

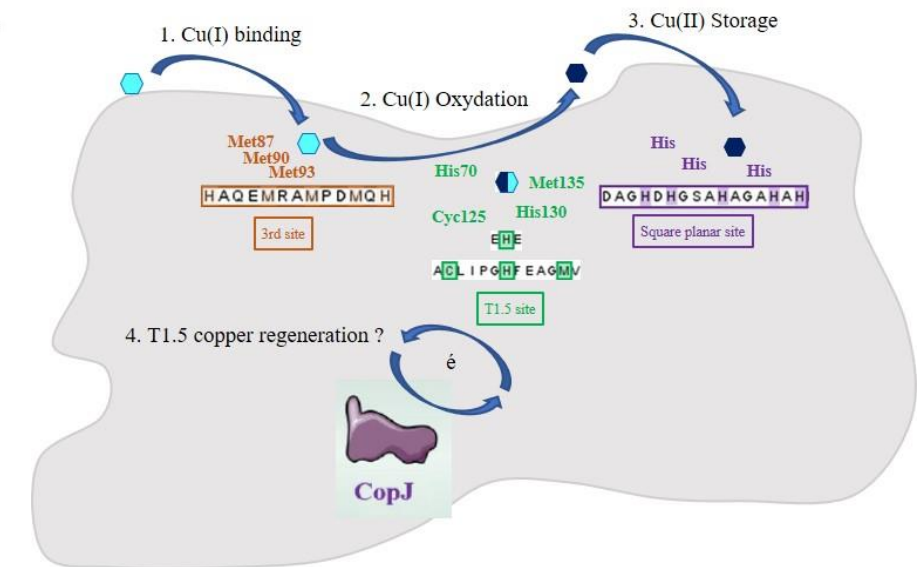
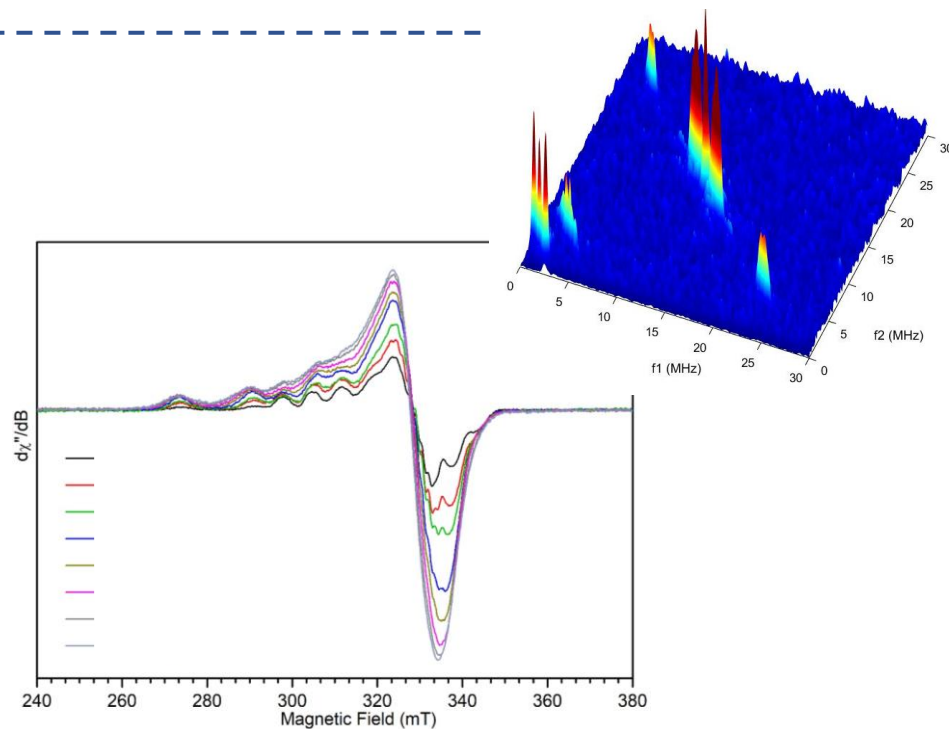
Combined EPR and NMR study of a novel periplasmic protein involved in bacterial copper resistance.



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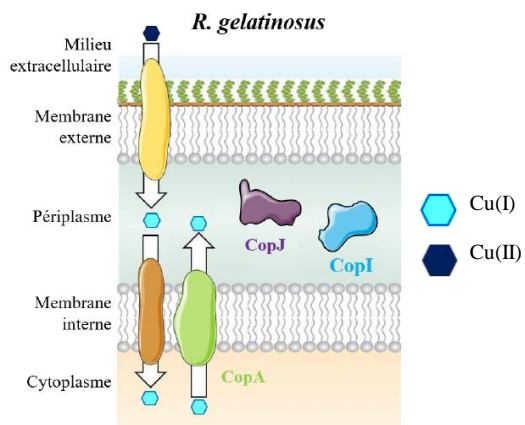
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Context

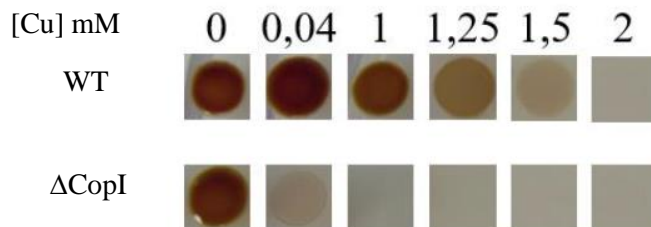
Copper Homeostasis



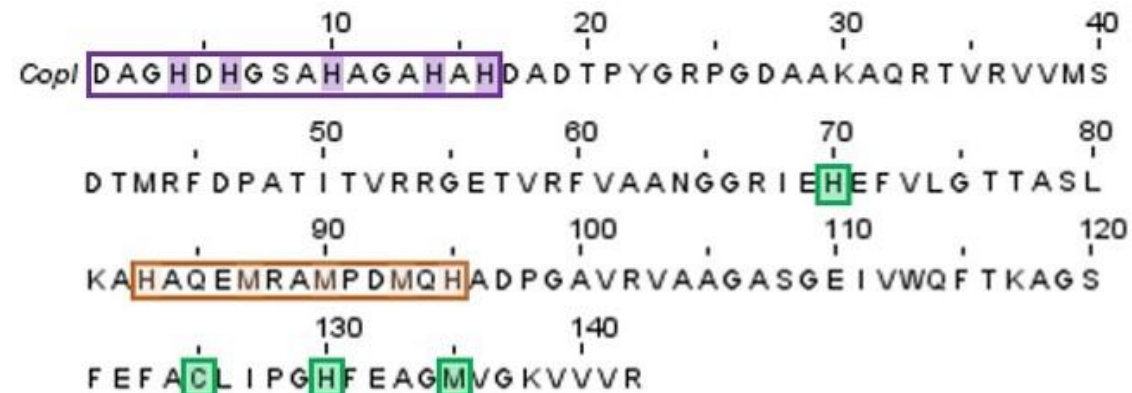
- **Role of the proteins CopJ (purple) and CopI (blue) is unknown.**

Physiological studies

Photosynthesis

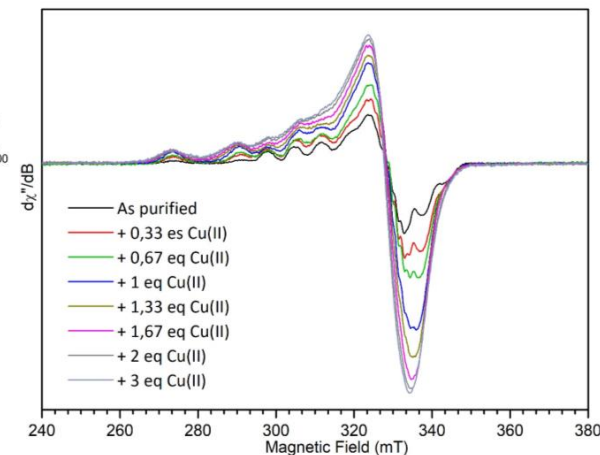
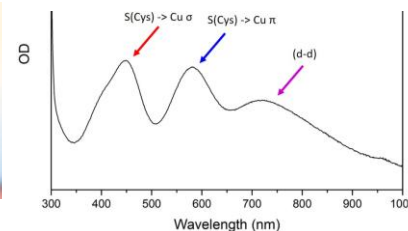
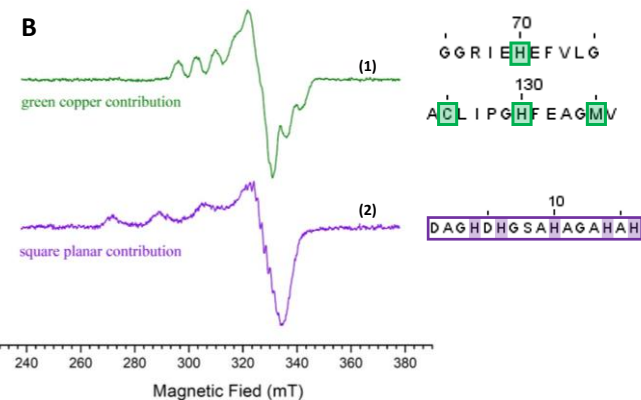
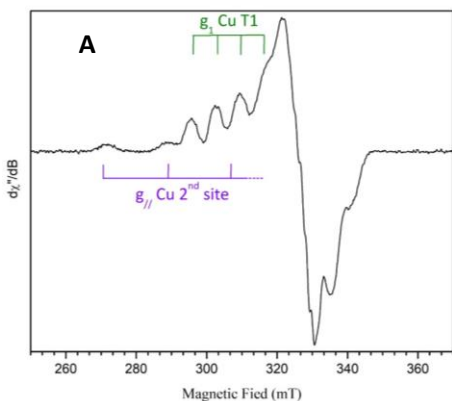


- **CopI involved in the copper resistance of the bacteria**

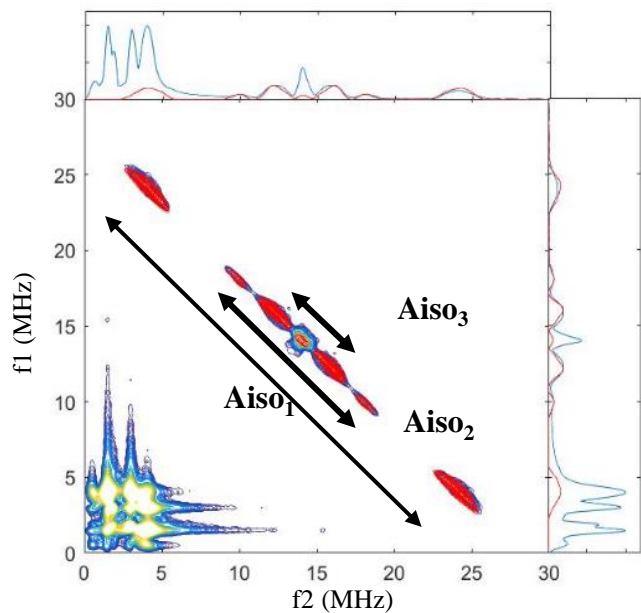


- 3 potentials Cu binding site

- T1 cupredoxin site: **His70-X54-Cys125-X4-His130-X4-Met135**
- Highly conserved **Met region and bis His** (H83 & H95)
- A **His rich** N term domain



Hyperfine spectroscopy



Simulation parameters:

$g = [2.196 \ 2.060 \ 2.016]$

A1 (Cu) = [210 25 149] MHz

A1 (^1H) = 19 MHz ; T1 (^1H) = 2.2 MHz

A2 (^1H) = 8.3 MHz ; T2 (^1H) = 1.3 MHz

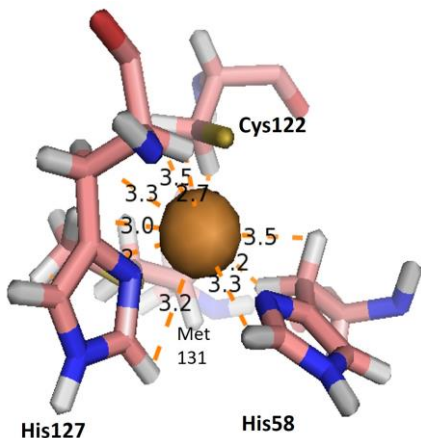
A3 (^1H) = 1.65 MHz ; T3 (^1H) = 2.6 MHz

$$T = \rho_0 g_c g_n \beta_c \beta_n / h r^3 = \rho_0 (b/r^3)$$

$d_{\text{Cu(II)-H}_1} = 3,9 \text{ \AA}$ avec $\rho_0 = 1$

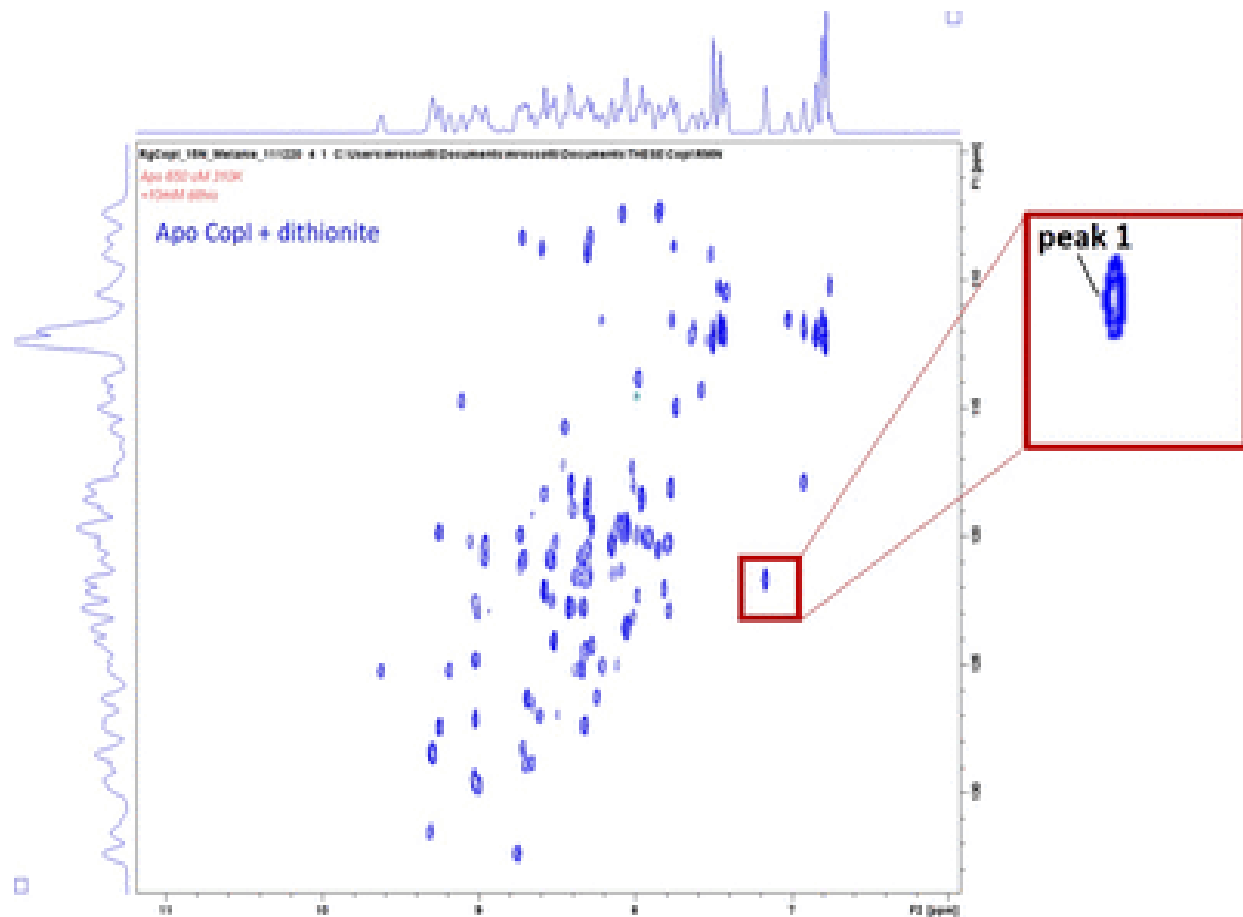
$d_{\text{Cu(II)-H}_2} = 3,3 \text{ \AA}$ avec $\rho_0 = 1$

$d_{\text{Cu(II)-H}_3} = 3,1 \text{ \AA}$ avec $\rho_0 = 1$

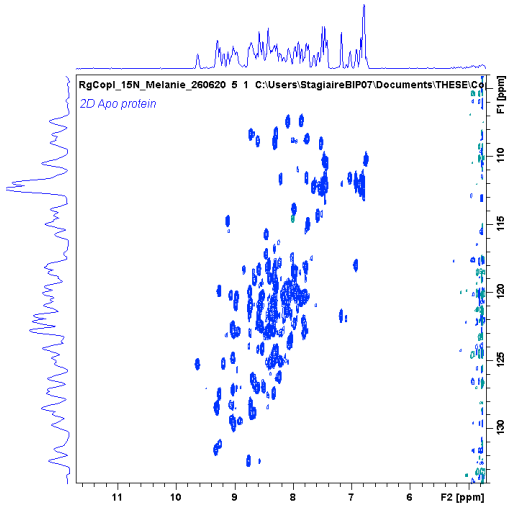


T1.5 binding site
in auracyanin

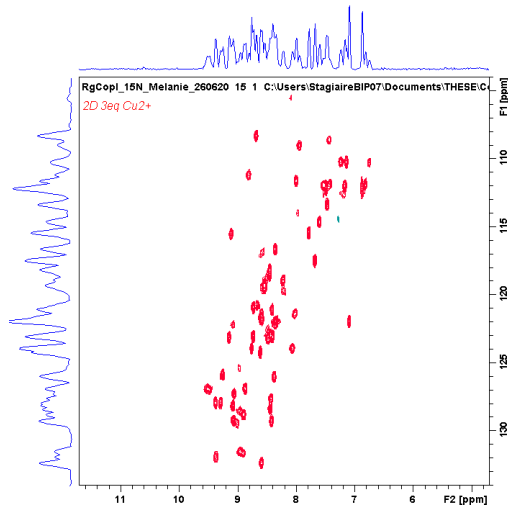
Titration by Cu(I) followed by NMR



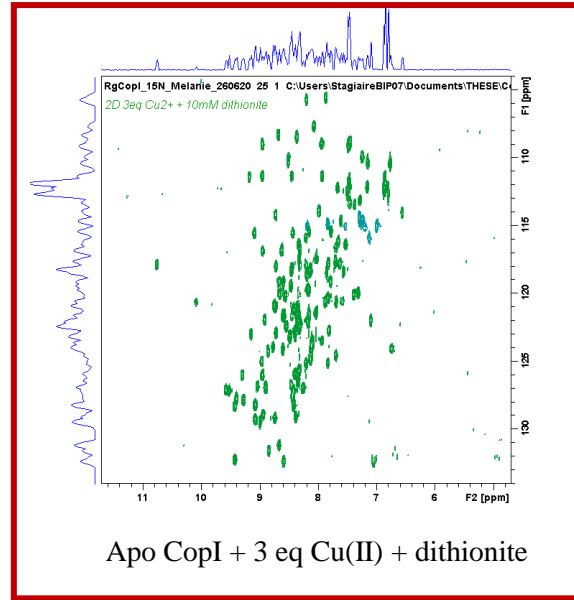
Structure Determination by NMR



Apo CopI



Apo CopI + 3 eq Cu(II)

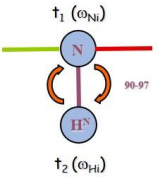


Apo CopI + 3 eq Cu(II) + dithionite

^{15}N - ^1H HSQC experiment

1 correlation peak per H-N bond

1 amino acid = 1 amide bond = 1 peak in the ^{15}N HSQC spectrum



3D NMR experiment

1. Acquisition



2. Assignment



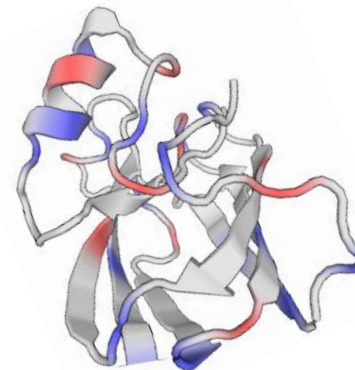
3. Secondary structures
calculation



4. Distance constraints
calculation



Structure determination



Characterisation of a mutant

CopI

His₅

Cu^{T1.5}

His₂/ Met₃

CuT1.5

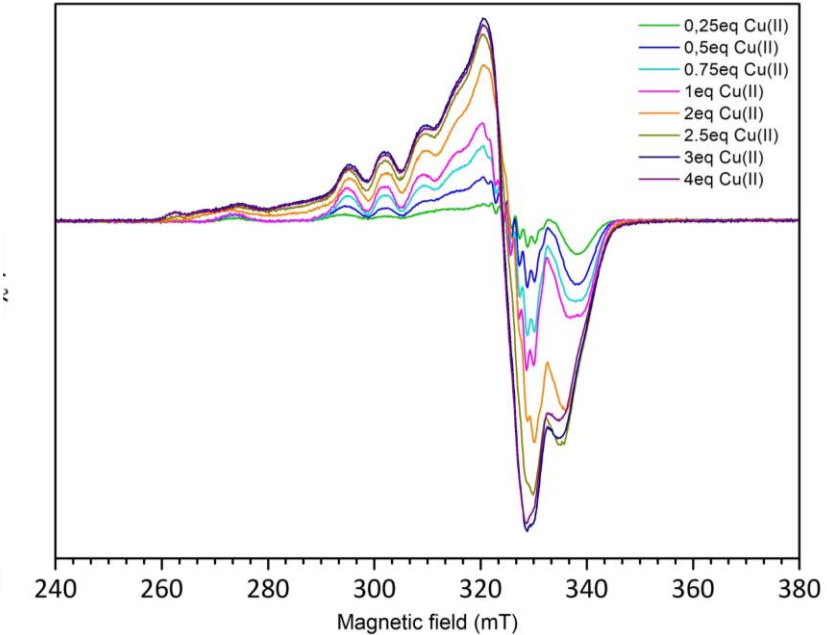
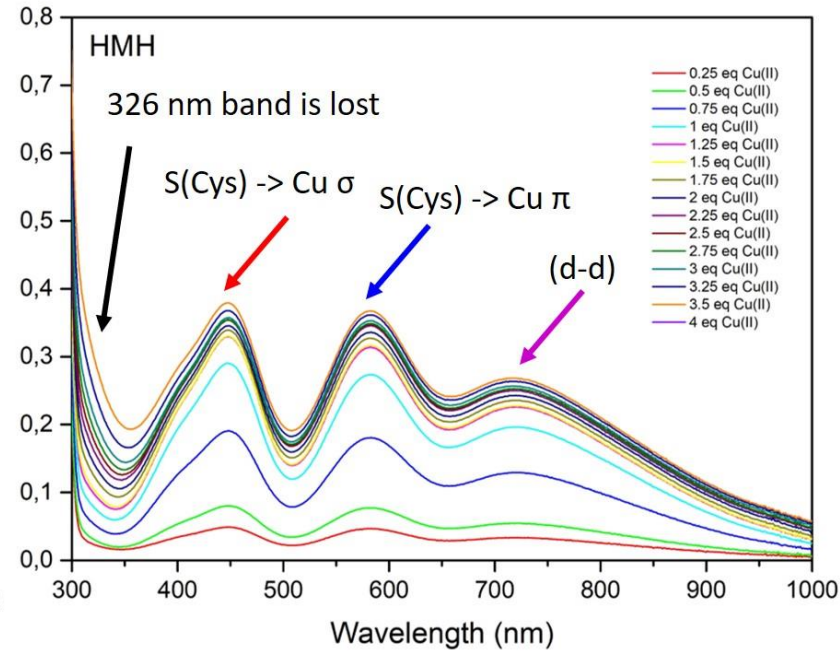
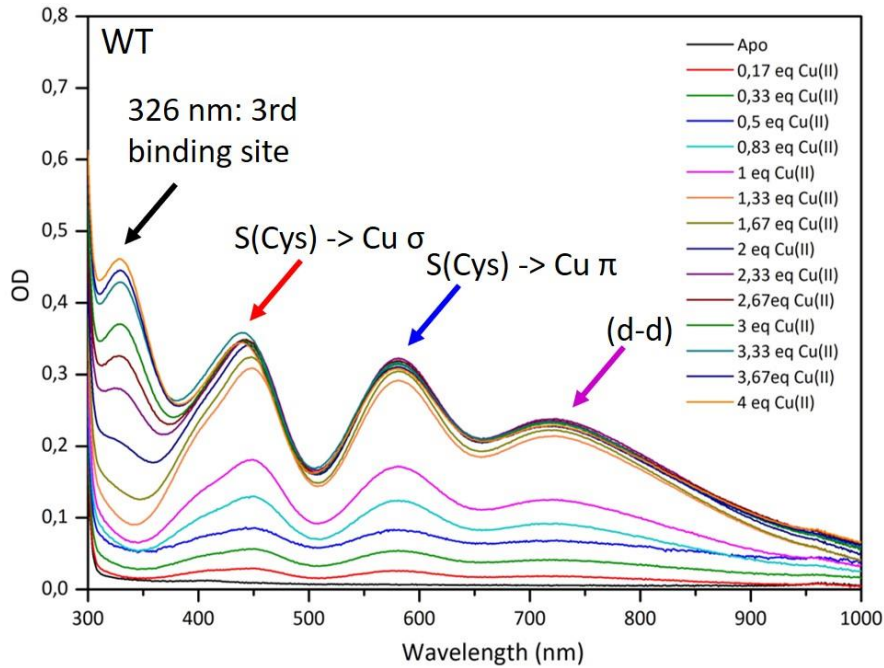
CopI_Met3His2->Ser5

His₅

Cu^{T1.5}

His₂/ Met₃ → Ser₅

CuT1.5



→ A 3rd copper site confirmed with the HMH mutant

→ It affects the other two Cu sites: it is in interaction with other regions of the protein