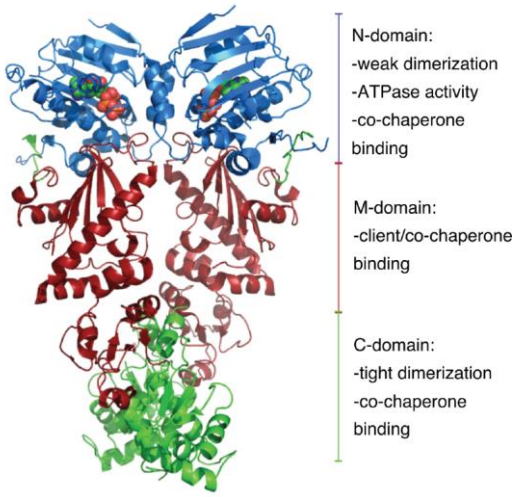
Lab Spotlight

Dr. Leo Spyracopoulos

History

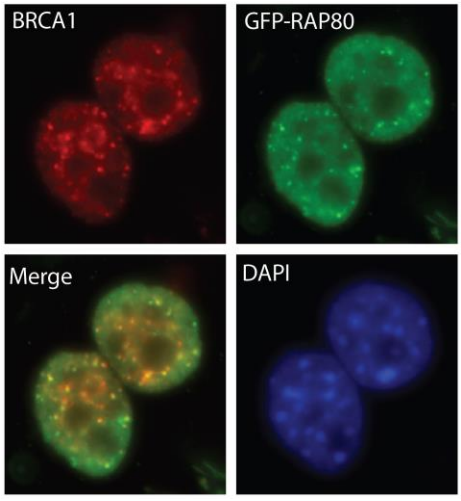
Leo has been interested in using protein NMR to understand the structures, dynamics, and biological functions of proteins since beginning his research career as an undergraduate and graduate student in the lab of Dr. Joe O'Neil at the University of Manitoba. Following a productive postdoc in Brian Sykes' lab at the Department of Biochemistry at the University of Alberta, Leo started his lab in the Department in 2000. Leo's lab has made important contributions to our understanding of the enzymatic synthesis of Lys63-linked ubiquitin chains, and how these chains are recognized in the context of double strand DNA repair processes. We highlighted our work recently in a special issue on ubiquitination in the Journal of Molecular Biology (http://dx.doi.org/10.1016/j.jmb.2017.05.029).

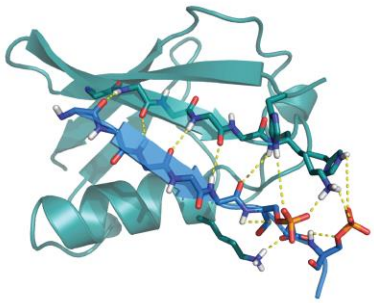
Research Overview



Hsp90 Chaperone

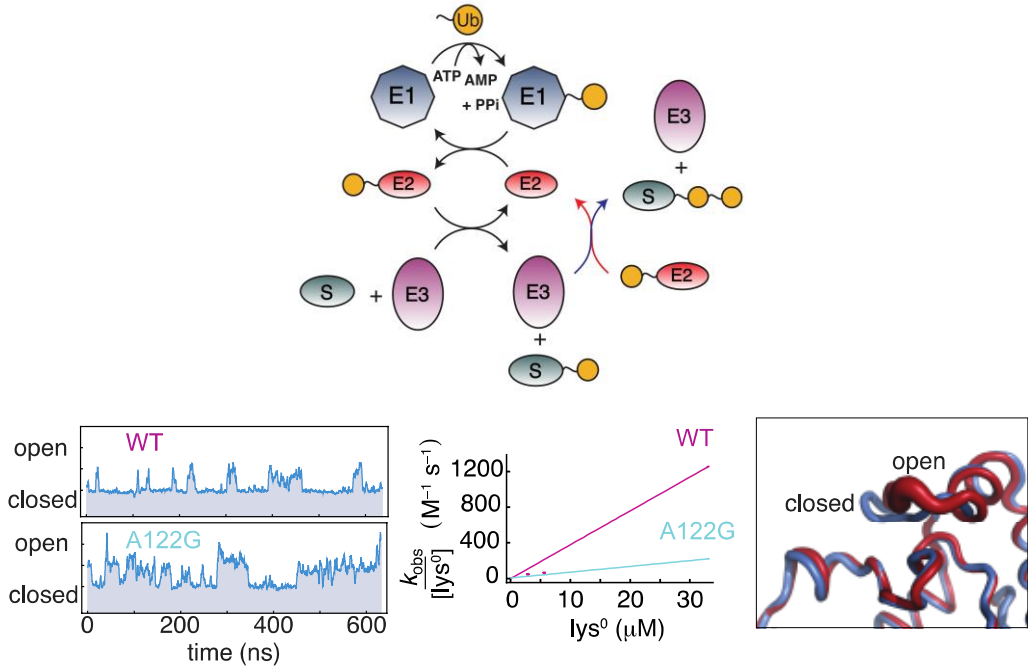
Chaperone enzymes fold and stabilize proteins using ATP as an energy source; this protects them from aggregation or non-biologically relevant interaction with other proteins. Hydrolysis of ATP releases bound protein and the cycle repeats until folding is complete. The chaperone Hsp90 is essential for proper cellular function; it binds fully or partially folded proteins, or "clients", to modulate their biological functions. There are numerous client proteins, including signaling kinases and transcription factors. The activity of these clients depends on hydrolysis of ATP by Hsp90. Furthermore, Hsp90 is tightly regulated through binding of numerous co-chaperone proteins, which either increase or decrease the ATPase/chaperone activity, thereby regulating interactions with clients. In collaboration with Dr. Paul LaPointe in the Department of Cell Biology, we are using NMR studies to build a molecular view of the function of the Hsp90 chaperone in the presence of client proteins and activating co-chaperones (funded by NSERC).





RAP80 DNA Repair Protein

One of the most severe types of DNA damage is a double strand DNA break (DSB). These breaks are repaired by a number of pathways; our focus is on the homologous recombination (HR) pathway, which is driven by binding of DNA repair scaffolding proteins and enzymes to the small proteins ubiquitin (Ub) and SUMO, or chains of these proteins that are attached to protein clusters known as damage foci. An early step in the formation of damage foci is synthesis SUMO and Ub chains near the damage sites. RAP80 binds these chains and recruits the BRCA1 tumor suppressor complex to the damage sites to start DNA repair. In collaboration with Dr. Michael Hendzel in the Departments of Experimental Oncology and Cell Biology, we are blending biophysical NMR methods with cell imaging studies to understand the dynamics and subcellular trafficking of DNA repair proteins like RAP80 in the nucleus (funded by CIHR).



Catalytic Mechanism of Ubiquitination Enzymes

The ubiquitin proteasome system (UPS) tags proteins with ubiquitin, signaling for their removal from the cell. Defects arising from mutations in the enzymes and proteins of the UPS are increasingly being recognized as key players in the development of diseases such as neurodegeneration, cancer, and viral infection. The UPS uses three enzymes to attach ubiquitin to target proteins. The central enzyme is known as a ubiquitin conjugation, or E2 enzyme, and it catalyzes the attachment of a target protein lysine to ubiquitin, with the help of an E3 accessory protein. In collaboration with Dr. Mark Glover in the Department of Biochemistry, and Dr. Michael Hendzel (Depts. of Experimental Oncology and Cell Biology), we blend NMR, X-ray crystallography, molecular dynamics simulations, cell imaging studies, and enzyme kinetics to study E2 active site gate dynamics. The rates of gate opening appear precisely tuned, and differences in the active site loops of different E2s may be selectively targeted by E2 inhibitors. This represents a novel therapeutic approach to treating diseases associated with UPS function (funded by CIHR).