The Gingras and Derry laboratories have been working together for the past several years to better understand the molecular mechanism of action of CCM proteins. Interest in CCM in the two groups has emerged independently, initially, from the discovery of the STRIPAK complex into which the bulk of CCM3 resides in mammalian cells (*Gingras*; Appendix 1), and from studies of the role of CCM1 (*kri-1*) in *C. elegans* germ cell apoptosis (*Derry*; Appendix 2). Since then, however, the co-applicants have combined their efforts in genetics, biochemistry and proteomics. These collaborative efforts have resulted in two recent publications (also with collaborator F. Sicheri) reporting the heterodimerization of CCM3 with kinases of the GCKIII family (Appendix 3), and the molecular organization of the STRIPAK complex (Appendix 4). These reports are described in the introduction to this proposal and help to frame the future goals of this work. Importantly, and as described in more detail in the preliminary data section of the grant, our two laboratories have been working together to assess the functional significance of the association of CCM3 with the GCKIII kinases and other STRIPAK components using cell biological assays in mammalian cells and phenotypical analysis and genetic screens in the multicellular organism *C. elegans*. In particular, the Derry lab has now identified important functions for CCM3, GCKIII kinases and other STRIPAK components in osmoregulation and morphology of the *C. elegans* excretory cell, the sole vascular structure in this organism. The phenotypes reported for CCM3 and CCM1 in *C. elegans* (including a synthetic lethal interaction between the two gene) will enable our team to harness the power of genetic screens to identify additional components of the CCM signaling pathway(s). The Derry lab has a very strong expertise in this area, having conducted genetic screens and phenotypical analysis for the past 8 years (i.e., Derry *et al*., 2007; Quevedo *et al*., 2007; Gao e*t al*., 2008; Ito *et al*., 2010; Ross *et al*., 2011).

Since starting her laboratory at the Lunenfeld, AC Gingras has established herself as a proteomics expert (as evidenced by her publications, multiple invitations as a guest speaker, participation to the editorial board of the leading proteomics journal and serving on the advisory board of the UCSF proteomics centre). She has been very active in the past several years in developing reagents and procedures to facilitate generation of high quality mass spectrometry data, in particular for interaction proteomics. For example, she has developed protocols to improve the yield of affinity-purified samples (e.g. Chen and Gingras, *Methods*, 2007), but also contributed to the development and implementation of a number of bioinformatics tools. In particular, she co-developed, with A Nesvizhskii, algorithms to identify true interaction partners in affinity purification/mass spectrometry (AP-MS) experiments. The SAINT (Significance Analysis of INTeractome) statistical tool compares the quantitative information associated with the identification of each protein in a specific experiment, with the quantitative information for the same protein across all other experiments in the dataset (Appendix 5). This strategy is applicable to very large datasets (including the yeast kinase and phosphatase interaction network published with M Tyers; *Science* 2010), but also to much smaller datasets (see, e.g. Skarra et al., *Proteomics*, 2011). In the laboratory, SAINT is supported by our robust bioinformatics pipeline ProHits which enables us to track our mass spectrometry data, annotate experiments, and view and analyze the experimental results (with M Tyers, *Nat Biotech*, 2011). We have an open source policy and are distributing both and ProHits freely to the scientific community. In the recent years, new postdoctoral fellows with expertise in identification of kinase substrates and quantitative proteomics have joined the group and a research collaboration with mass spectrometry vendor AB-SCIEX has further cemented our leadership in quantitative phosphoproteomics; this is enabling the realization of the challenging experiments proposed here.

While the Gingras and Derry labs are supported by grants from the CIHR for unrelated projects this application will support efforts to combine their distinct but complementary strengths in proteomics and genetics to define the mechanisms by which CCM proteins function.