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Protein structure and function

My research interests are diverse, but mostly involve the structure and function of proteins (1-5). The aim of all projects is to find, by the study of molecular interactions, novel ligands that will serve as templates for drug design, or explain an important biological process. In our laboratory you will gain skills in basic molecular biology, protein expression and isotope labeling using bacterial and cell-free systems, protein purification, site-directed mutagenesis and functional assays. To understand structure function relationships we use bioinformatics and an array of biophysical methods which include NMR spectroscopy, fluorescence, circular dichroism, X-ray crystallography, mass spectrometry, Surface Plasmon Resonance, and small-angle X-ray scattering. We have a strong interest in how protein motion affects function, and aspects of our projects include the analysis of protein motion (5).

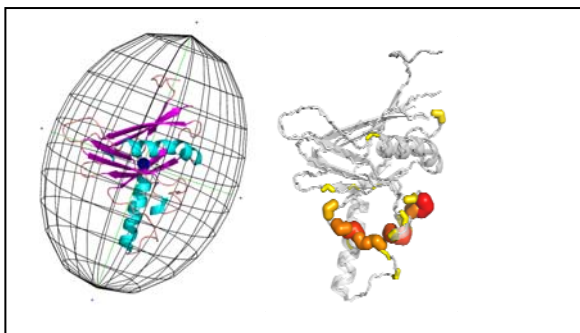


Fig. 1. Analysis of the dynamics of proteins. Protein dynamics is critical for all aspects of protein structure and function. To speed up analysis we have coded and developed novel software (5).

New drugs for diabetes: targeting carbohydrate binding of AMP-activated protein kinase

AMP activated protein kinase (AMPK) is a central metabolic enzyme, and a drug target for the treatment of diabetes. The enzyme consists of three subunits that can exist as multiple isoforms: the catalytic α , and the regulatory β and γ subunits. The β -subunit contains a carbohydrate-binding module that binds glycogen, thus regulating the activity of AMPK by sequestration and localization. Preventing the binding of glycogen might therefore offer new avenues for the treatment of diabetes. We have made two key discoveries: the muscle-specific β 2-isoform binds glycogen and small oligosaccharides with up to 16-fold greater affinity than the ubiquitous β 1-isoform (1); and the differences in affinity can be explained by differences in protein dynamics (unpublished). We are taking a novel approach to finding new drug leads by looking for small non-carbohydrate molecules that can disrupt the flexibility of the β 2-isoform and, in doing so, prevent carbohydrate binding. We will also explore molecules that directly inhibit carbohydrate binding.

Collaborators: Dr David Stapleton (Dept. of Physiology) and Prof Michael Parker (St Vincent's Medical Research Institute).

Molecular interface of hormone binding and activation of relaxin G-protein-coupled receptors

The hormone relaxin and its homologues INSL3 and Relaxin-3 have essential biological roles in pregnancy and parturition, and the renal, cardiovascular and central nervous systems. The receptors for these peptides are all G-protein coupled receptors (GPCRs), the largest class of cell surface signaling molecules

and major drug targets. Consequently, the relaxin family of hormones and their small molecule mimics are important drug leads especially for heart failure, dementia and treatment of addiction and stress. Indeed, relaxin is currently being used in a Phase III clinical trial for acute heart failure. GPCRs contain a seven-transmembrane (7TM) helical domain. In addition the receptors for relaxin and INSL3 contain a leucine rich-repeat (LRR) ectodomain with an LDLa module essential for activation (2). Relaxin-3 interacts with a GPCR that lacks the LRR ectodomain. Knowledge of the precise structures, and how individual domains and specific residues are involved in activation, ligand binding and signaling is needed to understand how these proteins function and to assist in drug design. We are taking many different approaches to solving these problems: cell-free expression of the 7TMs, protein engineering to generate “soluble receptor” mimics for ligand binding analysis, site-directed mutagenesis of the LDLa domain, and ligand screening studies to whole cell receptors.

Collaborator: Assoc Prof Ross Bathgate (Howard Florey Institute).

Importation of proteins into mitochondria

Proteins are imported into mitochondria through the translocase of the outer membrane (TOM) complex. This incredible process requires unfolding of the targeted protein, recognition by components of the receptor, and passage of the targeted protein through the receptor. TOM is a molecular machine, consisting of several components: Tom5, Tom6, Tom7, Tom20, Tom22, and Tom40.

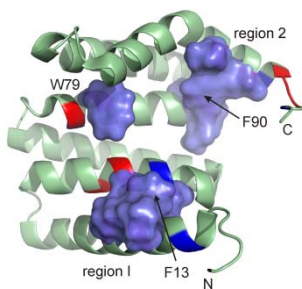


Fig. 2. Presequence binding sites of the cytosolic domain of plant Tom20

We have solved the structure of the plant Tom20 (Fig. 2) and compared it to the known structure of rat Tom20. Our results indicate that plant and animal Tom20s converged in evolution independently (3). We also now know that plant Tom20 has two binding sites for presequence and that presequences have two or more regions that recognize these sites (4). We also found that unlike yeast Tom22, the plant Tom22 cytosolic domain not bind presequence, but binds plant Tom20. Tom22 might therefore have the role of preventing binding of non-mitochondrial proteins that contain presequence-like sequences or of assisting preproteins to leave Tom20, thereby enabling their progression along the import pathway (4).

We propose to use “soluble membrane” mimics (leucine zippers) that hold Tom20 and Tom22 together in a membrane-like environment so we can examine how preproteins interact with the complex rather than the individual domains. We think that the two subunits may cooperate in the import process (4). We will use standard bacterial expression systems, as well as the cell-free system that we have developed, and biophysical methods to characterize the interaction.

Collaborator: Dr Terry Mulhern (Department of Biochemistry and Molecular Biology).

Recent publications

1. Koay A, Woodcroft B, Petrie EJ, Yue H, S, Bieri M, Bailey MF, Hargreaves M, Park J-T, Park K-H, Ralph S, Neumann D, Stapleton D, Gooley PR. (2010) ‘AMPK Beta Subunits Display Isoform Specific Affinities for Carbohydrates’, *FEBS Letters*, 584, 3499–3503.
2. Hopkins EJ, Layfield S, Ferraro T, Bathgate RAD, Gooley PR. (2007) ‘The NMR solution structure of the Relaxin (RXFP1) receptor LDLa module and identification of key residues in the N-terminal region of the module that mediate receptor activation’, *J Biol Chem*, 282, 4172–4184.
3. Perry AJ, Hulett JM, Likić VA, Lithgow T, Gooley PR. (2006) ‘Convergent evolution of receptors for protein import into mitochondria’, *Current Biol*, 16, 221–229.
4. Rimmer, K.A., Foo, J.H., Ng, A., Petrie, E.J., Shilling, P.J., Perry, A.J., Mertens, H.D.T., Lithgow, T., Mulhern, T.D., and Gooley, P.R. “Recognition of mitochondrial targeting sequences by the import receptors Tom20 and Tom22.” *Journal of Molecular Biology* (2011) 405, 804-818. (Cited by Faculty of 1000 as must read).
5. d’Auvergne EJ, Gooley PR (2008) ‘Optimisation of NMR dynamic models II. A new methodology for the dual optimisation of the model-free parameters and the Brownian rotational diffusion tensor’, *Journal of Biomolecular NMR*, 40, 121–133.