



# International Cancer Study & Therapy Conference

April 4-6, 2016 Baltimore, USA

## ***In meso* crystallization characterization of butyrate receptors, GPR41 and GPR43 and new developments in X-ray Free Electron Laser sources to further structure-based drug design**

Connie Darmanin<sup>1,2</sup>, Yi-Lynn Liang<sup>3</sup>, Charlotte E. Conn<sup>4,5</sup>, Calum J Drummond<sup>5</sup>, Brian Abbey<sup>1</sup> and Keith Nugent<sup>1</sup>

<sup>1</sup>ARC Centre of Excellence in Advanced Molecular Imaging, La Trobe University, Australia

<sup>2</sup>CSIRO Materials Science and Engineering (CMSE), Australia

<sup>3</sup>Monash University, Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Australia

<sup>4</sup>CSIRO Materials Science and Engineering (CMSE), Australia

<sup>5</sup>RMIT, School of Applied Sciences, College of Science, Engineering and Health, Australia

G protein-coupled receptors (GPCRs) are key pharmaceutical targets in a number of biological diseases and until the recent introduction of Lipidic Cubic Phase (LCP) crystallization, otherwise known as *in meso* crystallization, these protein structure have been extremely difficult to solve. LCP crystallization has been successful in solving the structures of a number of GPCRs. However, understanding the process of LCP crystallization is limited. The butyrate G-protein coupled receptors (GPCRs), GPR41 and GPR43, have been implicated in colorectal cancer. To date their function has not been elucidated as low levels of protein expression and difficulties in producing diffraction quality crystals have hindered their structural determination. *In meso* crystallization, which uses an artificial lipid membrane matrix to facilitate crystal growth, is becoming an increasingly successful crystallization technique, particularly for GPCRs. We report herein the lipid membrane matrix structural characterization for GPR41 and GPR43 within two lipid self-assembly systems (monoolein and phytantriol) commonly used for *in meso* crystallization and comment on their suitability for crystallizing these GPCRs. Synchrotron small angle X-ray scattering (SAXS) studies were used to determine the initial phase and uptake of these receptors within the lipid matrix and investigate the role of cholesterol in this process. The self-assembled lipid nanostructure was retained in the presence of GPR43 for both lipids but was destabilized for GPR41 in the phytantriol lipid system. The structural changes to the lipid matrix upon protein incorporation were greater for cholesterol-doped systems, potentially indicative of increased receptor uptake.

Elucidating GPCRs structures using conventional crystallography can be challenging due to the size of the crystals grown during *in meso* crystallization. An alternative and new method of development is serial femtosecond crystallography at an X-ray Free Electron Laser (XFEL) source. These sources are able to generate data from nano-crystals enabling structure determination to be carried out on such finite crystal sizes. This has led the way for new nano-crystallography developments.

### **Biography:**

Dr. Darmanin completed her PhD at Monash University, Melbourne in 2006 where she focused on protein crystallography solving ultra high resolution structure of Aldose Reductase. After completion of her PhD she moved to CSIRO, Melbourne in 2007 and during her post doctorate she setup a G protein coupled receptor laboratory and gained expertise in Synchrotron Science and Electron Crystallography specializing in developing new methods for solving structures of GPCRs and *in meso* crystallization. She continued on at CSIRO after her Post Doc as project leader and continued to grow the area of *in meso* crystallization of GPCRs in Australia developing protocols and trying to understand the process of *in meso* crystallization to help solve GPCR structures. Recently, 2015 she has moved to La Trobe University, Melbourne to continue her work in *in meso* crystallization and led the X-ray Free Electron Laser group providing expertise in this new exciting field for structure determination of difficult proteins.