

FA5-MS04-O1**XAFS Study of Actinides in Natural Minerals Analogues of Ceramics for Nuclear Waste.**

Messaoud Harfouche^a, François Farges^b. ^aPaul Scherrer Institut, Villigen, Switzerland. ^bMuseum National d'histoire Naturelle, Paris, France.

^cStanford 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. Extended 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 (ThO₂, UO₂). 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. ^aNational Institute for Biotechnology and Genetic Engineering (NIBGE) Faisalabad Pakistan. ^bNational Centre

for Physics, Quaid-i-Azam University Campus, Islamabad, Pakistan. ^cAstbury Centre for Structural Molecular Biology, University of Leeds, Leeds, UK. ^dSchool 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. ^aSabancı University, Faculty of Engineering and Natural Sciences, Orhanlı, Tuzla, 34956, Istanbul, Turkey.

E-mail: filizy@sabanciuniv.edu

Metallothioneins (MTs) are small proteins with high