Applying superresolution microscopy to elucidate protein localisation and the mechanism of filopodia formation

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Here we use superresolution microscopy, both SIM (structured illumination microscopy) and dSTORM (direct stochastic optical reconstruction microscopy) together with complementary live-cell imaging to study filopodia. Filopodia are actin rich dynamic structures that emerge from the leading edge and apical surface of cells. They have been shown to be involved in many cellular process including cell migration, cell invasion, phagocytosis and axonal guidance. The Cdc42 effector I-BAR protein, IRSp53 and its –SH3 domain interactors, play a major role in filopodial formation. The I-BAR domain of IRSp53 binding with actin and lipids within the plasma membrane is responsible for membrane deformation and resultant curvature. IRSp53 induces filopodia formation by coupling the membrane deformation and actin polymerisation.

We have applied superresolution microscopy, SIM and dSTORM, to localise IRSp53 protein and its I-BAR domain in relation to F-actin. IRSp53 generates dynamic protrusions 300 nm in width with a distinct gap between IRSp53 and F-actin. In contrast, protrusions induced by the I-BAR domain alone are non-dynamic with diameters between 100-200 nm within which the I-BAR domain more closely localises to the F-actin.

The data suggest that IRSp53 localisation is restricted to certain areas of the membrane, in contrast to the I-BAR domain which is uniformly distributed throughout filopodia. In addition, we also investigated the IRSp53–SH3 domain interactors Mena, Eps8 and Dynamin within filopodia and measured their localisation relative to actin. Actin de-polymerization, using Cytochalasin D, during live-cell imaging experiments reveals that F-actin is required for the dynamic extension and retraction of filopodia generated by IRSp53 but is not necessary to maintain the static protrusions generated by I-BAR domain. Taken together, the data explains the mechanism of filopodia formation and their dynamic activity.