

Project #19: **Todd Sulchek** and **Chunyang Xiong**: *Biophysicochemical Effects on Cell Endocytosis*

The goal of this research proposal is to determine the biophysicochemical impact on receptor mediated endocytosis. For this study, we will fabricate and functionalize nano-microparticles with integrin-binding RGD mimetics (e.g. Fibronectin). We will explore the effect of four experimental variables on particle endocytosis. First, we will determine the role of ligand density, which determines the number of molecular bonds that form at the interface. Second we will determine the impact of ligand affinity, which affects the strength of each bond. Third we will study the impact of the spatial dimension of particle size, which affects the number of molecular bonds that forms at the interface as well as determines the extent of cell deformation required to endocytose a particle. Fourth we will fabricate the nano/microparticles with different Young's modulus, to investigate the stiffness effect on cell endocytosis. To accomplish the fourth aim, we will make the polymer beads of controllable size and stiffness using a microfluidic technique and functionalize with RGD mimetics. All ligands will contain the RGD binding motif, but will have different affinity for the integrin receptors due to slight structural and charge variations in the molecule. The RGD mimetics will bind to cell surface integrin membrane receptors and produce dynamic and specific molecular responses within the cell, such as local receptor clustering, effector protein activation, and cytoskeletal protein rearrangements. We will quantify the results of these processes through adhesion and endocytosis studies with optical fluorescence microscopy, flow cytometry, and atomic force microscopy (AFM) adhesion measurements. Our hypothesis is that by spatially sequestering high-density and high-affinity cell adhesion ligands upon the appropriate stiffness substrate, we can create microparticles that can actively control the dynamics of endocytosis, as well as facilitate the targeting of therapeutics.