Probing Spatiotemporal Mechanical Heterogeneities and Gradients in Cell Membranes

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Tension at the cell surface regulates key cellular processes, yet its spatial and temporal heterogeneities remain underexplored. Using a correlative, multi-technique approach, we reveal an vertical gradient in membrane tension in adherent cells above the mid-plane. Optical trap–based sequential dual-tether force measurements identify an actin-dependence, with midplane regions exhibiting higher tension. Flipper-TR imaging confirms these variations, while super-resolution imaging of actomyosin and endocytic components highlights the competing roles of endocytosis and cytoskeletal contractility. In parallel, we examine the rates of tension equilibration using interference reflection microscopy. We propose that the cell membrane displays long length-scale gradients shaped by competing effects of active processes like contractility and endocytosis made possible due to slow equilibration of tension also regulated by known players like Caveolae.