FA5-MS04-O1

XAFS Study of Actinides in Natural Minerals Analogues of Ceramics for Nuclear Waste. Messaoud Harfouche[a], François Farges[b]. [a]*Paul Scherrer Institut, Villigen, Switzerland.* [b]*Museum National d'histoire Naturelle, Paris, France.* [c]*Mstandford University, USA.*
E-mail: messaoud.harfouche@psi.ch

Natural actinides (U and Th) are harmful for the crystalline structure of natural minerals, due to their irradiation. Natural minerals can then become amorphous to x-ray diffraction ("metamict") after being irradiated throughout a long period of time (10^8 years). Then, they are used as natural analogues of ceramics for nuclear waste storage. Natural minerals may model long-term radiation effects by receiving very small amounts of radiation during hundreds of million years [1]. These mineral analogues offer a possibility to study the real effect of "in-situ" damage on the structure of ceramics receiving severe amounts of radiation. We have selected, from different localities throughout the world (Sri Lanka, Japan, Norway etc.), various samples that contain significant amounts of natural actinides (Th and U from ~0.1 to ~10 wt.%). These samples were thoroughly characterized using various methods (x-ray diffraction, electron microprobe, etc.).
X-ray Absorption Spectroscopy (XAS) studies were performed in zircon, monazite and titanite to understand the radiation damage effect on the local structure around Th and U. In zircon, a local expansion around actinides substituting for Zr cation is found. The radial expansion is a function of the metamictisation degree: up to ~4Å in crystalline zircon and larger in the metamict counterparts. Extend X-ray Absorption Fine Structure (EXAFS) spectra calculated based on a molecular dynamic simulation of the radiation damage in crystalline zircon [2] confirm this expansion. Moreover, tetravalent actinides were found to be 8-coordinated in the undamaged structure, whereas their coordination drops to 7 in the damaged structures. In contrast to zircon, no local expansion around actinides in monazite was detected, despite some polymerization around P, related to radiation damage, is measured. Finally, in some phases (such as titanite), actinides are found as oxide-type clusters (ThO_2, UO_2). Consequently, actinides do not "systematically" substitute for major actions in these structures, in contrast to the common belief in mineralogy.

[1] Weber et al., *Radiation Waste Mgmt* 2(3), 295-319,**1982**. [2] Crocombette et al., *J. Nucl. Mater.*, 257, 282-286,**1998**.

Keywords: EXAFS; actinides; ceramics

FA5-MS04-O2

Efforts for Structural Biology of Membrane Proteins in a SESAME Member Country. Moazur Rahman[a], Hafeez R. Hoorani[b], Michael J. McPherson[c], Stephen A. Baldwin[c], Samar Hasnain[d]. [a]*National Institute for Biotechnology and Genetic Engineering (NIBGE) Faisalabad Pakistan.* [b]*National Centre for Physics, Quaid-i-Azam University Campus, Islamabad, Pakistan.* [c]*Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, UK.* [d]*School of Biological Sciences, University of Liverpool, Liverpool, UK.*
E-mail: moazur@yahoo.com

Membrane transport proteins are of central importance to living cells and have been implicated in a large number of diseases. High-level expression of membrane proteins typically remains a stumbling bottle-neck for their structural analysis. In this project, over-expression and purification of a number of membrane proteins was achieved using a systematic approach of affinity-tagging which takes into account transmembrane topology. Using a set of bacterial transporters (NupG, ZitB, HI0736, GltP, UhpT, MntH, PitA, PutP and YchM) from nine distinct families with known and differing topologies, the efficacy was tested of a panel of conventional and Gateway™ recombinational cloning vectors. These were designed for protein expression under the control of the *tac* promoter and for the addition of differing N- and C-terminal affinity tags. For transporters in which both termini were cytoplasmic, C-terminal oligohistidine tagging by recombinational cloning typically yielded functional protein at levels equivalent to or greater than those achieved by conventional cloning. In contrast, recombinational cloning was not effective for examples of the substantial minority of membrane proteins that have one or both termini located on the periplasmic side of the membrane, possibly because of impairment of membrane insertion by the tag and/or *att*-site-encoded sequences. However, fusion either of an oligohistidine tag to cytoplasmic (but not periplasmic) termini, or of a *Strep*-tag II peptide to periplasmic termini using conventional cloning vectors did not interfere with membrane insertion, enabling high-level expression of such proteins. In conjunction with use of a C-terminal Lumio™ fluorescence tag, which was found to be compatible with both periplasmic and cytoplasmic locations, these findings offer a system for strategic planning of construct design for high throughput expression of membrane proteins for structural genomics projects. The structural and functional characterization of the *E. coli* glutamate/aspartate transporter will be described as an example. Further, efforts made to establish molecular structural biology research at NIBGE with the help of national and international scientists will be discussed.

Keywords: membrane proteins; over-expression; structure

FA5-MS04-O3

Use of Modified Metallothioneins for Biosensor Application. Filiz Yeşilirmak[a], Zehra Sayers[a]. [a]*Sabanci University, Faculty of Engineering and Natural Sciences, Orhanli, Tuzla, 34956, Istanbul, Turkey.*
E-mail: filizy@sabanciuniv.edu

Metallothioneins (MTs) are small proteins with high

cysteine content and high binding capacity for metals essential (e.g. Zn and Cu) and toxic (e.g. Cd and Hg) metals. MTs consist of two metal binding domains (α and β) that are assembled from cysteine clusters. Cysteine sulfhydryl groups participate in the coordination of heavy metals. Due to their high binding capacity for different metals, MTs are suitable for detoxification, remediation and recycling in applications in agricultural areas. Their potential use for development of metal biosensors for environmental and therapeutic purposes is also recognized. A Type 1 MT from *Triticum durum,* dMT, was expressed in *E. coli* cells as a GST-fusion protein (GSTdMT) [1]. Due to the aggregation propensity, instability in the presence of oxygen and susceptibility to proteolytic degradation applications involving native MTs are impractical. Some of these difficulties were circumvented with the GST fusion partner. In the present study structure of the model system GSTdMT was investigated with view of biosensor applications. GSTdMT was purified with Cd as a dimer in monodisperse solutions [2]. Structure of GSTdMT was investigated by small angle X-ray scattering (SAXS), circular dicroism (CD) and UV-vis spectrophotometric measurements. Inductively coupled plasma optical emission spectroscopy (ICP-OES) and EXAFS measurements showed that GSTdMT binds about 4 Cd^{2+}/protein in a tetrahedral arrangement. SAXS measurements revealed that GSTdMT has an elongated shape with a radius of gyration of 3.57 nm. dMT structure appears to be independent of GST in the GSTdMT fusion. In the investigations for biosensor activity the fusion protein (apo- and holo-foms) was immobilized onto epoxy- and thiol-modified surfaces. Immobilization was verified and attempts for quantification of the bound protein were carried out by GST antibody labeling. Results on detection and quantification of Cd-binding to the apo- and holo-protein will be presented.

[1] K. Bilecen, Ü.H. Öztürk, A.D. Duru, T. Sütlü, M. Petoukhov, D.I. Svergun, M.H.J. Koch, U. Sezerman, I. Cakmak and Z. Sayers, *The Journal of Biological Chemistry*, vol. 280, no. 14, pp. 13701-13711, **2005**. [2] F. Dede, G. Dinler, and Z. Sayers, Proc. of the NATO Advanced Research Workshop, Published by Springer Verlag, Heidelberg, pg 135-146, **2006**.

Keywords: metal-binding proteins; SAXS; biosensors

FA5-MS04-O4

Aspects of Crystallinity of High Grad Quartz Ores Using X-Ray Diffraction and Infrared Spectroscopy Diagnostic Their Chemical Reactivity. Mervat S. Hassan[a], Tafuk R. Boulos[a], Alia Adam[b]. [a]*Central Metallurgical R & D Institute, Egypt.* [b]*Physics Department, Faculty of science, Al-Azhar University, Girls Branch, Egypt.*
E-mail: mervathassan@hotmail.com

This work aimed to find relationship between structural imperfection, physical properties and chemical reactivity of two Egyptian quartz ores employed in local production of sodium silicate. Quartz (A) is known to be the highly reactive sample in sodium silicate production than quartz (B). The powder diffraction data of the two quartz samples were refined on the basis of Rietveld method using the Fullprof program. Peak profile analysis of the single and multiple lines were performed applying the winFit program based on the Fourier methods of Waren-Averbach to calculate the crystal size and strain in the two samples. Infrared spectroscopy has been used to study the structure of quartz. Investigation is based on the assignment of infrared bands to certain structural groups of SiO_4 tetrahedra. Systematic investigations of structure have been carried out in between 1000 cm^{-1} and 500 cm^{-1} bands of silicates The crystallinity of samples has been ascertained by comparing the ratio of intensity of the characteristic peak at 778 and 695 cm^{-1}. For analytical band wavenumber close to 797cm^{-1} it was observed that the absorbance decreased as particle size increased. The opposite effect was noted for analytical bands wavenumber 693 and 506-513 cm^{-1}. For strongest diffraction lines of the two quartz samples, it was observed that the intensity increased with the diameter of the particles.

Keywords: quartz; crystallinity; structure imperfections

FA5-MS04-O5

Protein Crystallography: Counter Diffusion Crystallization Method and Its Potential for Room-Temperature Data Collection. Mehmet Aslantas[a], Engin Kendi[b], Vivian Stojanoff[c], Tuba Büyükdemirkıran[a]. [a]*K.S.U., Department of Physics, Kahramanmaras, Turkey.* [b]*Hacettepe University, Department of Physics Engineering, Ankara, Turkey.* [c]*BNL, NSLS, 11973 Upton, NY, USA.*
E-mail: aslantas@ksu.edu.tr

High-resolution cryogenic synchrotron X-ray data collection is valuable to protein crystallography compare to room temperature data collection leading to serious radiation damage to the crystals, cryo-induced structural changes and freezing problems. However counter-diffusion method for crystallization can be applied to a wide range of molecules and complexes, and might be very useful for SESAME synchrotron users in Middle East. In this presentation, the potential benefits of Counter Diffusion technique, data collection at the optimum wavelength of lysozyme derivative crystals at room temperature, data quality and structure refinement results will be discussed.

Keywords: macromolecular synchrotron X-ray crystallography; crystallization; room-temperature data collection