Subtomogram averaging of Arp2/3 complex-mediated branches in human macrophage podosomes

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Human macrophages form protrusive adhesion structures called podosomes which are involved in mechanosensing [1]. They consist of an adhesion ring surrounding an F-actin rich core in which actin polymerization occurs through branching mediated by the Arp2/3 complex. How branches are spatially organized and contribute to force production in podosomes remains unknown. Following up on a recent study on actin waves [2], we employed cryo-electron tomography and subtomogram averaging to identify branches in human macrophage podosomes and analyze their spatial organization. The initial approach on *in situ* tomograms of native podosomes did not allow confident identification of true-positive branches. Simulations ruled out the missing wedge as a limiting factor. Instead, the high density in the podosome core combined with a low signal-to-noise ratio in the data prevented the detection of branches. Improvement of the processing workflow and higher data quality enabled the identification of branches within three tomograms. A low-resolution branch structure was obtained and preliminary analysis showed that ~50% of the branches in the core point toward the cell membrane. Mother and daughter filaments have similar orientations, suggesting that mother filaments may originate from earlier branching events.

[1] Labernadie, A. et al. Protrusion force microscopy reveals oscillatory force generation and mechanosensing activity of human macrophage podosomes. Nat Commun 5, 5343 (2014)

[2] Jasnin, M. et al. The architecture of traveling actin waves revealed by cryo-electron tomography. Structure 27,1211 (2019)