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## XAFS experiment on metalloprotein

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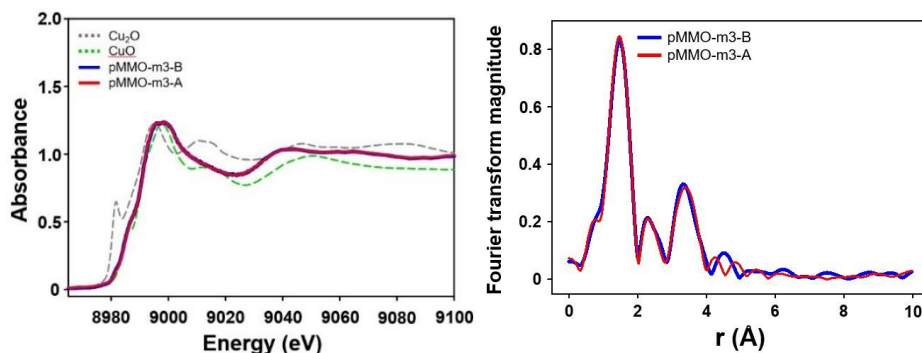
### 1. Introduction

Native MMO is an industrially promising enzyme owing to its significant potential impact on the future of industrial biomanufacturing. Despite potential importance, the low cell productivity and low MMO yield of methanotrophs has been a major obstacle for the researches. To overcome this obstacle, we attempted a molecular design for structural and functional mimicry of pMMO using ferritin scaffold, and eventually succeed in high-yield synthesis of a catalytically active, novel MMO particles in *E. coli*. In this work, we are trying to confirm the nuclearity of Cu ions of the synthesized pMMO.

### 2. Experiment

FZ-3P, FZ-3A were prepared as a freeze-dried powders. These samples were analyzed at the Aichi Synchrotron center in order to confirm the nuclearity of Cu ions in the synthesized pMMOs. The collected XANES and EXAFS data were analyzed by using *Athena* software.

### 3. Results and Discussions



The absorption peaks of XANES spectra of FZ-3P and FZ-3A in Figures for pMMO catalytic reaction before and after are appeared at the same absorption energies  $\sim 8982$  eV (Cu(I),  $1s \rightarrow 4p$  transition) and  $\sim 8990$ - $9000$  eV (Cu(II),  $1s \rightarrow 3d$  transition). These two absorption peaks of XANES spectra confirmed the presences of a Cu(I) and a Cu(II) species in pMMO. The radial distribution results of EXAFS shows three peaks at  $1.47 \text{ \AA}$ ,  $2.29 \text{ \AA}$  and  $3.36 \text{ \AA}$ , which are best fitted to the di-copper model. Therefore, the first, 2<sup>nd</sup>, third shell for the Cu center are to be nitrogen ligands( $1.47 \text{ \AA}$ ), Cu atom ( $2.29 \text{ \AA}$ ), and carbons( $3.36 \text{ \AA}$ ) in sequence. Consequently, the nuclearity of copper ions is di-nuclear.

### 4. References

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