

**MS03-P06****Unreduced enzyme intermediate structure caught by X-ray Free Electron Laser**

Peter Moody<sup>1</sup>, Hanna Kwon<sup>1</sup>, Emma Raven<sup>1</sup>, Takehiko Toshi<sup>2</sup>, Hiroshi Sugimoto<sup>2</sup>, Keitaro Yamashita<sup>3</sup>

1. Leicester Institute for Structural & Chemical Biology, University of Leicester, Leicester, United Kingdom
2. Riken SPring-8 Center, Japan
3. University of Tokyo, Japan

**email:** [peter.moody@le.ac.uk](mailto:peter.moody@le.ac.uk)

The heme peroxidases have high valent ferryl (Fe(IV)) intermediates, these intermediates can be spectroscopically monitored, formed and cryo-trapped in the crystal, and thus the structures determined. However, X-rays are strongly reducing and therefore standard X-ray crystallographic data collection methods are likely to perturb the chemical nature of these intermediates. We are particularly interested in the “Compound II” intermediate, and determining if its identity is Fe(IV)=O or Fe(IV)-OH. These should be distinguishable from the Fe-O distance, but direct or indirect photo reduction into the ferric state would make these measurements invalid. By using fs flashes of X-rays from the free electron laser SACLA at Spring-8 to record diffraction data before photoreduction can take place, we have been able to determine the structure of the unreduced Compound II intermediate of Ascorbate Peroxidase at 1.5Å. The data collection methodology will be presented. The preliminary refinement results will be discussed in the context of our results from neutron crystallography and previous multiple crystal approaches.

**MS04 - Biophysical characterization and crystallization**

**Chairs:** Dr. Andrzej M. Brzozowski,  
Dr. Pavlina Rezacova

**MS04-P01****Magnetic crystallization proof-of-concept: Lysozyme and trypsin case study**

Raquel dos Santos<sup>1</sup>, Iana Lychko<sup>1</sup>, Maria João Romão<sup>1</sup>, A. Cecília A. Roque<sup>1</sup>, Ana Luísa Carvalho<sup>1</sup>

1. Ucbio, Requimte, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

**email:** [rar.santos@campus.fct.unl.pt](mailto:rar.santos@campus.fct.unl.pt)

Iron oxide magnetic particles (MPs) affinity fishing is a proven method for target protein separation from crude extract [1]. Given the virtual infinite surface modifications that can be made on magnetic particles surface, we investigated the MPs influence in protein crystal growth as nucleation points. Functionalized and non-functionalized MPs were used as additives in lysozyme and trypsin crystallization in the presence and absence of an external magnetic field. A rational design for MPs functionalization was achieved, having MPs functionalized with chitin for lysozyme crystallization, and MP functionalized with casein for trypsin. The physico-chemical properties of the MPs were studied by Fourier transform infrared spectroscopy, dynamic light scattering, zeta potential and transmission electron microscopy. The assay was developed to overcome some crystallization drawbacks as crystal growth kinetics, yield and morphology. Improvement of some of these factors were observed, notably in the presence of functionalized MP. The presence of functionalized MP led to a faster crystal growth kinetics, still improving crystal yield and morphology without hampering crystal diffraction. The new magnetic crystallization method enables the possibility to overcome some protein crystallization difficulties, but also, due to the MP functionalization system, has the potential to be integrated in protein purification methods involving crystallization/precipitation steps. For this purpose, a high throughput screen in the presence of MP functionalized with an affinity ligand towards antibodies was designed showing protein crystal growth in different crystallization conditions.

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