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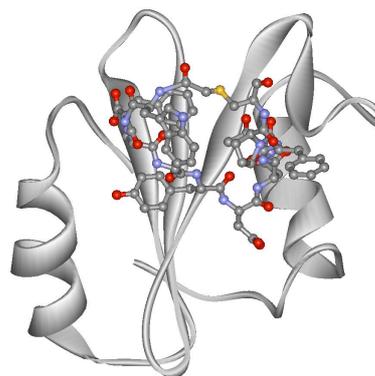


Research interests: the basis of molecular recognition. I am investigating several protein-peptide and protein-oligonucleotide interactions using structural, biophysical and computational tools to understand their bases of recognition. These include x-ray crystallography and NMR spectroscopy to obtain structural information, surface plasmon resonance, isothermal titration calorimetry and analytical ultracentrifugation to determine the affinity of interaction and self association, and molecular dynamics simulations to explore the predicted dynamics of the system.

Structure and peptide binding of the Grb7-SH2 Domain
- in collaboration with A/Prof Matthew Wilce (Monash University) and Prof Peter Leedman (UWA) and PhD student Corrine Porter.

Growth factor receptor bound protein-7 (Grb7) is a member of a family of SH2 domain containing adaptor proteins. SH2 domains are present in a diverse group of proteins which are implicated in tyrosine kinase signalling. The SH2 domain of Grb7 binds to phosphorylated tyrosine residues located in the cytoplasmic domain of several growth factor receptors including the epidermal growth factor receptor-2, erbB2. Grb7 is over-expressed with erbB2 in a subset of human breast cancer cell lines and breast tumours, suggesting erbB2 signaling via Grb7 may be increased in these cancers. As it has been shown that breast tumours that over-express the erbB2 receptor are generally estrogen-receptor negative and have a poor prognosis, the Grb7-erbB2 complex provides an attractive target for the development of novel therapeutics in the treatment of breast cancer.

We have determined the crystal structure of the Grb7-SH2 domain to 2.1 Å resolution. This structure provides insight into the specificity of the Grb7-SH2 domain for binding phosphotyrosines contained within the pYXN sequence motif. We have also synthesized a cyclic, non-phosphorylated peptide reported to have binding specificity for the Grb7-SH2 domain.



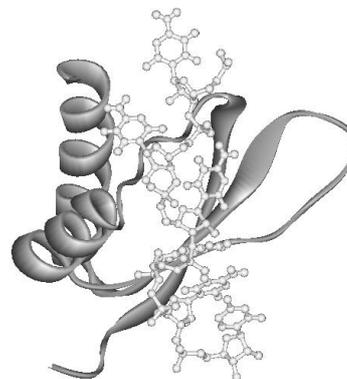
We are currently examining the interaction between the peptide and the Grb7-SH2 domain using biophysical techniques. We hope this will reveal how tightly the peptide binds and why it is specific for Grb7 and not, for example, the similar Grb14 molecule. It is anticipated these studies will serve as a starting point in the design of therapeutics to target the erbB2-Grb7 interaction.

Structure and RNA binding of poly(C)-binding protein

- in collaboration with A/Prof Matthew Wilce (Monash University) and Prof Peter Leedman (UWA), Dr Andrew Barker and PhD student Mahjooba Sidiqi.

α CP1 is a member of the poly(C)-binding protein family of proteins which include α CP1, α CP2, α CP3, α CP4 (also known as hnRNP E or PCBP) and the earliest member to be characterized, the heterologous ribonucleoprotein K, hnRNP K. These proteins contain a triplet K homology (KH) RNA-binding motif, as first identified in hnRNP K, which confer specificity for single-stranded poly(C) tracts of both RNA and DNA. Upon binding to RNA, they are involved in a diverse range of functions affecting post-transcriptional regulation of specific genes. These include the shuttling of mRNA between the nucleus and the cytoplasm, the stabilization of specific mRNAs, translational silencing and translational enhancement. Our interest follows on from the finding that androgen receptor mRNA is bound by α CP1 in a specific region of its 3'UTR which affects translation.

We have shown that the third KH domain of α CP1 binds pyrimidine-rich RNA using surface plasmon resonance (using BIACORE) and RNA electrophoretic mobility-shift assays (REMSA). In addition we have solved its structure to 2.1 Å resolution using x-ray crystallographic techniques. This has allowed us to model the interaction between this domain and poly(C)-RNA using NAMD molecular dynamics simulation software (<http://www.ks.uiuc.edu/Research/namd/>).



Future work will involve the pursuit of the structure of the protein:RNA complex and other KH domains using NMR spectroscopy and x-ray crystallography. We also will measure how tightly the complexes form with RNA in the presence of other proteins which also bind mRNA. The structural and affinity measurements will help us understand the role α CP1 plays in affecting the translation of mRNA.

Selected Publications

J.P.Vivian, J.A.Wilce, A.F. Hastings and M.C.J. Wilce (2001) Crystallization of the B. subtilis RTP/DNA complex Acta Crystallographica D57 421-424.

J. A. Wilce, J. P. Vivian, A.F. Hastings, G. Otting, R. Folmer, I.G. Duggin, R.G. Wake and M.C.J Wilce, (2001) Structure of the RTP/DNA complex and the mechanism of polar replication fork arrest. Nature Structural Biology 8, 206-210.

J.Wilce, S.Love, S. Richardson, P. Alewood and D.Craik (2001) Synthesis of an analog of the thyroid-hormone-binding protein transthyretin via regioselective chemical ligation. J. Biol. Chem 276, 25997-26003.

B.B. Yeap, D. Voon, J.Vivian, R. McCulloch, A.M. Thomson, M.F. Czyzyk-Krzeska, H. Furneaux, M.C.J Wilce, J.A.Wilce and P.J. Leedman (2002). Novel Binding of

HuR and Poly (C) Binding Protein to a Conserved UC-rich Motif within the 3' Untranslated Region of the Androgen Receptor messenger RNA. *J. Biol. Chem.* 277, 27183-27192

J.A. Wilce, Peter J Leedman and Matthew CJ Wilce (2002) RNA-binding proteins that target the androgen receptor mRNA *IUBMB Life* 54, 345-349.

J.P. Vivian, A.F. Hastings, I.G. Duggin, R.G. Wake, M.C.J. Wilce and J.A. Wilce, (2003) The impact of single cysteine residue mutations on the replication terminator protein. *Biochem. Biophys. Res. Comm.* 310, 1096-1103.

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M. Sidiqi, J.A. Wilce, J.P. Vivian, C.J. Porter, P.J. Leedman and M.C.J. Wilce (2005) Structure and RNA binding of the third domain of poly(C)-binding protein. (accepted - *Nucleic Acids Research*).

M. Sidiqi, J.A. Wilce, C.J., Porter, A. Barker, P.J. Leedman and M.C.J. Wilce (2005) Formation of CP1-KH3 complexed with UC-rich RNA (accepted- *European Biophysics Journal*)

Porter CJ, Wilce MCJ, MacKay JP, Leedman PJ, Wilce JA (2005) Grb7-SH2 domain dimerisation is affected by a single point mutation. (accepted - *European Biophysics Journal*).