

## **Structural and Functional Studies of the 67kDa form of Human Glutamic Acid Decarboxylase (GAD67)**

**C. G. Langendorf<sup>1</sup>, R. H.P. Law<sup>1</sup>, G. Fenalti<sup>1</sup>, K. Tuck<sup>2</sup>, C. J. Rosado<sup>1</sup>, N. G. Faux<sup>1</sup>, K. Mahmood<sup>1</sup>, M. Rowley<sup>1</sup>, A. M. Buckle<sup>1</sup>, J. C. Whisstock<sup>1</sup>**

*<sup>1</sup>Department of Biochemistry and Molecular Biology, Monash University, Melbourne, VIC, Australia*

*<sup>2</sup>School of Chemistry, Monash University, Melbourne, VIC, Australia*

In humans, the 67kDa form of the PLP dependent enzyme GAD67 is responsible for basal production of the inhibitory neurotransmitter GABA. The essential nature of GAD67 is highlighted by the observation that murine knockouts of GAD67 die of cleft palate at birth, and display markedly reduced GABA levels. Further, human mutations of GAD67 have been shown to result in spastic cerebral palsy. In order to understand the structural basis for GABA production by GAD67 we embarked on a program of structural studies. An N-terminally truncated, enzymatically active form of GAD67 was produced in yeast and subjected to crystallisation trials. Initial crystals were obtained and a 2.3Å dataset (P21, a = 84.05 Å, b = 62.74 Å, c = 101.35 Å,  $\beta$  = 106.7 °, consistent with two molecules per asymmetric unit) collected at the Advanced Photon Source (IMCA-CAT beamline, Chicago). Despite limited (17%) sequence identity, the structure was determined by molecular replacement, using the structure of DOPA-Decarboxylase as an MR probe and the PHASER algorithm. The crystal structure of GAD67 reveals a tethered catalytic loop covers the active site and brings a catalytic tyrosine residue into close proximity to the co-factor PLP. Furthermore, we obtained a product (GABA) complex of GAD67 that revealed how the catalytic tyrosine residue functions in protonation of a GABA-PLP intermediate and the subsequent release of GABA.