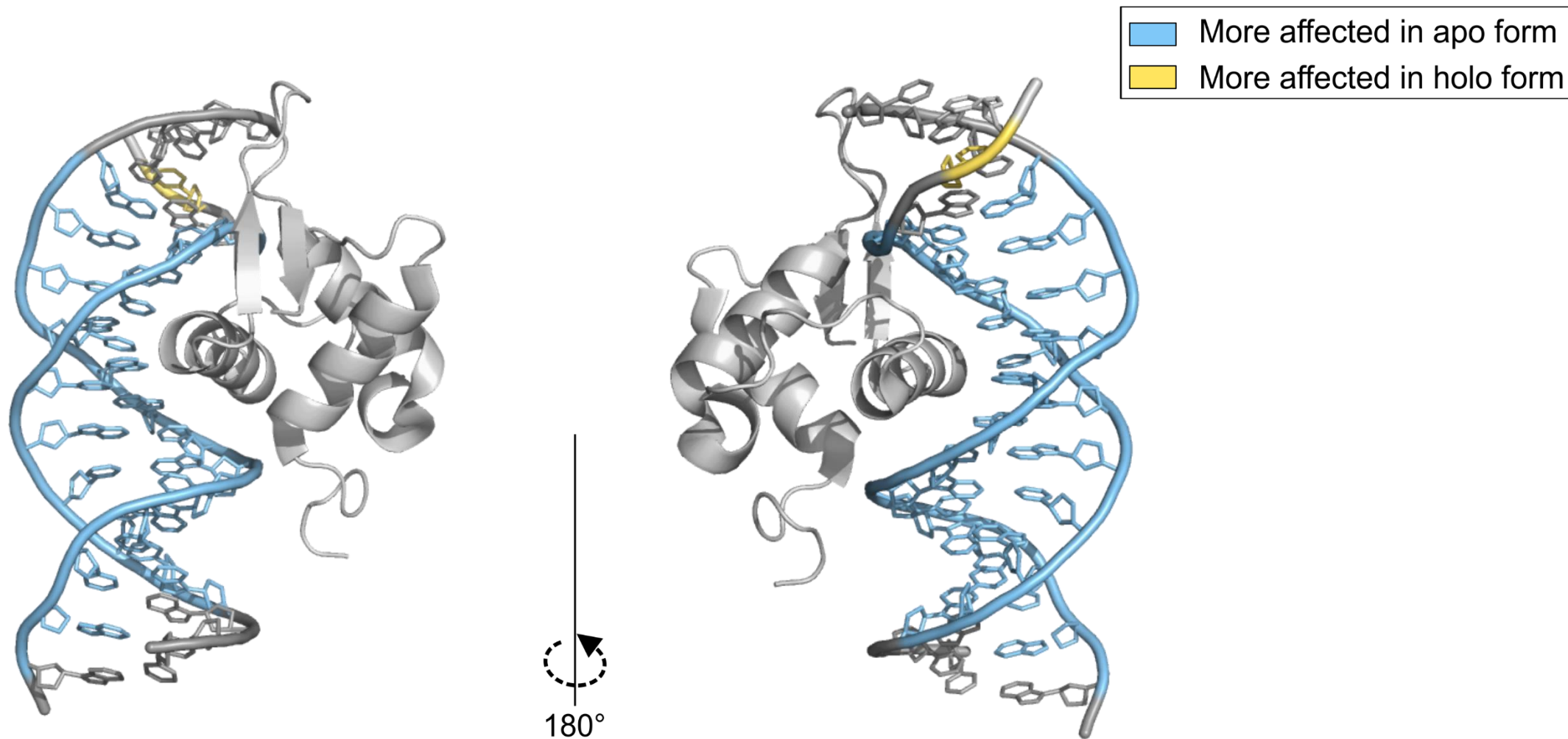
## DNA damage of Forward strand



## DNA damage of Reverse strand



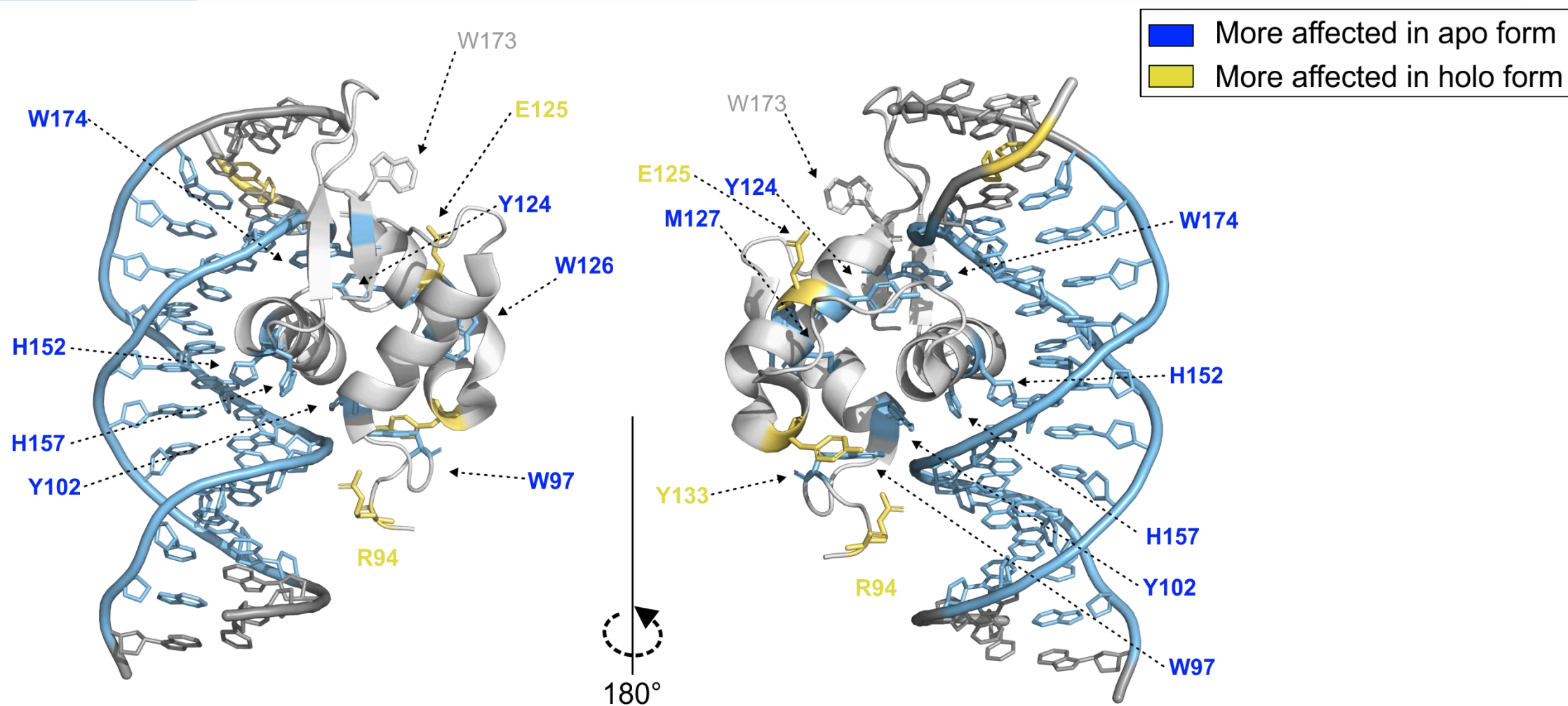
# Plotting residues into a crystal structure model



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

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

# Conclusion





# Acknowledgements

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