MS03-P06

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

Peter Moody[1], Hanna Kwon[1], Emma Raven[1], Takehiko Tosha[2], Hiroshi Sugimoto[2], Keitaro Yamashita[3]

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

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

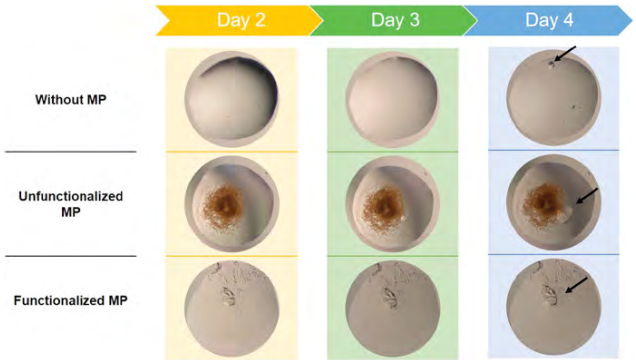
Raquel dos Santos[1], Iana Lychko[1], Maria João Romão[1], A. Cecília A. Roque[1], Ana Luísa Carvalho[1]

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

email: 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.

Acknowledgments: R. dos Santos acknowledges FCT-MCTES for the research fellowship PD/BD/105753/2014 within the scope of the PhD program Molecular Biosciences PD/00133/2012. This work is supported by UCIBIO, financed by FCT/MEC (UID/Multi/04378/2013) and co-financed by the ERDF under the PT2020 Partnership Agreement (POCI-01- 0145-FEDER- 007728).



References:

[1] dos Santos, R. et al. (2016). J. Chromatogr. A. 1457, 50–58.

Keywords: Magnetic particles, Novel crystallization method, protein crystallization

MS04-P02

Synthesis and Characterization of Cross-Linked Lysozyme Crystals filled with Single-Walled Carbon Nanotubes Bionanomaterials

Guillermo Escolano[1-2], Rafael Contreras-Montoya[1], Juan J. Díaz-Mochón[3], Luis Álvarez de Cienfuegos[1], José A. Gavira[4]

1. Departamento de Química Orgánica, Facultad de Ciencias (UGR), Granada, Spain
2. Laboratorio de estudios cristalográficos, Instituto Andaluz de Ciencias de la Tierra (CSIC-UGR), Granada, Spain
3. Departamento de Química Farmacéutica y Orgánica, Facultad de Farmacia, (UGR), Centre for Genomics and Oncological Research, Pfizer/UGR/Andalusian Regional Goverment, PTS Granada, Granada, Spain
4. Laboratorio de estudios cristalográficos, Instituto Andaluz de Ciencias de la Tierra (CSIC-UGR), Granada, Spain

email: gec@ugr.es

Novel bionanomaterials are hybrid materials that include the combination of biomolecules and inorganic substances to generate, enhance or support relevant properties. Bionanomaterials have useful applications in bio- and nanotechnology applications [1,2] .Among the biomolecules used to prepare hybrid materials, proteins have shown to be versatile materials thank to their capacity to self-assembly in crystalline form generating a porous network of nanometer size. The internal cavities of the protein have the ability to act as template[3, 4] and it gives the material the possibility to extrapolate nanoscale properties to macroscopic materials for practical applications.

In this work, we present a new methodology to homogenously incorporate inorganic particles within protein crystals using dipeptide hydrogels as growth media. To exemplify this methodology, we have obtained lysozyme crystals incorporating single wallet carbon nanotubes at different concentration. Crystals were grown in Fmoc-PhePhe-OH hydrogels[5]. The influence of the nanotubes on the diffraction properties, hardness, enzymatic activity and conductivity will be presented and discussed, as well as a full characterization of these new materials.

References:

[1] Ruiz-Hitzky, E.; Darder, M.; Aranda, P.; Ariga, K. Advances in biomimetic and nanostructured biohybrid materials. Adv. Mater. 2010, 22, 323–336.
[2] Lu, Y.; Liu, J. W. Smart nanomaterials inspired by biology: Dynamic assembly of error-free nanomaterials in response to multiple chemical and biological stimuli. Acc. Chem. Res. 2007, 40, 315–323.
[3] M. Liang, L. Wang, R. Su, W. Qi, M. Wang, Y. Yua and Z. He. Synthesis of silver nanoparticles within cross-linked lysozyme crystals as recyclable catalysts for 4-nitrophenol reduction. Catal. Sci. Technol., 2013, 3, 1910-1914.
[4] O. L. Muskens, M. W. England, L. Danos, M. Li, and S. Mann Plasmonic Response of Ag- and Au-Infiltrated Cross-Linked Lysozyme Crystals. Adv. Funct. Mater. 2013, 23, 281–290.
[5] Conejero-Muriel, et al, Chem. Commun. 51, (2015), 3862 & M. Conejero-Muriel, et al., CrystEngComm, 17, (2015), 8072.

Keywords: bionanomaterials, crystallization, carbon nanotubes