Poster Number

42 **The zebrafish scale as a possible model for studying bone / plasma Ca^{2+} exchange.**

Jacky T.S. Hung[1], Alessandro Rubinacci[2], Joseph Kunkel[3], Alan Shipley[4], Sarah E. Webb[1] and Andrew L. Miller[1,5]

[1]Division of Life Science and State Key Laboratory of Molecular Neuroscience, HKUST, Clear Water Bay, Hong Kong, PRC.; [2]Bone Metabolism Unit, San Raffaele Hospital, Milan, Italy; [3]University of New England, ME, USA; [4]Applicable Electronics Inc., New Haven, CT, USA and [5]Marine Biological Laboratory, Woods Hole, MA 02543, USA.

In teleosts such as zebrafish, the elasmoid scales are known to be important reservoirs of Ca^{2+} via the presence of a significant amount of hydroxyapatite, (which acts as the primary store of Ca^{2+} in both bones and scales) although the extent of their contribution to either short or long-term regulation of Ca^{2+} homeostasis, when compared with the bony skeleton, is not yet clear. The scleroblasts that ensheath the scales are proposed to be responsible for controlling Ca^{2+} mineralization (influx) and mobilization (efflux). Indeed, it has been suggested that the scales may play a more significant role in Ca^{2+} homeostasis in fish, than the axial skeleton. In this project, the scanning ion-selective electrode technique (SIET) was employed to measure Ca^{2+} fluxes in zebrafish scale samples at different extracellular [Ca^{2+}]. The SIET system makes use of a self-referenced vibrating microelectrode, thus, it is able to non-invasively measure ion fluxes at the pmole/cm^2/sec range from samples in real-time and with a spatial resolution of ~5 µm. On the episquamal side of scales exposed to a hypercalcemic (3 mM) or hypocalcemic (0.01 mM) bathing medium, a steady Ca^{2+} influx and efflux were measured, respectively. On the other hand, on the hyposquamal side of scales, a steady Ca^{2+} efflux was measured at all concentrations of Ca^{2+} in the bathing medium. These preliminary data suggest that the zebrafish scale might be a useful model for studying mammalian (and ultimately human) Ca^{2+} exchange and plasma homeostasis.

43 **Inorganic Polyphosphate as a Forgotten Molecule in Osteoblasts - From Synthesis to Function**

Khong Mei Li (HKU)
(Supervisor: Dr. Julian A Tanner, HKU)

Mineral and organic constituents crucial for cellular growth, development and survival are generally conspicuous with the exception of inorganic polyphosphate (polyP). PolyP, a linear polymer of many phosphate residues linked by energy-rich phosphoanhydride bonds, remained a largely 'forgotten' molecule albeit being ubiquitous and significantly crucial to the survival of living organisms. In the last two decades, major clues about the role polyP have in a plethora of prokaryotic biological processes unravelled with the introduction of novel enzyme-based assays. However, the origin and precise machinery of polyP in humans remain largely elusive. This research aims to discover the mechanisms of polyP as a fundamental molecule in osteoblasts, from its synthesis to function. The classic Clark's polyP extraction method was modified to successfully extract polyPs of different chain lengths from osteoblast-like SaOS-2 cells, for study of differential physiological effects exerted by varying polyP chain lengths. Unexpectedly, addition of standard-length polyphosphates into SaOS-2 cell extracts yielded a pull-down of polyP-interacting proteins which were subsequently identified using peptide mass fingerprinting and MS/MS sequencing. As a secondary line of evidence, future work is underway towards identification of polyP-interacting proteins by the development of a polyP-specific affinity column. This method will incorporate a facile approach of chemically cross-linking the terminal phosphate group of polyP with a primary amine-linked biotin, via phosphoramidate linkage, for attachment to streptavidin-coated beads. This work will not only present a comprehensive overview of the multi-functional roles polyP has in osteoblasts but also provide a broader perspective of polyP's functions in humans.