With the advent of next generation DNA sequencing technologies, the pace of discovery of cancer-associated sequence variants has greatly accelerated, leading to the realization that tumours (especially solid tumours) harbour hundreds of mutations. Ongoing statistical analysis across multiple laboratories worldwide is progressing rapidly, helping to identify which of these mutations are likely drivers of the cancer phenotype. However, in spite of rapid progress in mapping of cancer related signaling and interaction networks, there has been an increasing disconnect between the identification of a cancer variant, and the mechanistic understanding of transformation induced by this mutation. While the downstream effects of many of the mutations may be straightforward to explain (e.g., abrogation of protein expression or function for tumour suppressors), what happens at the molecular level as a consequence of a point mutation is generally not well understood. Such molecular understanding is crucial for developing therapeutic interventions. Frequent consequences of cancer mutations (especially point mutations in the coding sequence) are specific alterations of protein-protein interactions. As we are developing both appropriate structural data and technology to assess and modulate such interactions, an analysis of specific protein-protein interactions altered by cancer-associated mutations is timely and could offer novel therapeutic avenues.

To ideally exploit this novel venue, we should be able to quantitatively measure both loss and gain of interactions, be able to rapidly measure different sequence variants, and have a robust, sensitive and accurate approach. In order to develop such a methodology, we have coupled affinity purification (AP) to a novel data-independent mass spectrometric acquisition (SWATH). We have then tested this strategy for the quantification of the changes imparted by melanoma-associated mutations in the kinase CDK4. In addition to confirming known interaction loss, we were able to also identify interaction gains in the same experiment, namely in the recruitment of HSP90 network components to the mutated proteins. We next used AP-SWATH to monitor the consequence of pharmacological inhibition of HSP90 on the recruitment of chaperones to the wild type and mutated CDK4 proteins and rapidly demonstrated that the mutant proteins are resistant to the inhibitor. This finding has obvious implications for patient care.

Based on these results, we are proposing to develop an experimental and computational pipeline to assess interactions changes. We will make this pipeline scalable to optimally make use of ongoing genomic sequencing efforts. We have access through our collaborations to a large collection of disease alleles for human proteins that will be prioritized for analysis by AP-SWATH based on the structure-based predictions of mutations most likely to disrupt protein-protein interactions (this is the area of expertise of co-applicant Kim). In Aim 1, selected mutants (and their wild type counterparts ~100) will be analyzed by AP-SWATH to identify deregulated interactions. This will be followed in Aim 2 by a deeper analysis of the changes imparted in interactions for phosphatases (an intense area of research in the Gingras lab). In Aim 3, we will explore the consequences of treatment with pharmacological inhibitors on the interactions for wild type and mutant proteins (as for HSP90 above).

Throughout the duration of the grant, we will optimize the platform to enable higher throughput analysis and provide cancer researchers with annotation of interactome changes imparted by mutations, and when possible, by the treatment with pharmaceuticals. In addition, we will be addressing yet unresolved questions: for how many (and which) of the point mutations are interaction profiles altered and how should the community embark on a systematic characterization of these changes? Our project will greatly enhance our understanding of the effects of cancer mutations both on a cellular and molecular level, and provide the research community with leads for biological follow-up, thereby accelerating the development of therapeutic options.