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Signal transduction defects in human diseases

Signal transduction is a complex web of activation and deactivation events mediated by molecular switches, such as phosphorylation and conformational change. Several NHMRC-funded projects in my laboratory are focused on developing an understanding of how defects in signal transduction proteins, such as the Src-family kinase contribute to human diseases including cancer, HIV, asthma and rheumatoid arthritis.

Mitochondrial import defects and metabolic disorders

Complex organisms are built from cells containing various organelles. Cargos are trafficked in and out of organelles using a range of intricate molecular machines. Our ARC-funded work on the protein import machinery of mitochondria is helping decipher the molecular basis of this fundamental aspect of cell biology. Furthermore, it has been suggested that subtle defects in mitochondrial protein import pathways contribute to numerous metabolic disorders.

Skills and techniques: protein biochemistry and structural biology

Honours projects in my lab will give you a taste of a variety of molecular and cellular biology techniques including: recombinant DNA manipulation, protein expression in bacterial and mammalian cell culture, protein purification, and the measurement of protein function using a variety of assays.

The relationship between protein structure and biological activity is very important to our work, so projects often include bioinformatics and use of biophysical methods, such as circular dichroism (CD), fluorescence spectroscopy and microscopy, mass spectrometry (MS), small angle X-ray and neutron scattering (SAXS and SANS), X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy.

We have excellent biophysical facilities based *in-house* at the Bio21 Institute (NMR, fluorescence, CD, MS and SPR) and also make extensive use of facilities at the Australian Synchrotron (X-ray diffraction and SAXS) and OPAL research reactor (SANS).

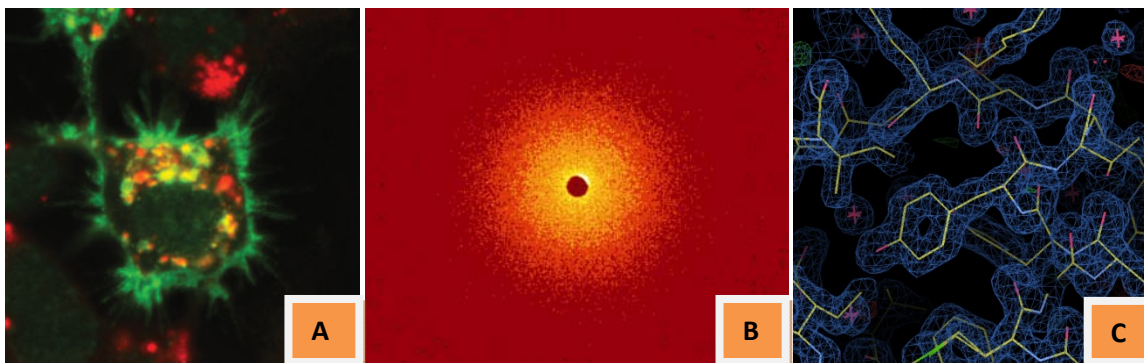


Fig. 1. Techniques used in the Mulhern lab. (A) Confocal microscopy of fluorescently tagged Src-family kinases in cells. (B) Small-angle X-ray scattering (SAXS) data from a mitochondrial import receptor. (C) X-ray crystal structure of kinase regulatory domain

Projects:

Regulation of immune system Src-family kinases

Several Src-family kinases (SFKs) have been implicated in diseases of the immune system, such as asthma, rheumatoid arthritis and lupus. We are interested in the structural, catalytic and regulatory properties of the immune system SFKs (Hck, Lyn, Lck, Blk and Fyn). We are investigating how these SFKs recognize their protein substrates and how their various functional domains interact with activators and inhibitors in macrophages, mast cells, B cells and T cells (1, 2).

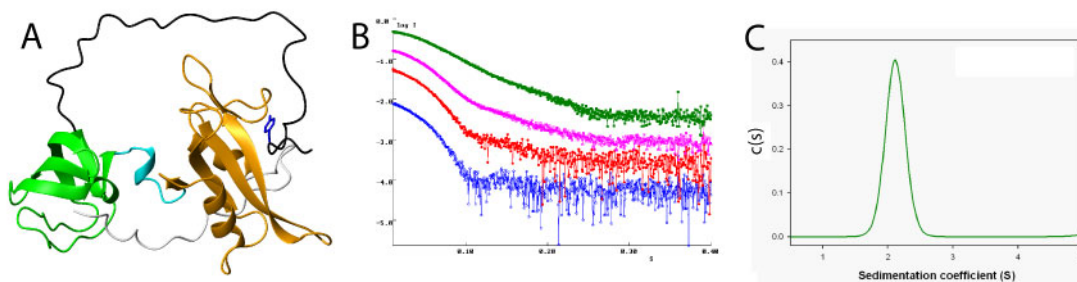


Fig. 2. Structural and functional studies of the hematopoietic cell kinase (Hck). (A) Molecular model of the Hck regulatory region. (B) Synchrotron SAXS data from complexes of the Hck regulatory region with viral peptides. (C) Analytical ultracentrifugation (AUC) analysis of the Hck regulatory region.

Crossing the “Great Divide”: Mitochondrial protein import machinery

The mitochondrial ADP/ATP carrier protein (AAC) is essential for cellular energy production; and defects in AAC function are linked to diseases including progressive external ophthalmoplegia, mitochondrial myopathy and cardiomyopathy. It is possible that up to 25% of patients suffering from apparent mitochondrial respiratory chain disorders actually have defects in AAC function. We are studying the interaction of AAC with Tom70, a receptor of the mitochondrial TOM complex, and the small Tim complex from the intermembrane space. These proteins evolved specifically to enhance import of the AAC and other inner membrane metabolite transporters (3, 4).

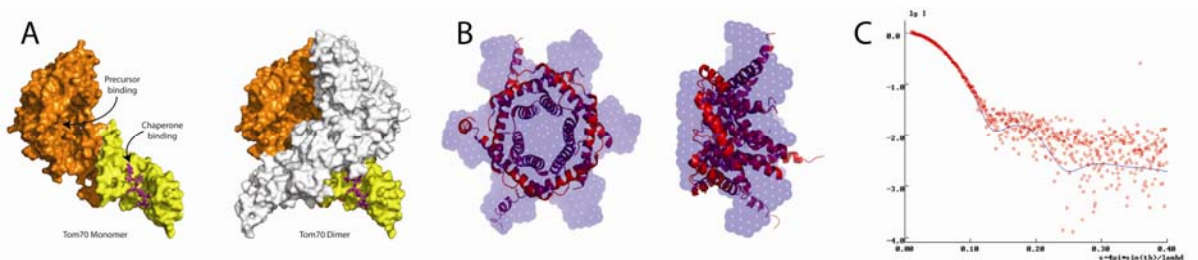


Fig. 3. Small-angle X-ray scattering (SAXS) analyses of mitochondrial import machinery. (A) SAXS models of Tom70. (B) Overlay of SAXS and crystallographic models of the small Tim complex. (C) Synchrotron SAXS data from the small Tim complex.

Recent publications

1. Kristelly R, Qiu TW, Gunn NJ, Scanlon DB & Mulhern TD (2011) “Bacterial expression and purification of active hematopoietic cell kinase” *Protein Expr Purif* 78, 14–21.
2. Gunn NJ, Gorman MA, Dobson RCJ, Parker MW & Mulhern TD (2011) Purification, crystallisation, small-angle X-ray scattering and preliminary X-ray diffraction analysis of the SH2 domain of Csk-homologous kinase” *Acta Cryst F* 67, 336–339.
3. Rimmer KA, Foo JH, Ng A, Petrie EJ, Shilling PJ, Perry AJ, Mertens HDT, Lithgow T, Mulhern TD & Gooley PR. (2011) “Recognition of mitochondrial targeting sequences by the import receptors Tom20 and Tom22” *J Mol Biol* 405, 804–818.
4. Mills RD, Trehwella J, Qiu TW, Welte T, Ryan TM, Hanley T, Knott RB, Lithgow T & Mulhern TD. (2009) “Domain organization of the monomeric form of the Tom70 mitochondrial import receptor. *J Mol Biol* 338, 1043–1058.