The proposal builds on concerted efforts from functional proteomics and experts at the Lunenfeld (Toronto; Gingras and Pawson) and a structural biologist at the Structural Genomics Consortium (Oxford; Filippakopoulos). The collaboration between the Pawson group and Dr. Filippakopoulos is longstanding, and has defined modes of molecular recognition (e.g. Filippakopoulos et al., *Cell* 2008); all co-applicants have a collaborative publication *in press* (Filippakopoulos et al., *Cell*, 2012; appended) that forms the basis of parts of Aims 2 and 4. By solving the structure of 29 bromodomains and characterizing their binding properties on peptides in isolation, this study began revealing important elements of bromodomain recognition. In particular, we found that the bromodomains of the BET family BRD4 exhibits a marked preference for distinct diacetylated peptides, suggesting a direct recognition of multiple marks by a single bromodomain containing protein.

As defined in the preliminary data, the Pawson and Gingras groups have established a strong and collaborative expertise in functional proteomics. We have defined efficient protocols for affinity purification coupled to mass spectrometry (AP-MS; e.g. Chen and Gingras, *Methods*, 2007; Dunham et al., *Proteomics*, 2011) that we have employed for the identification of interaction partners for multiple signaling proteins (e.g. Chen et al., *J Biol Chem*, 2008; Goudreault et al., *Mol Cell Proteomics*, 2009; Kean et al., *J Biol Chem*, 2011). We have also developed bioinformatics tools enabling the tracking of biological material (OpenFreezer, Olkovsky et al., *Nat Methods*, 2011) and mass spectrometry data (ProHits, Liu et al., *Nat Biotech*, 2010; Liu et al., *submitted*). Since identifying true interaction partners from AP-MS data is challenging because of non-specific background interactors, we have developed with collaborators statistical tools enabling the identification of true interaction partners (SAINT; Breitkreutz et al., *Science*, 2010; Choi et al., *Nat Methods*, 2011; Choi et al., *submitted*). All these resources are freely distributed to the scientific community and are subjected to active development.

Despite these advances, the protocols that we had developed for AP-MS analysis of soluble proteins were not appropriate for the analysis of chromatin-associated proteins (under standard cell lysis conditions, most of the chromatin-associated proteins precipitate as an insoluble pellet). Fortunately, the Pawson and Gingras lab recruited a joint postdoctoral fellow in Fall 2010 who had developed during his PhD a method he called mChIP for the solubilization of chromatin coupled to AP-MS (Lambert et al., *Mol Cell Proteomics*, 2009; Lambert et al., *Mol Sys Biol*, 2010). Since joining the SLRI, Jean-Philippe Lambert adapted the mChIP approach to mammalian cell AP-MS data, and showed that it enabled recovery of interactions which were otherwise lost. Jean-Philippe also developed methods to enrich acetylated peptides from affinity-purified samples; this method is an essential component of the current proposal. As described in the “preliminary data” section of the proposal, we have already cloned, expressed, purified, and analyzed by AP-MS the majority of the proteins composing the acetylome system using this protocol. In the past year, our laboratories have also made significant progress towards robust mass spectrometry-based quantification of AP-MS data, using intensity-based quantification (e.g. Kean et al., *J Biol Chem* 2010), SRM-based quantification (e.g. Bisson et al., *Nature Biotech*, 2011; Yong et al., under review at *Science*), SWATH quantification (Lambert et al., *in prep*), and isotope-based quantification (e.g. Jorgensen et al., *Science*, 2009). We recently used intensity-based quantification to measure the affinity of isolated bromodomains derived from BRD4 towards acetylated histone peptides (Filippakopoulos et al., *Cell*, *in press*), demonstrating the feasibility of our aims.

Lastly, we have engaged in key collaborations, which position us very well for the successful completion of our aims. We have access to a growing number of phage affinity reagents to target bromodomains through a collaboration with Sachdev Sidhu (U Toronto), and are working with the lead expert on NUT midline carcinoma, Christopher French (Harvard). Collaborations with statisticians, mass spectrometry vendors and computational biologists are also in place to ensure a timely generation and analysis of our data within the short period of this grant.