Mechanics of the cellular actin cortex using Atomic Force Microscopy

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The contractile actin cortex is a thin layer of actin, myosin, and actin-binding proteins that subtends the membrane of animal cells. The cortex is the main determinant of cell shape and therefore plays a fundamental role in processes such as cell division, migration, and tissue morphogenesis. During cancer metastasis, cells can migrate using a rounded amoeboid morphology that depends primarily on cortical contraction and misregulation of cortical contractility during division can lead to aneuploidy. Despite its importance, our understanding of cortex mechanics is poor and the proteic determinants of its function are not understood. We have recently determined the proteic composition of the actin cortex using mass spectrometry techniques. The goal of this project will be to examine the role of actin nucleating proteins, actin binding proteins, and myosin motor proteins in setting actin cortex mechanical properties. The mechanical properties of the cortex will be measured using Atomic Fore Microscopy microindentation. For each candidate protein, we will verify cortical localisation using transfection of GFP-tagged proteins and confocal microscopy. Then, we will perturb protein function using dominant negative mutants or deplete the protein using shRNA and ask how perturbation changes cortex mechanical properties. Finally, we will examine the change in cortical organisation induced by depletion using scanning electron microscopy for the proteins giving rise to the most dramatic mechanical changes.