

## W0122

**$\delta$ -Crystallin: A Model to Study the Enzymatic Mechanism of Argininosuccinate Lyase.** M. Tsai<sup>1,2</sup>, L. Sampaleanu<sup>1</sup>, L. Creagh<sup>3</sup>, C. Haynes<sup>3</sup>, P.L. Howell<sup>1,2</sup> <sup>1</sup>Hospital for Sick Children, Toronto; <sup>2</sup>Univ. of Toronto, Toronto; <sup>3</sup>Univ. of British Columbia, Vancouver, Canada.

$\delta$ -Crystallin is directly related to argininosuccinate lyase (ASL). In duck lenses, two  $\delta$ -crystallin isoforms exist,  $\delta 2$  and  $\delta 1$ , which are 94% identical.  $\delta 2$  is the duck orthologue of ASL, while  $\delta 1$  is enzymatically inactive. Chimeras of the two isoforms have shown that domain 1 of  $\delta 2$  is sufficient to recover activity in  $\delta 1$ . Structural comparisons of wild-type and mutant duck  $\delta 1$  and  $\delta 2$  crystallins reveal that conformational differences between the isoforms are localized to residues 23-32 (20's loop) and 74-89 (70's loop). As all the residues involved in catalysis are conserved in  $\delta 1$ , the amino-acid substitutions in these loops are hypothesized to prevent substrate binding in  $\delta 1$ . Contrary to expectations a  $\delta 1$  double loop mutant (DLM), where all residues in the 20's and 70's loops are replaced with those of  $\delta 2$ , was found to be inactive and binding of the substrate to the DLM could not be detected by ITC. To further investigate this result, crystal structures of the DLM with and without sulfate bound have been determined to 2.2Å and 2.5Å resolution, respectively. Structural comparisons and mutagenesis of additional residues in domain 1 provide further insight into the requirements for recovery of catalytic activity in  $\delta 1$ .