Radiolysis of concentrated myoglobin by accelerated ions

Q. Raffy[1]

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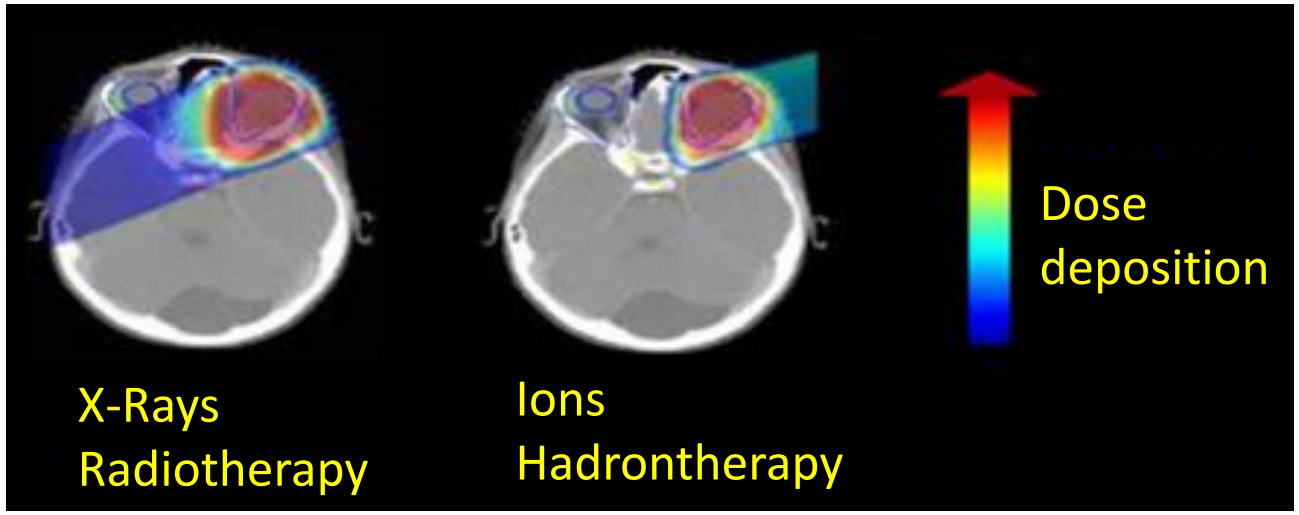
[3] ICube, Strasbourg, France

Context: Hadrontherapy

Hadrontherapy / Particle therapy : Cancer therapy with accelerated ions

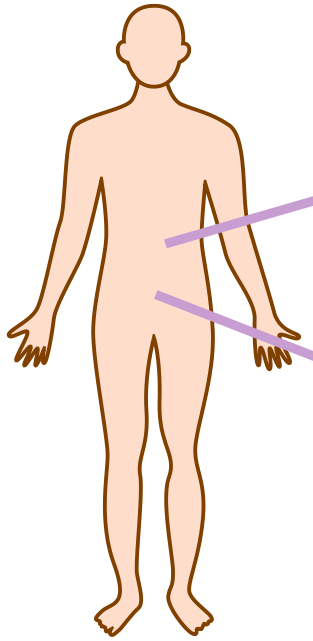
Ions/ X Rays:

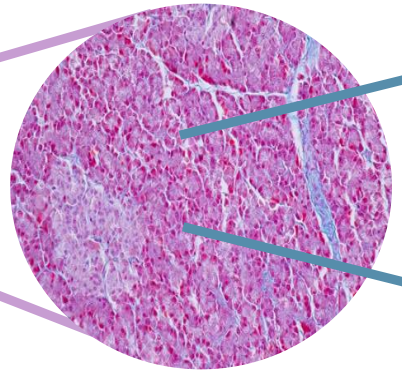
- Better ballistic
- Higher Relative Biological effect (RBE)
  (Density of energy deposition)



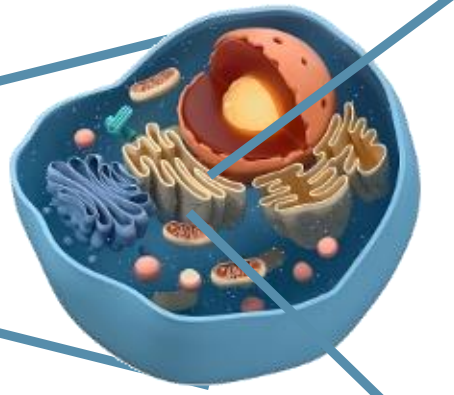
From ARCHADE project

Context: Hadrontherapy

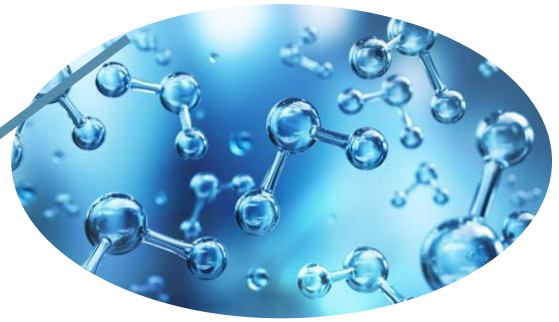




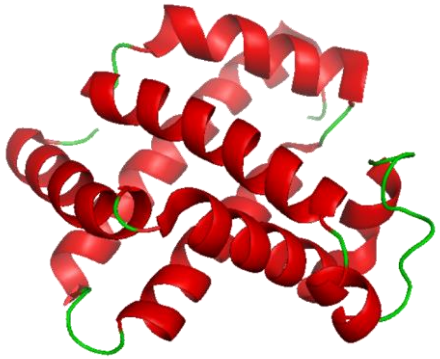
Biological Tissue



Cell



Water ~65%

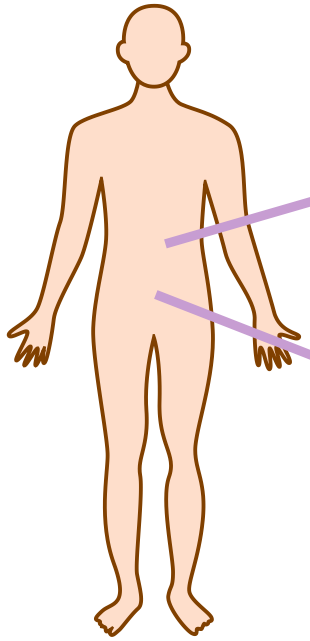


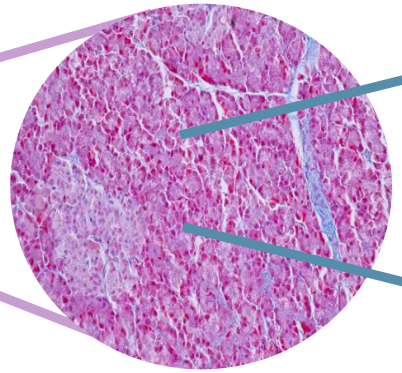
Proteins 20%

DNA <0.1%

Molecular

Context: Hadrontherapy





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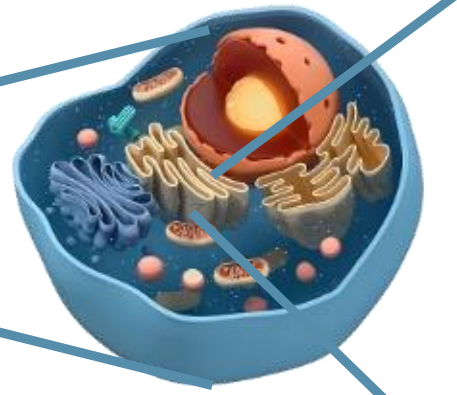

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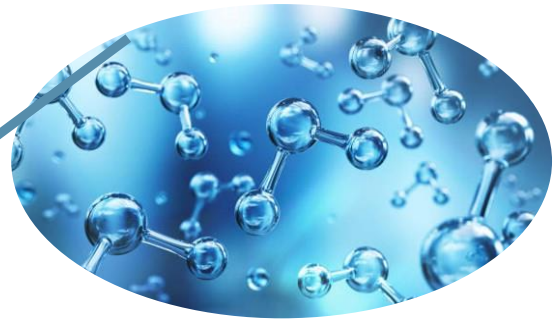

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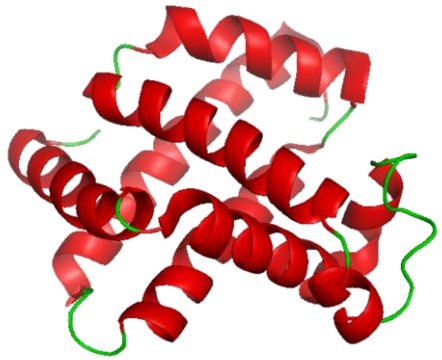
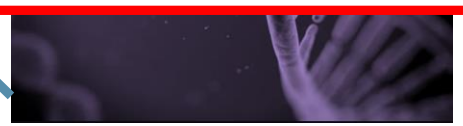


Proteins
20%

Lack of data
Irradiation with ions



<0.1%

Molecular

Radiolysis of Myoglobin by 2 MeV Protons

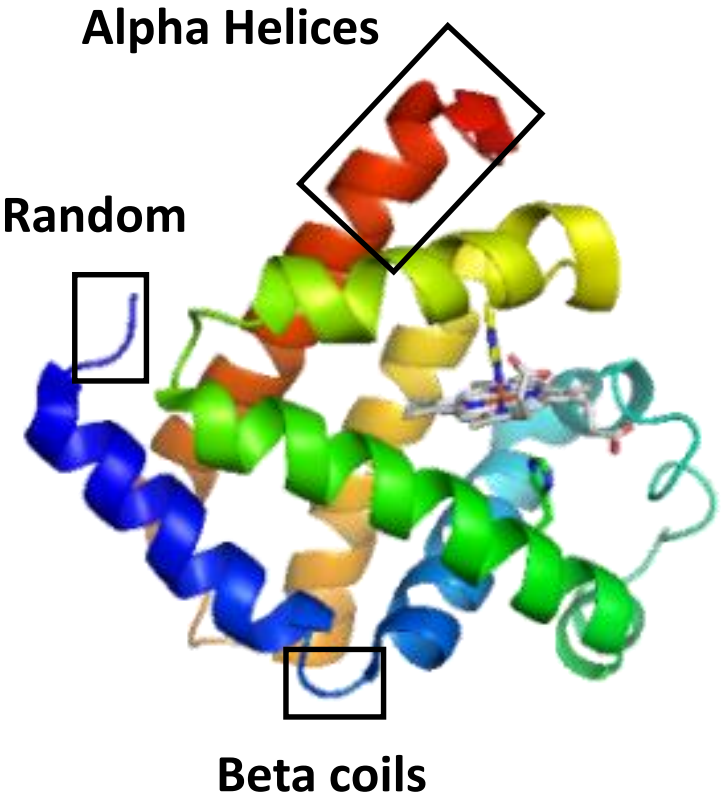
Myoglobin

Small **heme protein**
153 residues

Secondary structure

Mainly Alpha Helices
(73 %)

Proteins : biological activity
closely linked
to secondary structure



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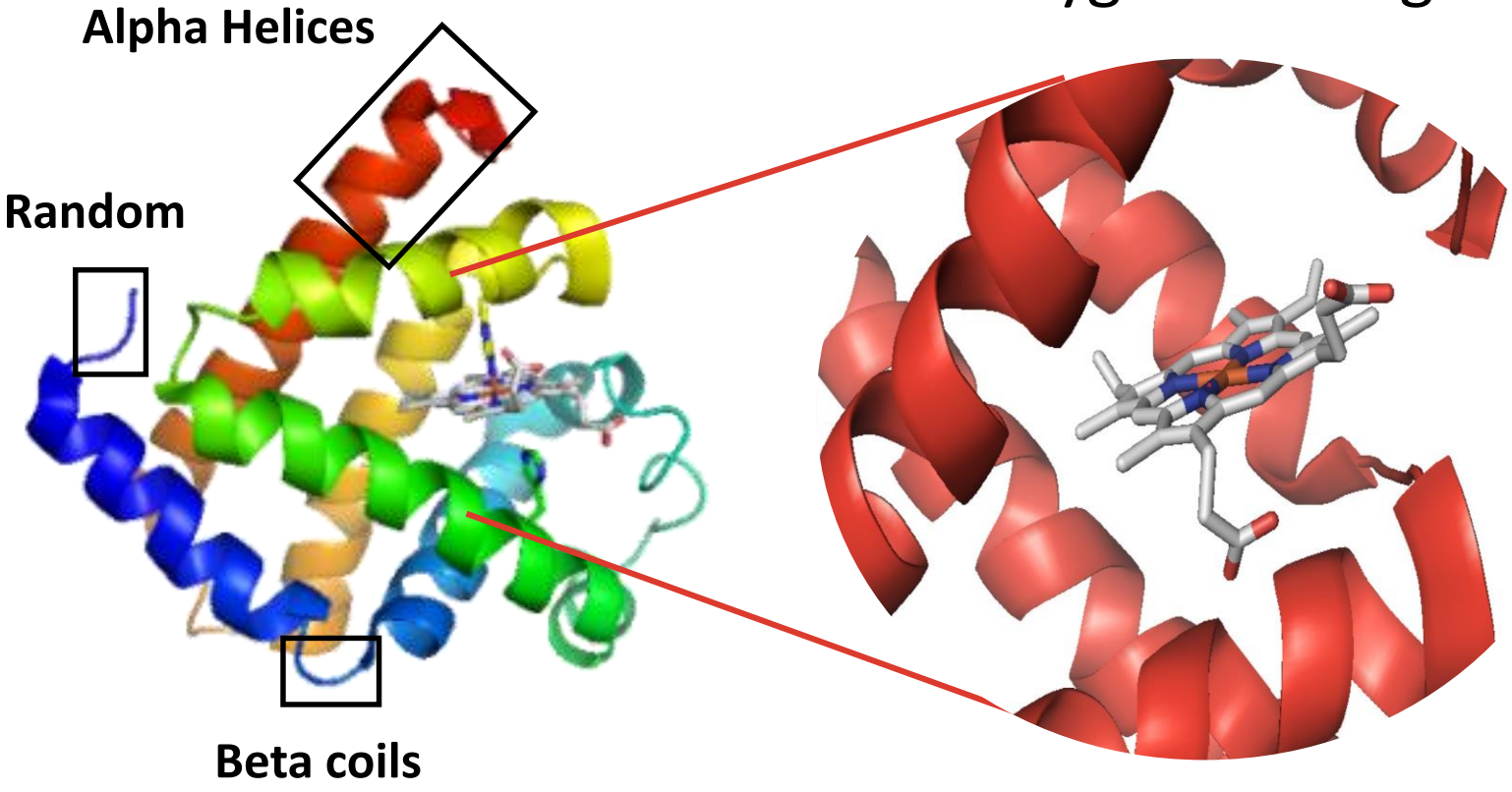
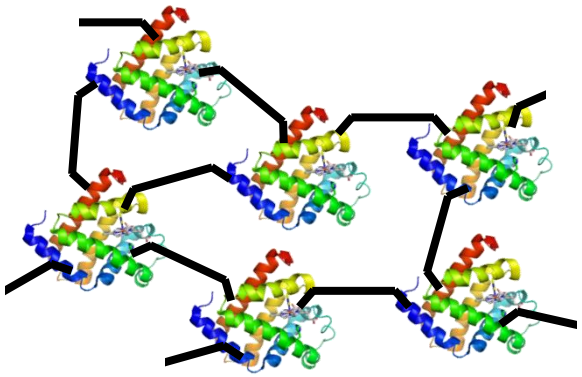
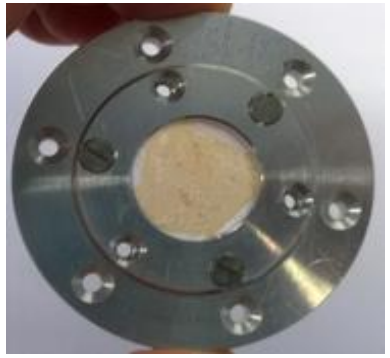


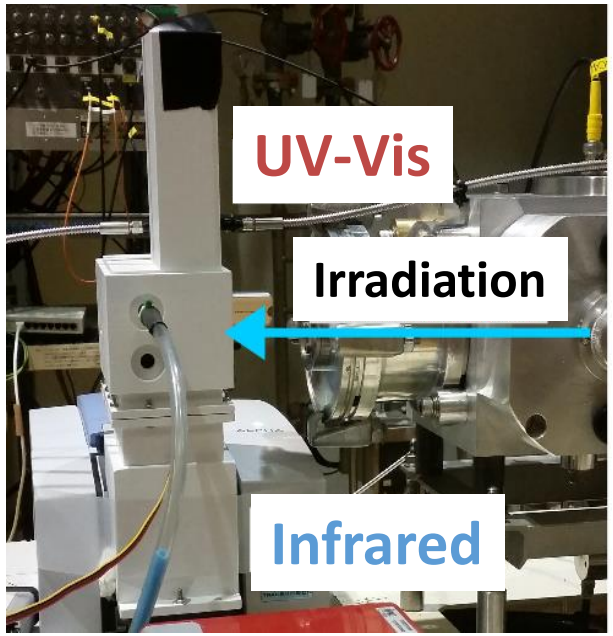
Radiolysis of thin hydrogels

Concentrated **native protein gels: 25 % in D_2O**
10-20 µm thickness
Cross-linked
Between PP sheets





Myoglobin gel



3D printed
Automated
irradiation cell

Irradiation
2 MeV protons

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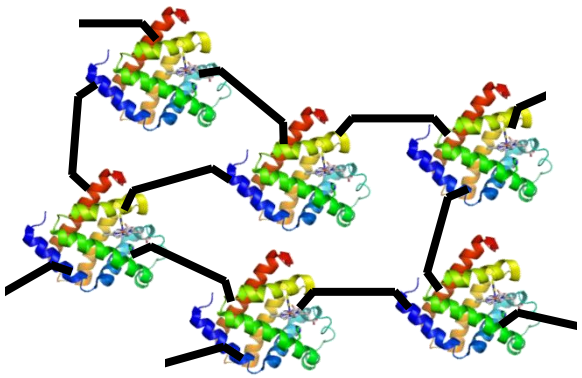

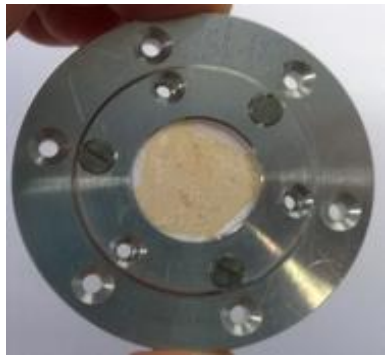

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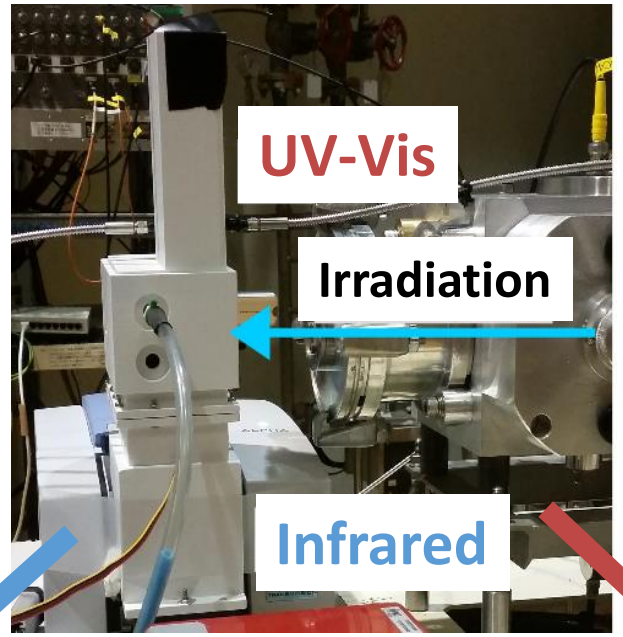
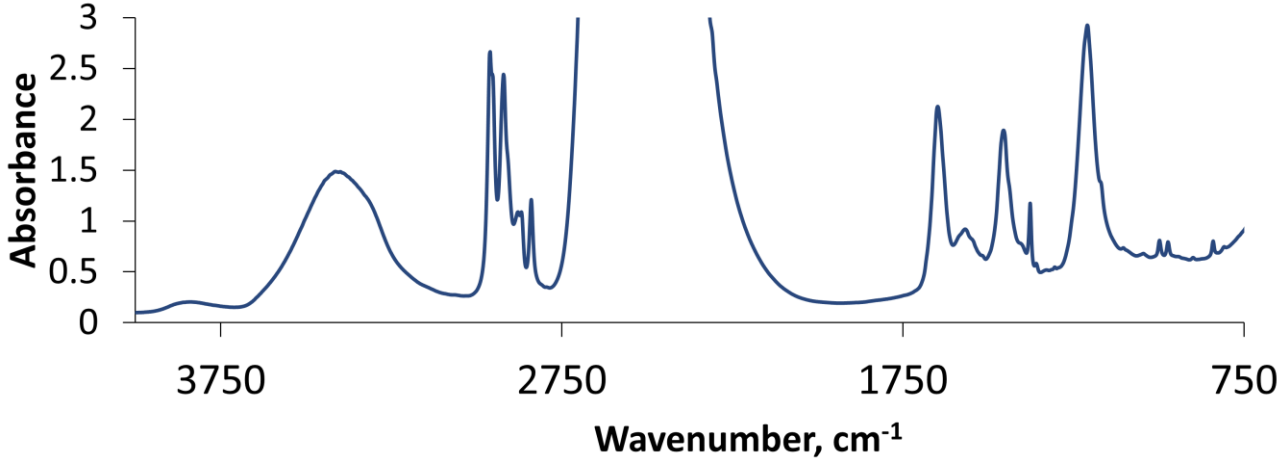


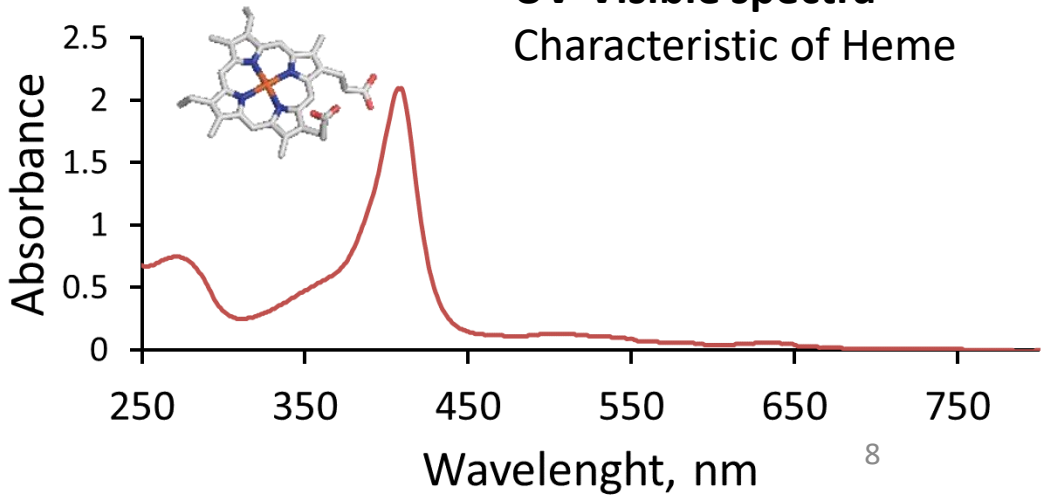
3D printed
Automated
irradiation cell

Irradiation
2 MeV protons

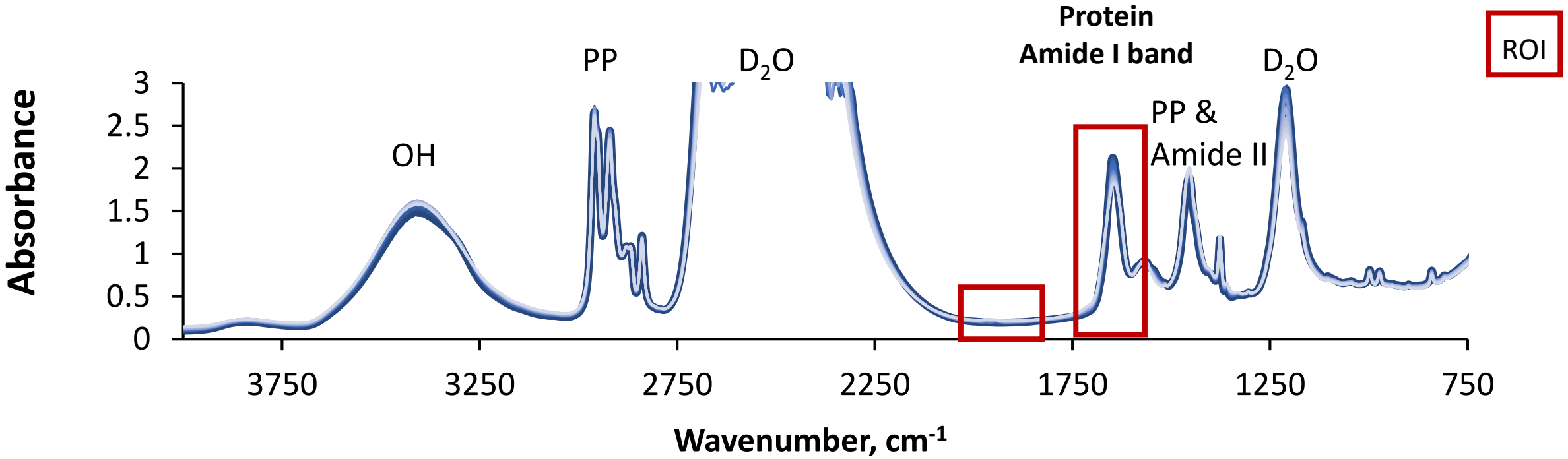
Infrared Spectra



UV-Visible spectra
Characteristic of Heme



Infrared spectra: secondary structure



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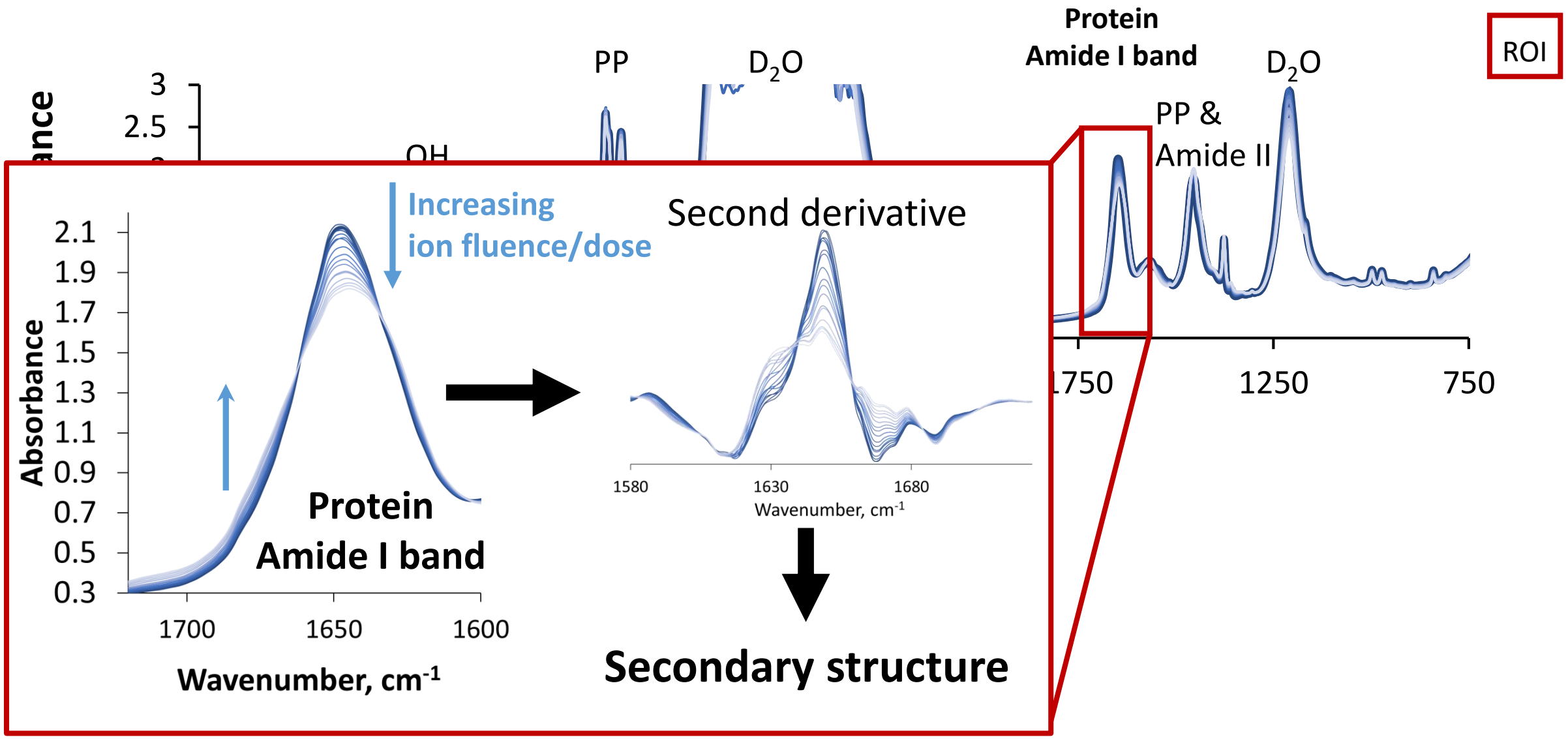

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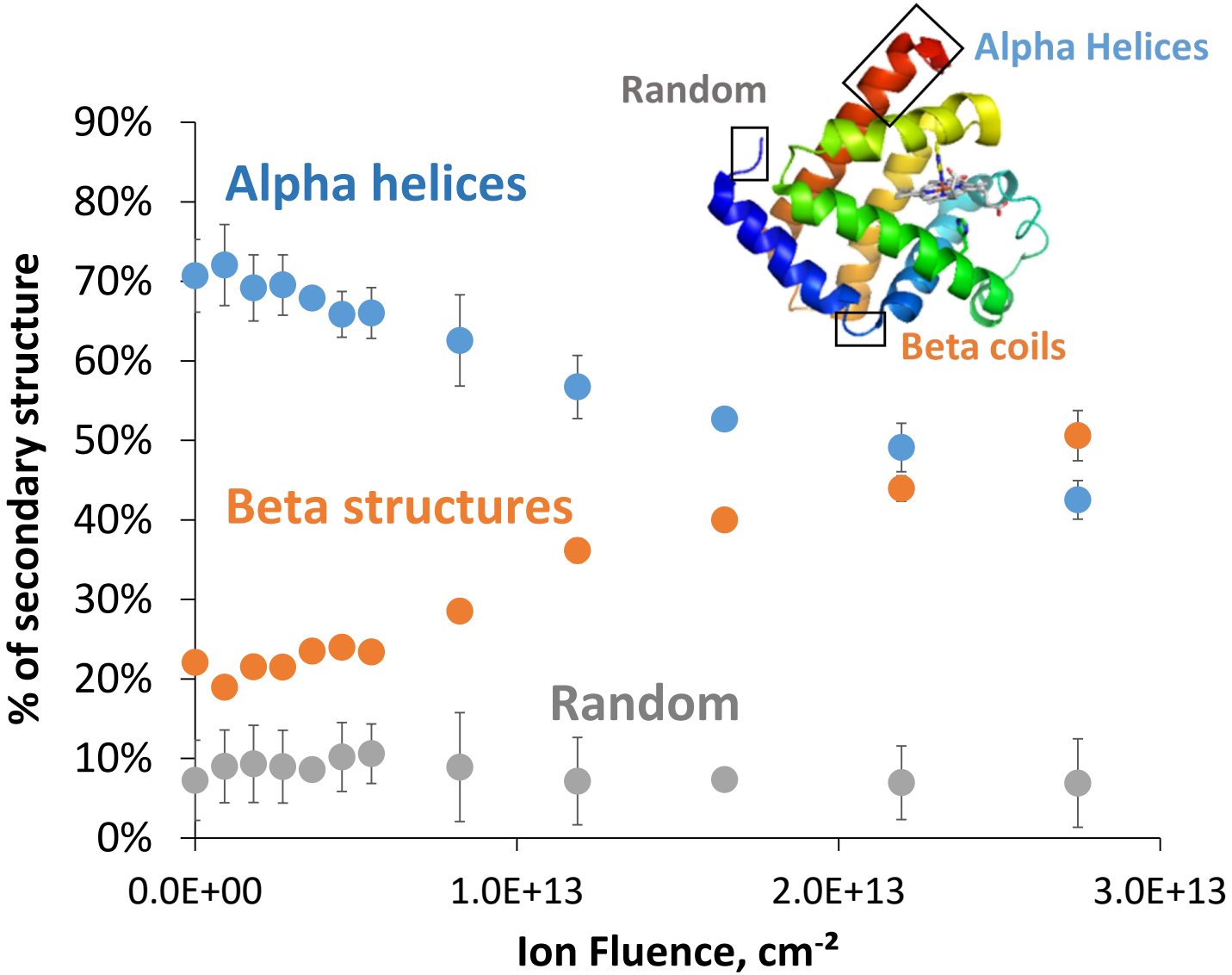


Under irradiation:

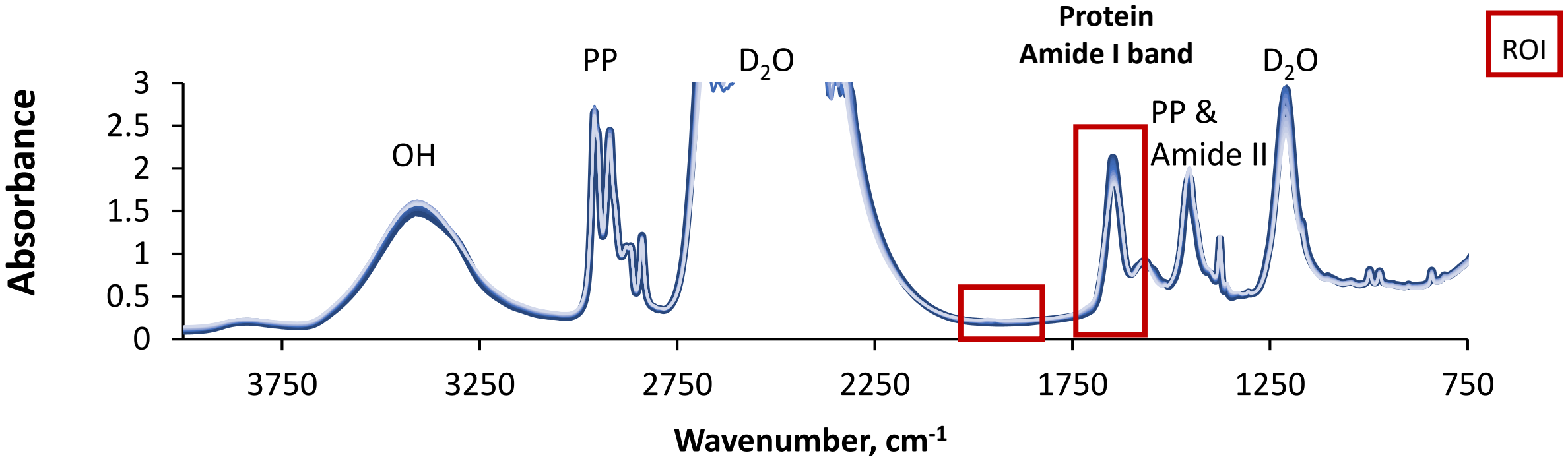
- Conversion of alpha helices to beta structures
- Random → stable

→ **New defined structure of the protein**

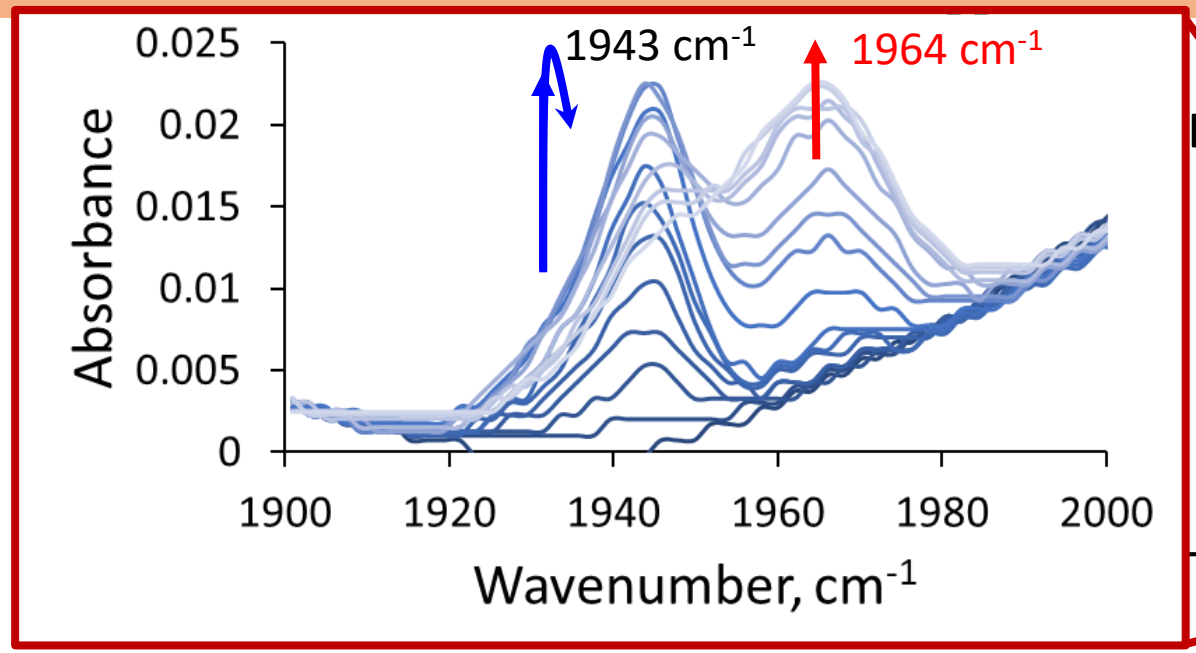
Final conformation :

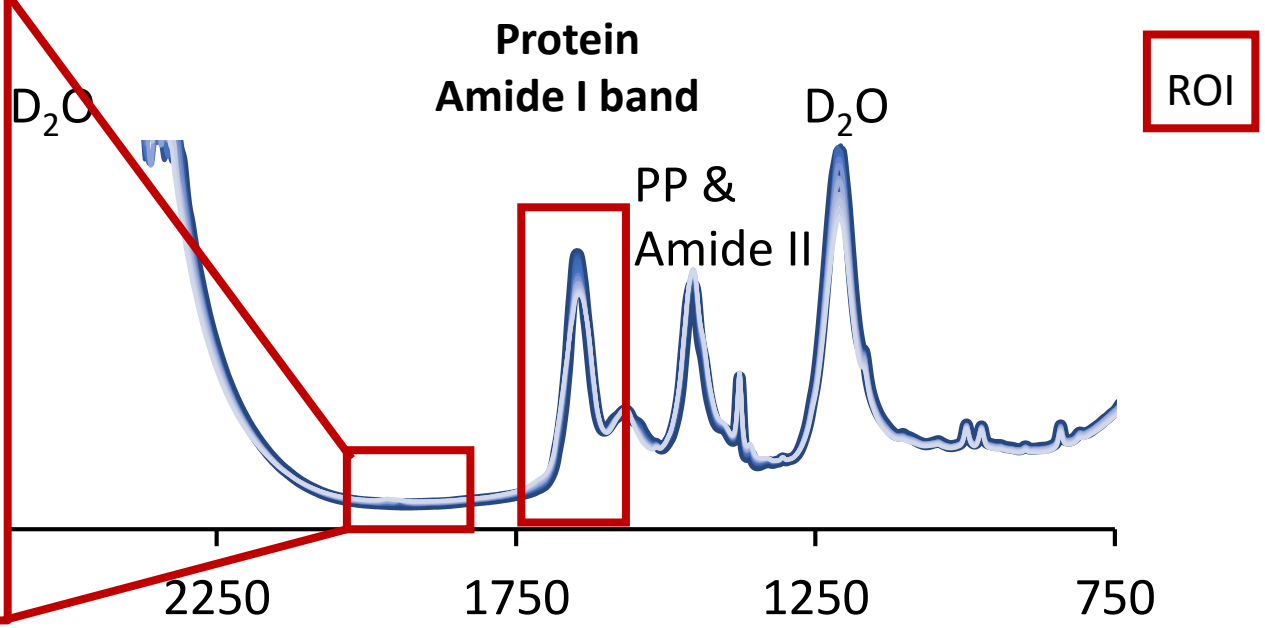
41% ± 3 % Alpha helices
48% ± 4 % beta
11 % ± 6 % random coils

Infrared spectra: secondary structure



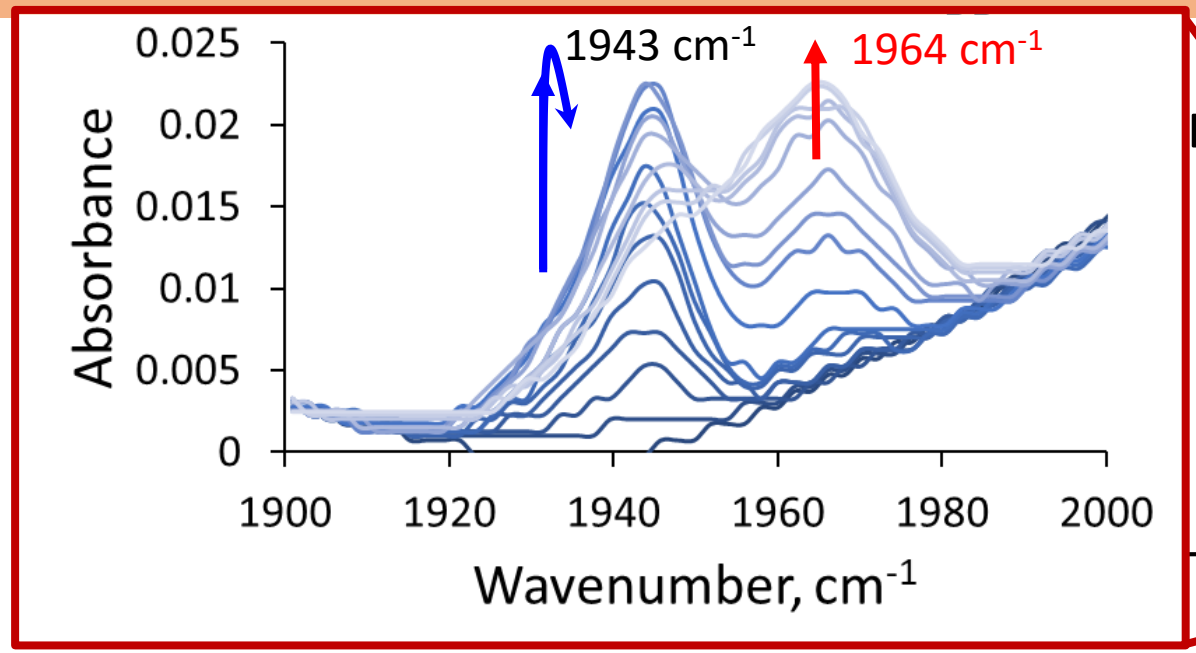
Infrared spectra: carbon monoxide

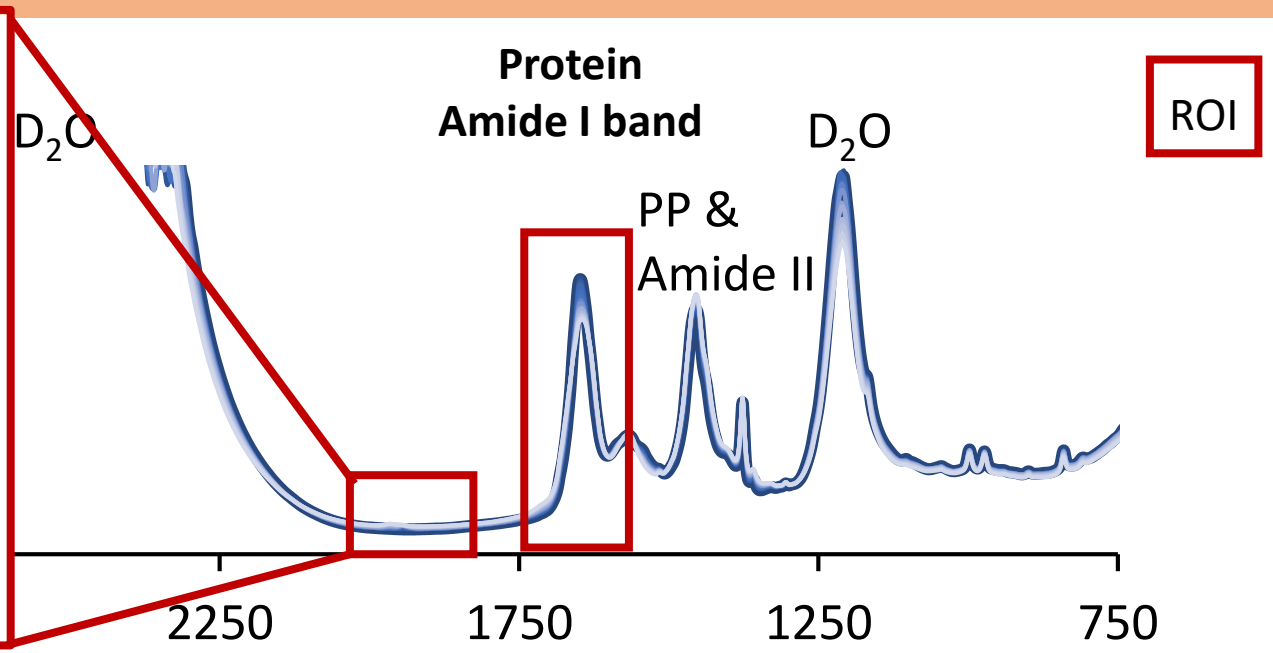


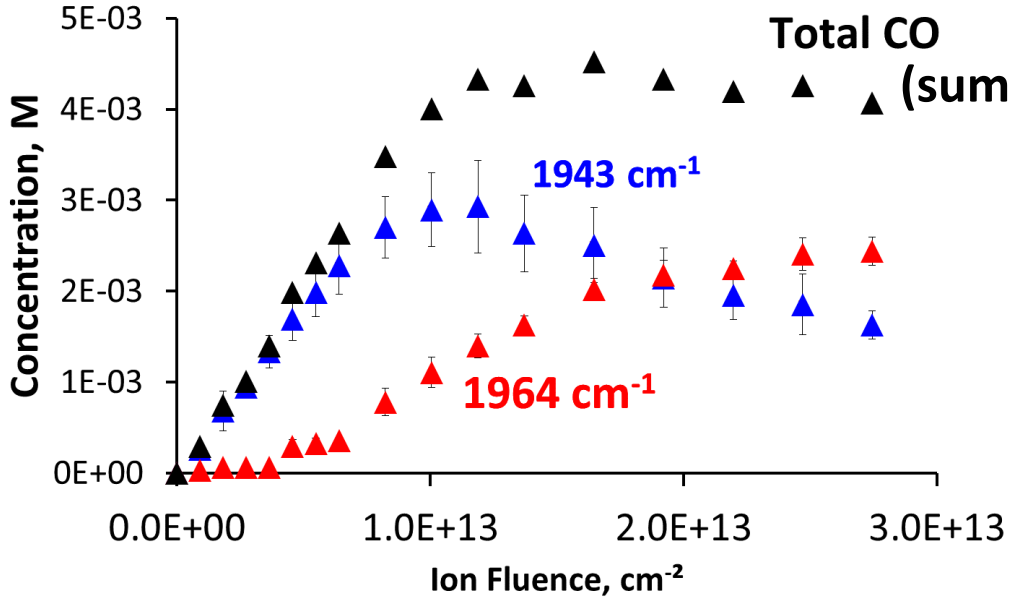


Carbon monoxide linked to
Heme or heme derivatives

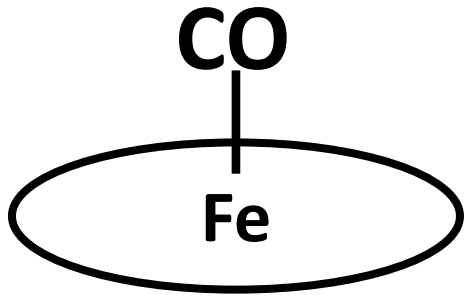
Infrared spectra: carbon monoxide





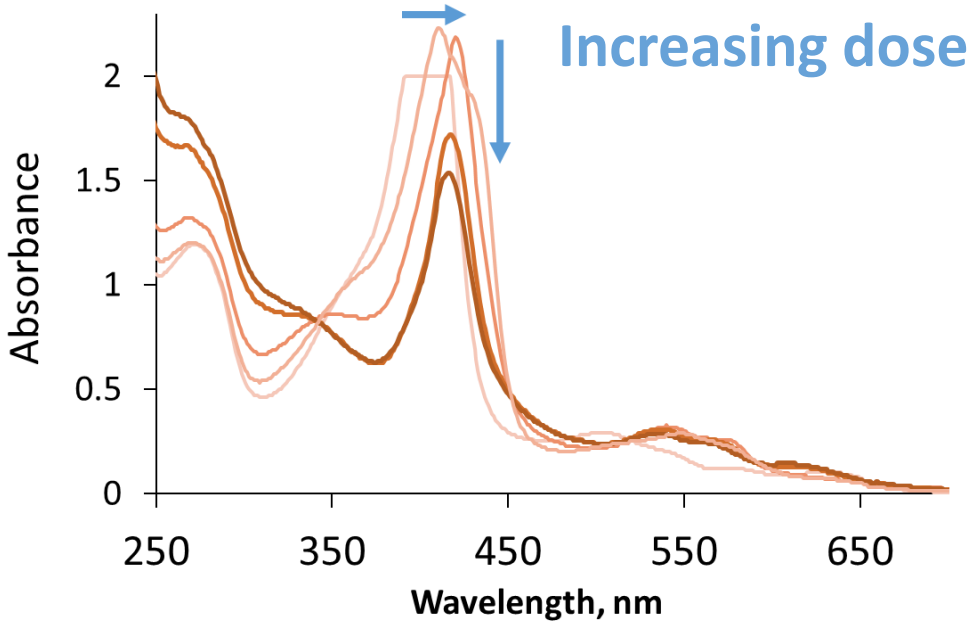


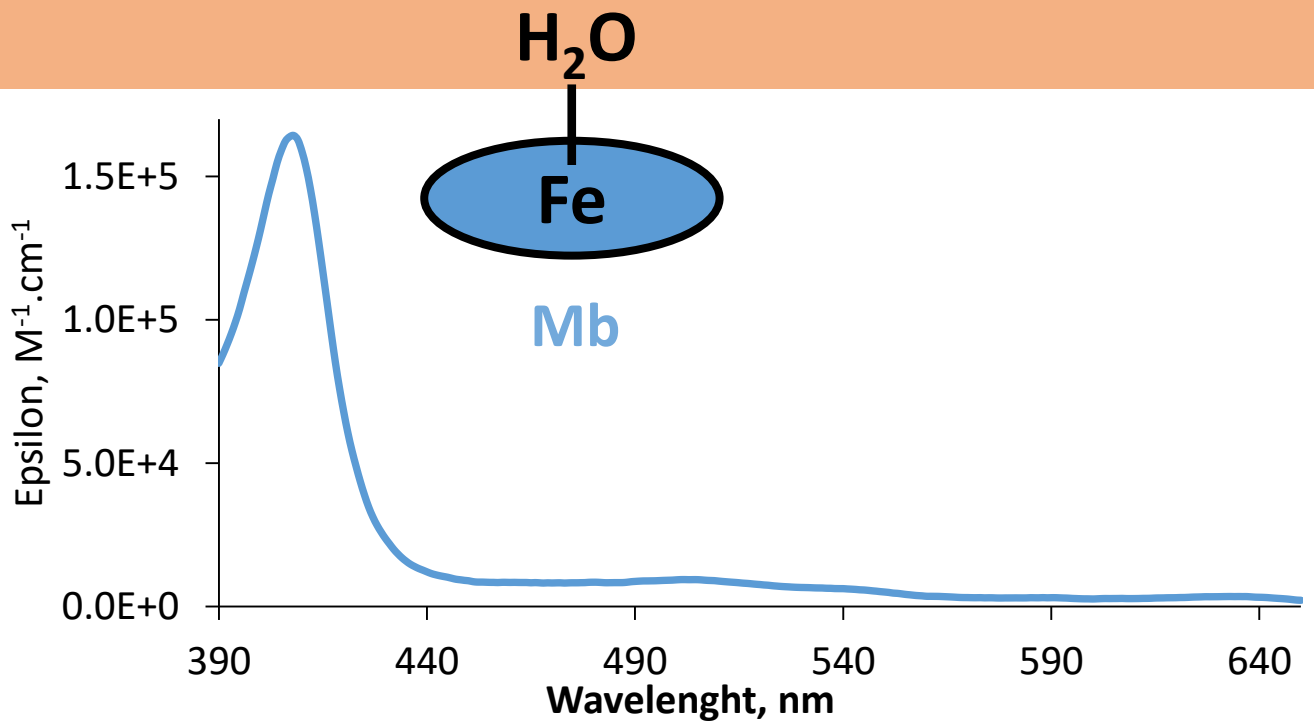
Carbon monoxide linked to
Heme or heme derivatives
→ 2 ≠ species
Identification ?



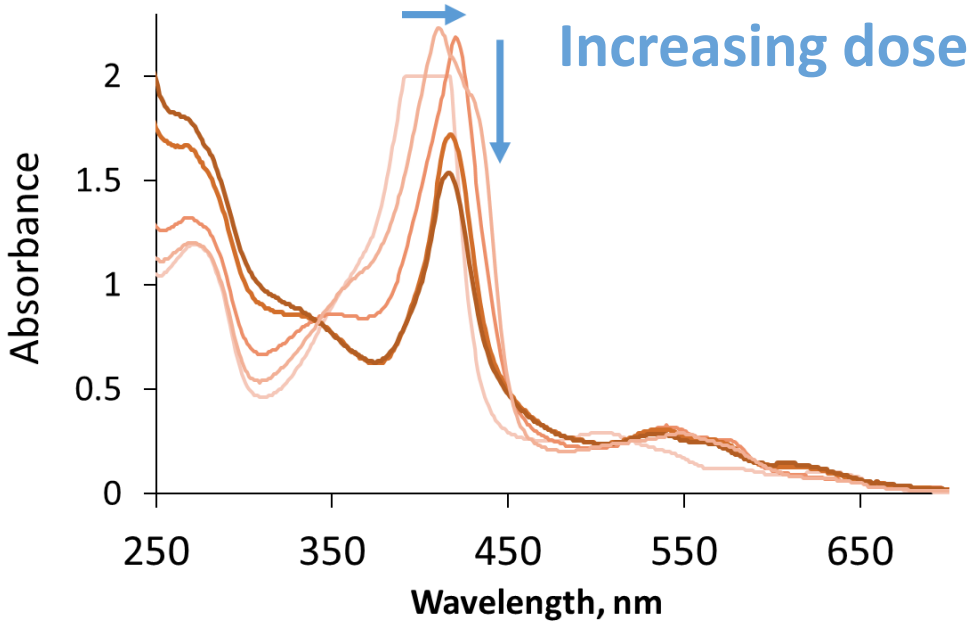
→ CO Formed under irradiation
Plateau

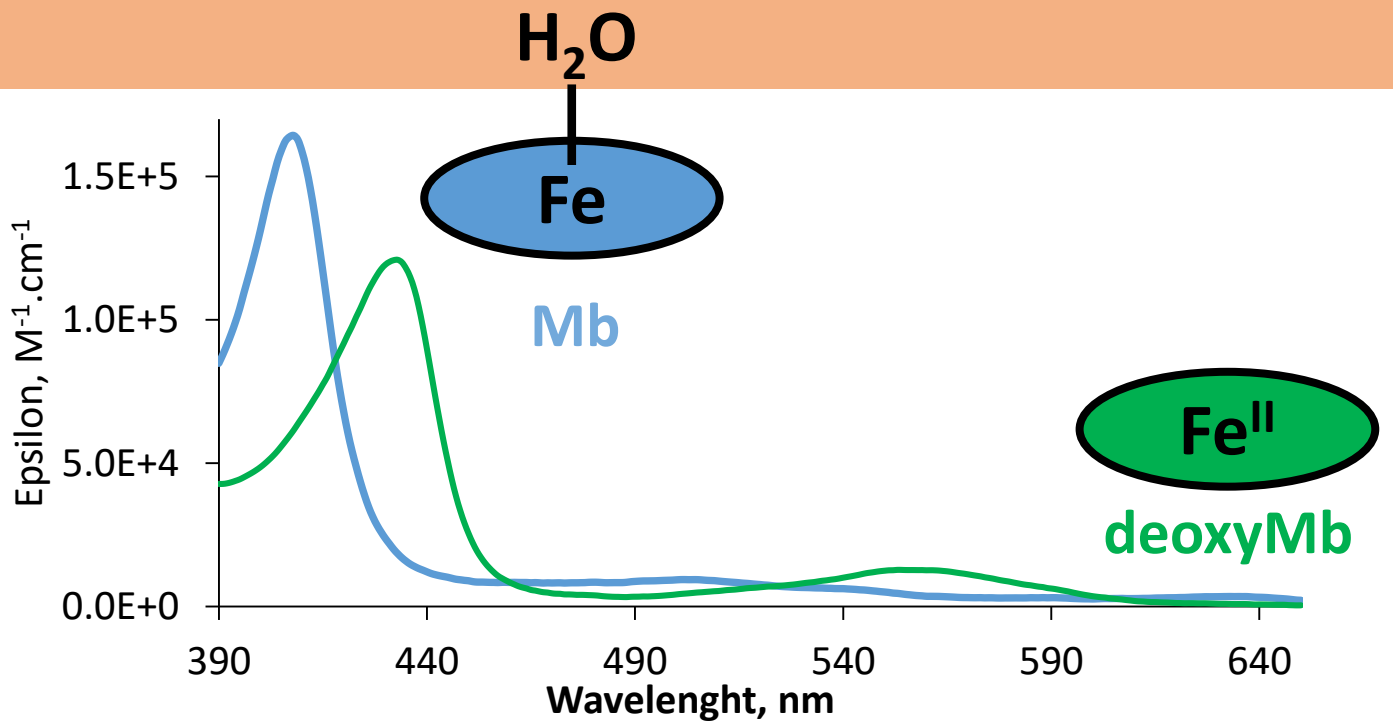
UV-Visible spectroscopy





UV-Visible spectroscopy





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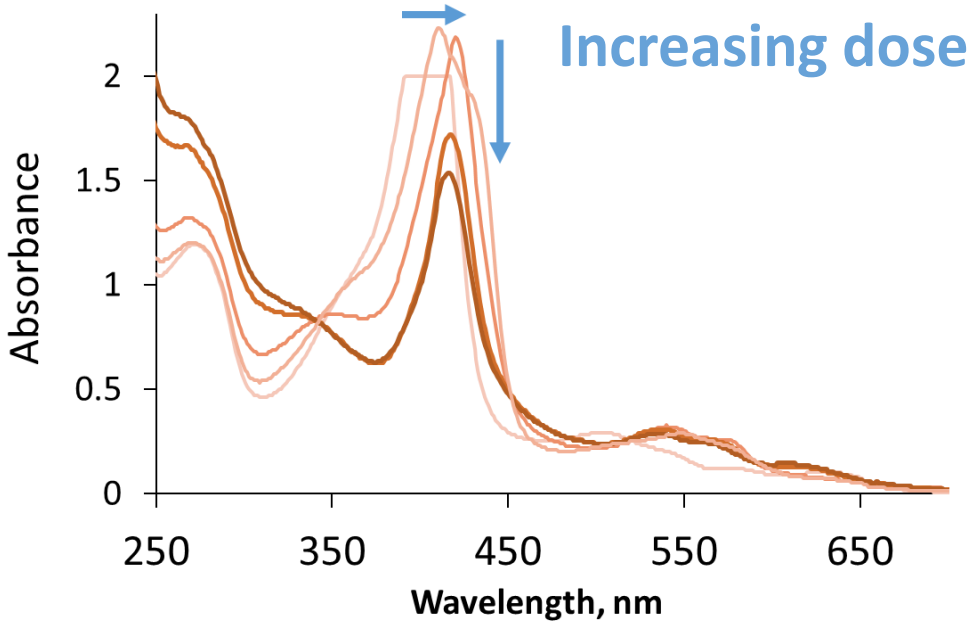

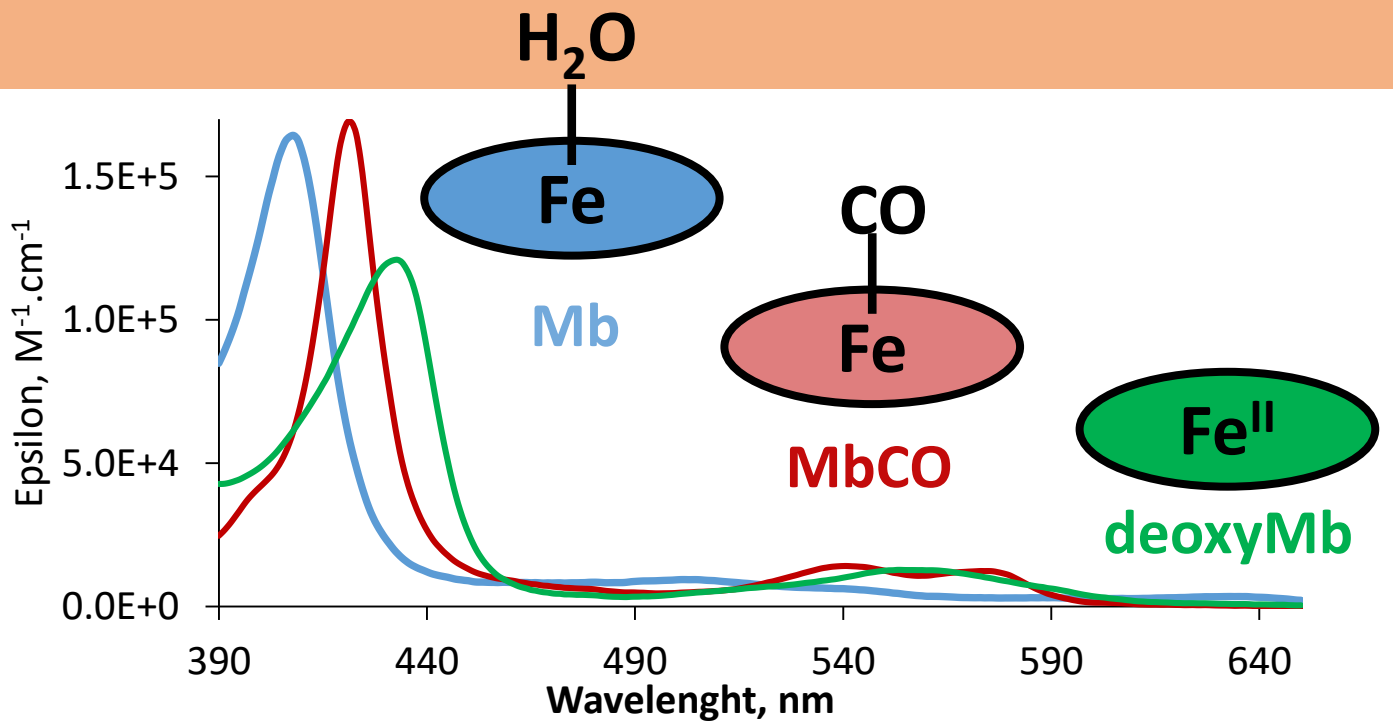

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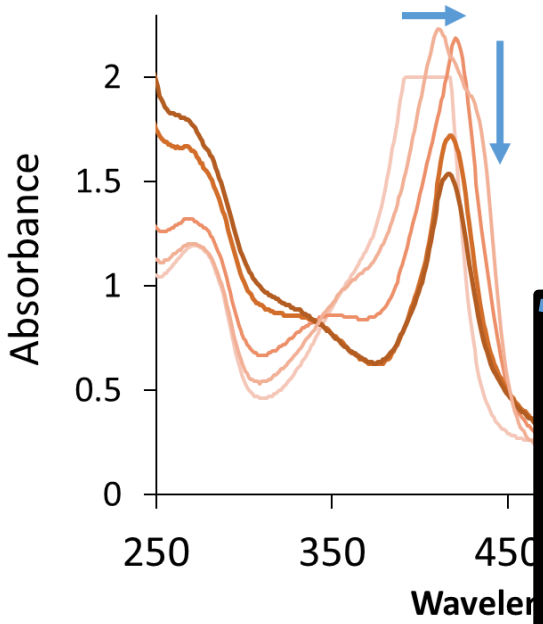
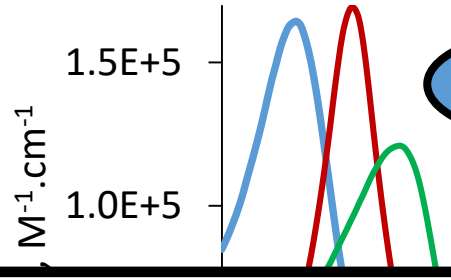
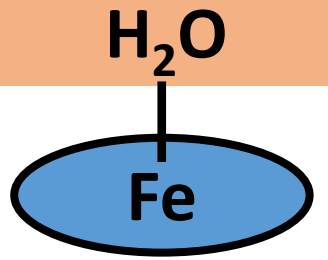
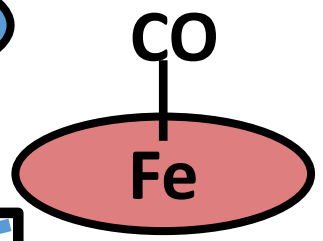


Increasing dose



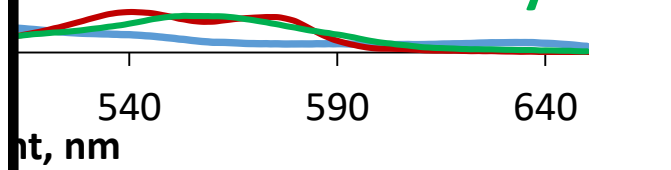


Mb

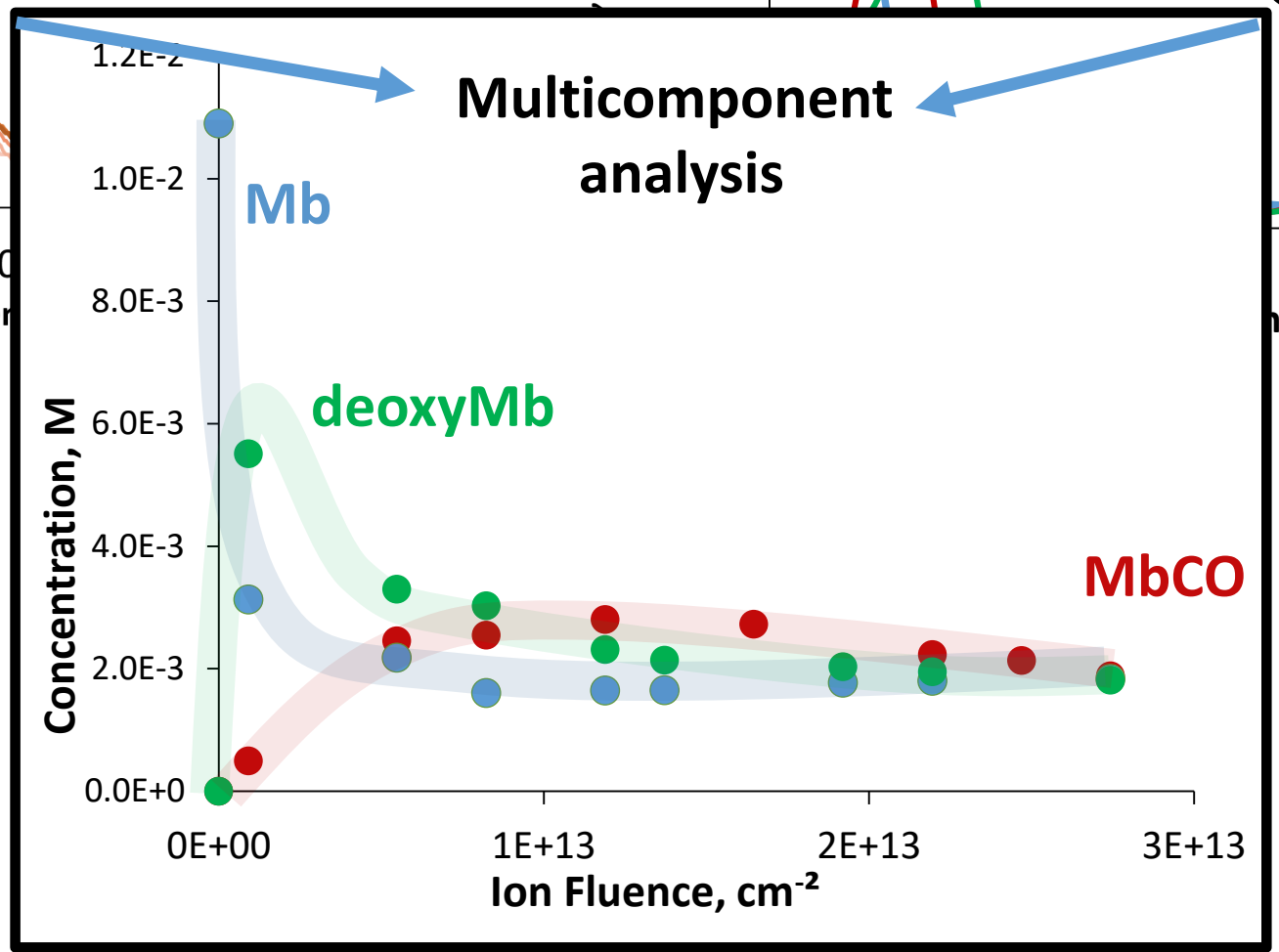


MbCO

deoxyMb

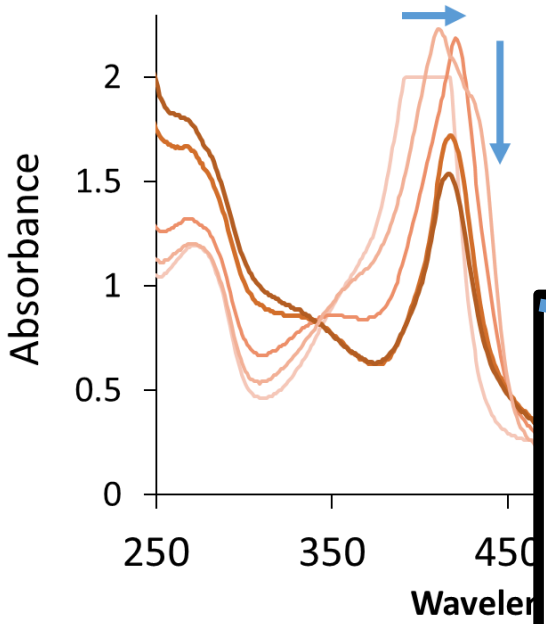


Multicomponent analysis



- Formation of deoxyMb
- Reaction with CO
  →MbCO

UV-Visible spectroscopy



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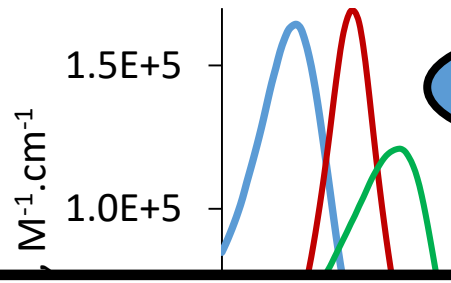

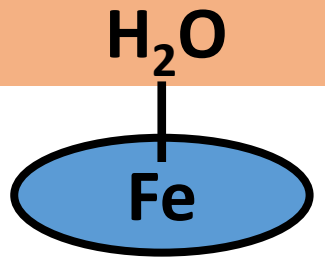

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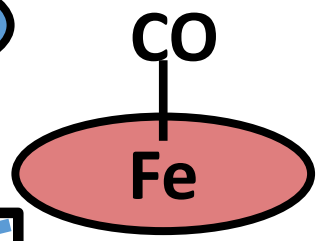

MbCO

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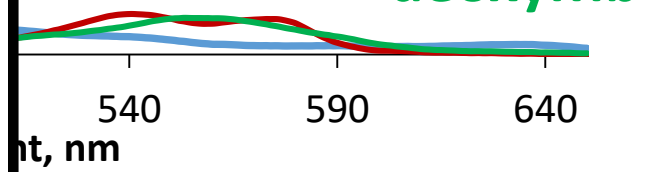
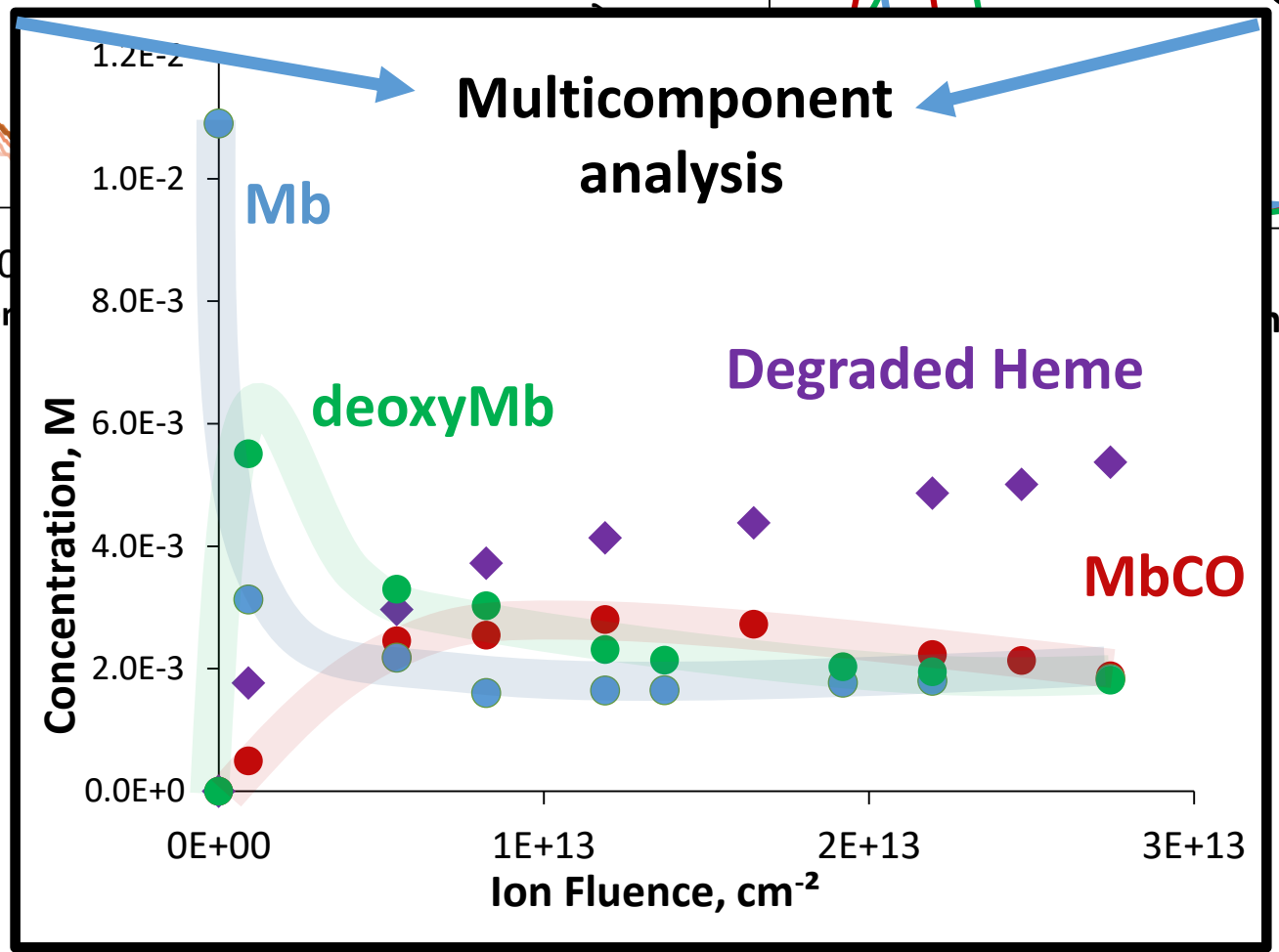
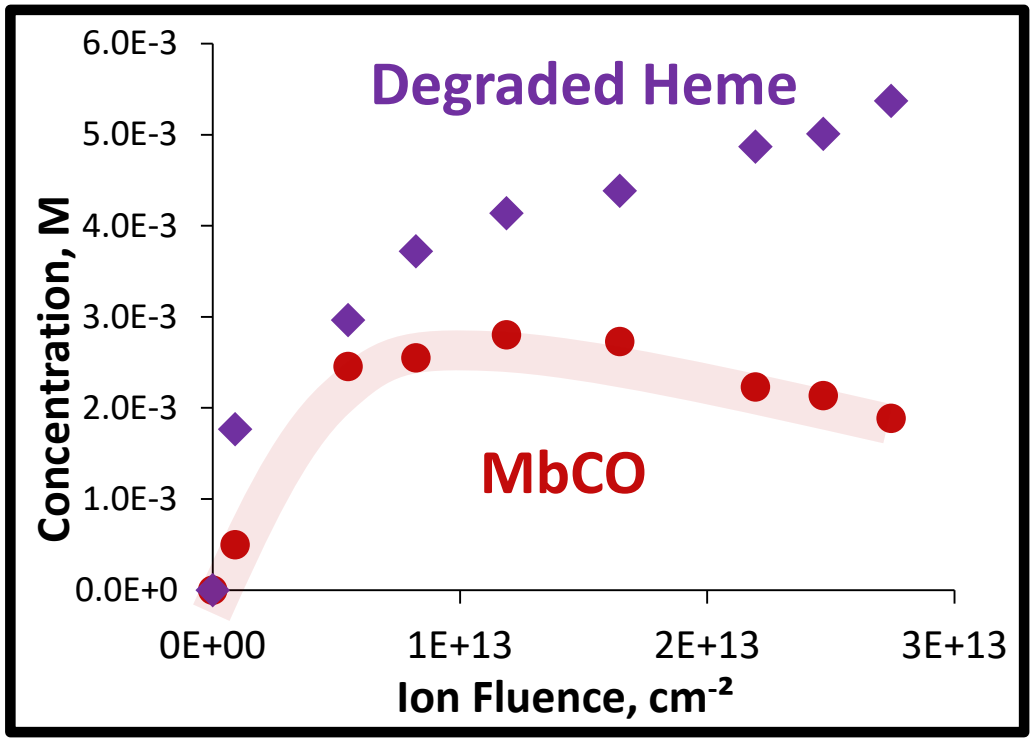




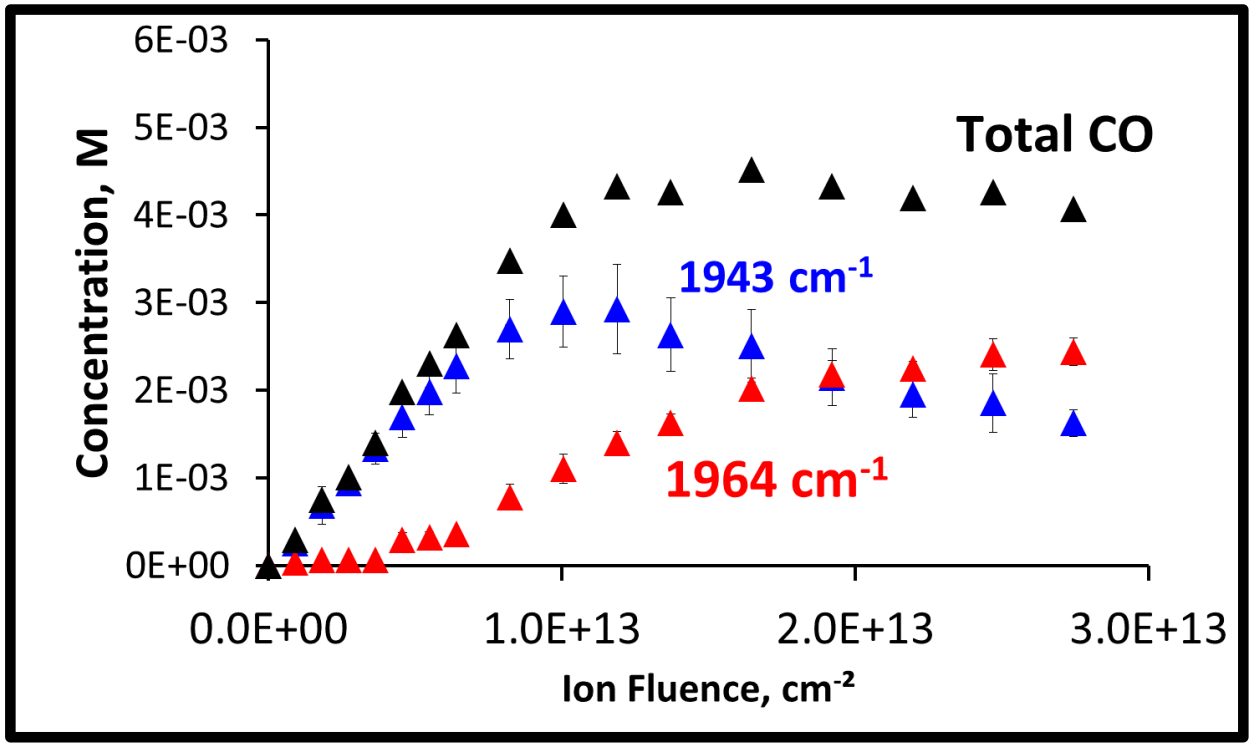
- Formation of deoxyMb
- Reaction with CO →MbCO
- Degradation of heme

Comparing data: source of CO

UV-Visible



Infrared



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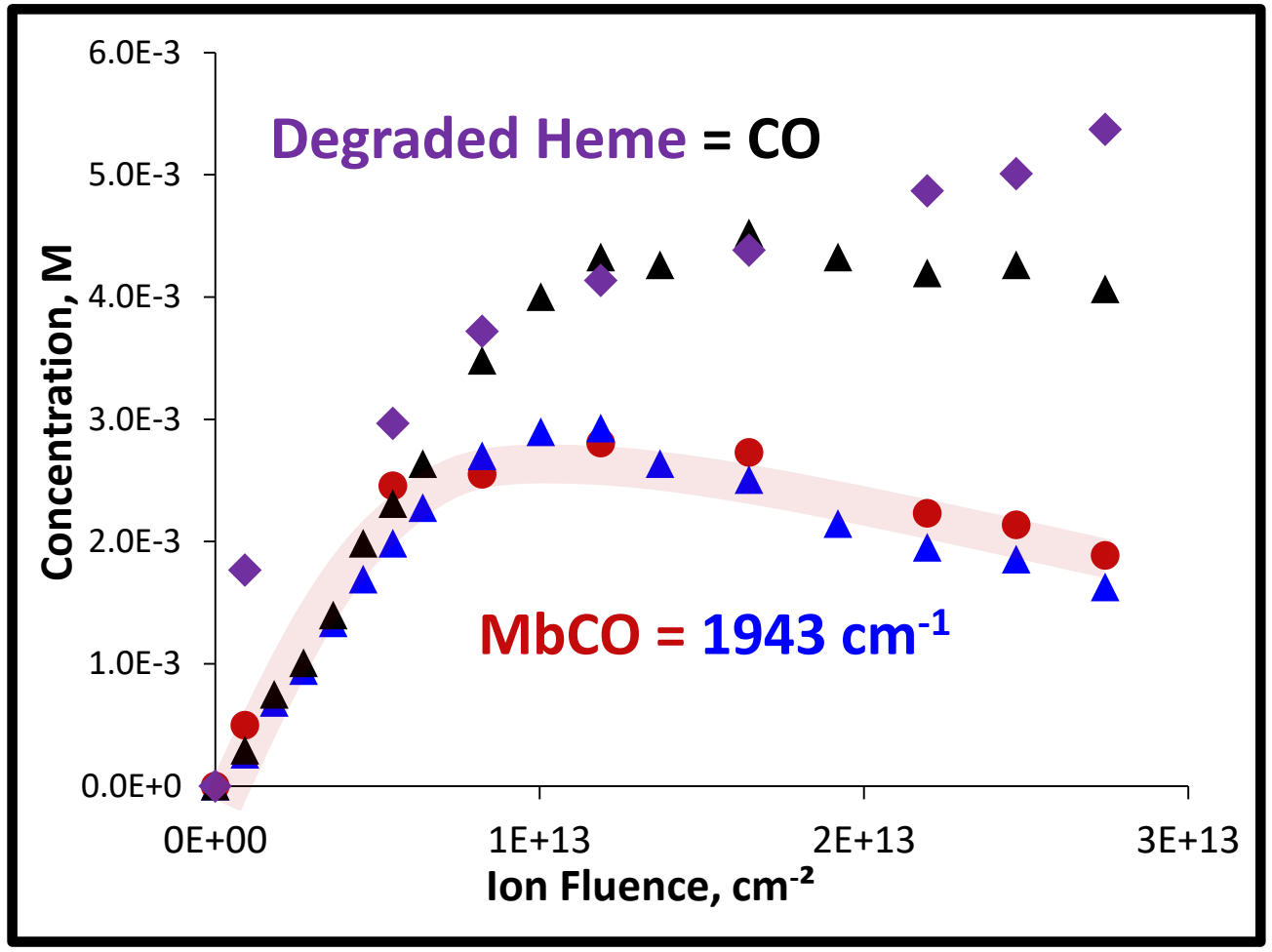

Absorbing species IR 1943 cm^{-1}

Same evolution as MbCO → same species

Same evolution degraded heme / total CO

Source formation CO = degradation of heme

Biological main mechanism **CO** production → heme degradation (heme oxygenases)

Absorbing species IR 1964 cm^{-1}

Heme degradation species

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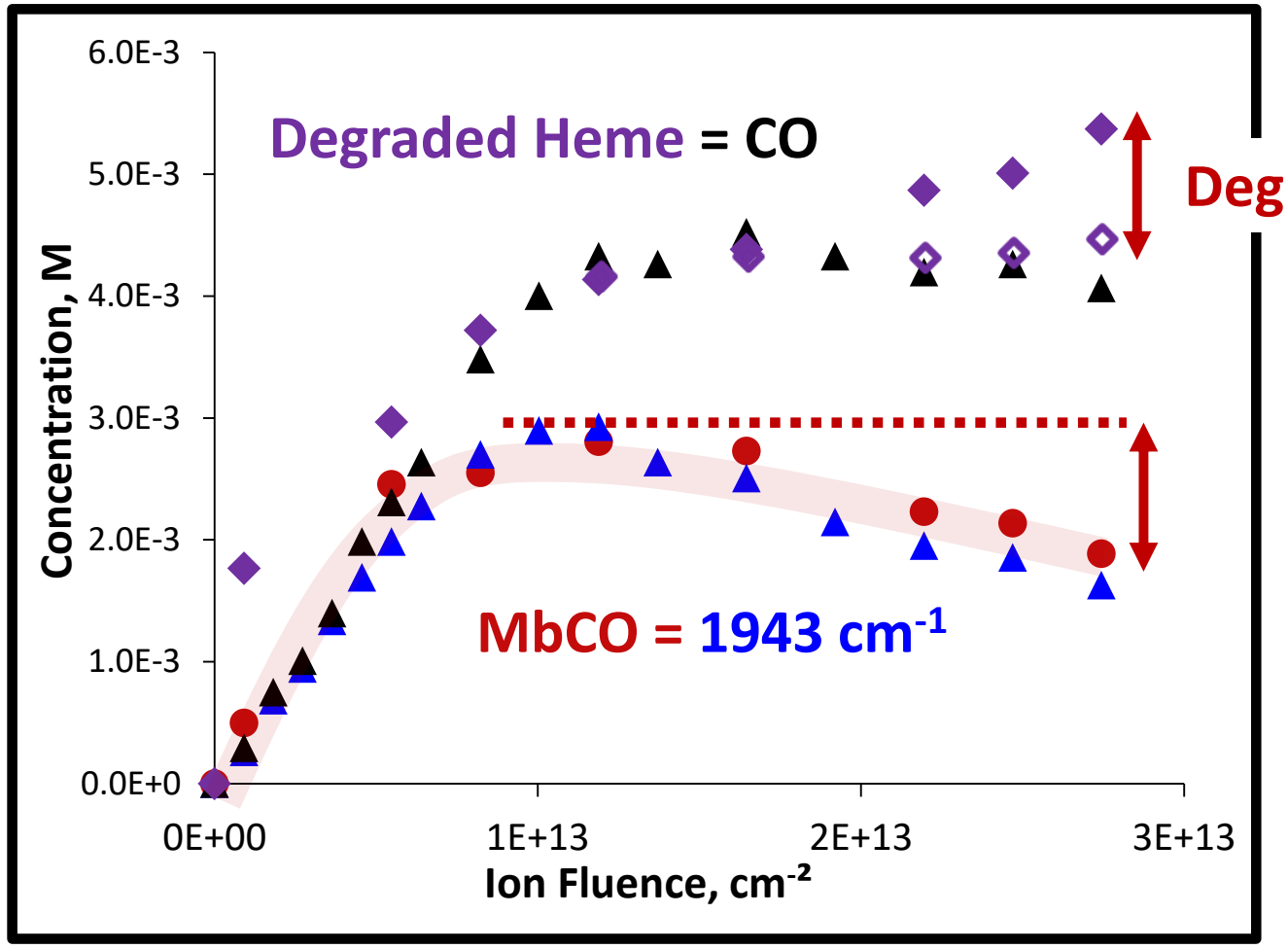

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Absorbing species IR 1964 cm^{-1}

Heme degradation species

Conclusions

First observation radiolysis of a **native** concentrated protein with **ions** (H^+)

Molecular study

Secondary structure: Well-defined final conformation
Mainly Beta structure

Carbon monoxide: Important formation of CO under irradiation
Source → heme degraded under irradiation
No clear correlation with structural changes

Carbon monoxide → strong influence on the metabolism of cells
Could play a role in biological response under ion therapy

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Source → heme degraded under irradiation
No clear correlation with structural changes

Carbon monoxide → strong influence on the metabolism of cells
Could play a role in biological response under ion therapy

Thank you for your attention !

Conclusions

First observation radiolysis of a **native** concentrated protein with **ions** (H^+)

Molecular study

Secondary structure: Well-defined final conformation
Mainly Beta structure

Carbon monoxide: Important formation of CO under irradiation
Source → heme degraded under irradiation
No clear correlation with structural changes

Carbon monoxide → strong influence on the metabolism of cells
Could play a role in biological response under ion therapy

Thank you for your attention !