MULTI-COLOR NANOSCOPY REVEALS MULTI-MODULE ACTIN MACHINERY DRIVING PODOSOME-MEDIATED CELL PROTRUSION

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Podosomes are cytoskeletal structures involved in protrusion, topography sensing and extracellular matrix degradation. They are formed by cells that need to cross and degrade tissue boundaries such as immune cells to extravasate or osteoclasts to degrade bone tissue. Furthermore, cancer cells form podosome-like structures called invadopodia to drive metastasis. We and others have shown before that podosome protrusion is regulated by myosin-mediated contractility and actin polymerization, but it remains elusive how continuous actin polymerization within the podosome cores is spatially confined and translated into a protrusive force. To address this specific question, we determined the nanoscale localization of actin nucleators and crosslinkers with respect to actin within podosomes by confocal microscopy, live cell imaging and multi-color nanoscopy. Image analysis revealed two podosome actin modules: 1) a dome-shaped module of ~500nm in height and width that appears to consist of linear filaments and 2) an inner core module with a diameter of ~250nm that appears to consist of branched filaments. To investigate the mechanosensing properties of this force-generating machinery we seeded cells on nanoscopy-compatible substrates (1-300kPa), revealing a remarkable structural plasticity of podosomes in response to substrate stiffness. Together, our novel insights provide for the first time a molecular basis to understand podosome-mediated cellular protrusiveness and mechanosensation.