

Intravital single molecule microscopy for studying nanoscale actin cytoskeleton organisation and dynamics

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Advances in single molecule detection (SMD) and tracking is revolutionising our understanding of underlying molecular and cellular processes. Significant differences in the actin cytoskeleton between 2D and 3D cell cultures have been observed, demonstrating that the actin cytoskeleton is exquisitely sensitive to the extracellular environment. Since cell culture conditions cannot recapitulate the *in vivo* environment, it is imperative to study cytoskeletal dynamics in live animals, under physiological conditions. However, current SMD methods are restricted by imaging depth (TIRF) or sample size (multiple objectives). By combining highly inclined and laminated optical sheet (HILO) microscopy and MATLAB/ImageJ algorithms, we could quantify the binding durations of fluorescently tagged molecules, generate super-resolution molecular density distributions and superimpose maps of molecular kinetics over finely resolved cellular structures. We used this novel imaging modality to compare dynamic features of the actin cytoskeleton via its associated protein tropomyosin (Tpm), in 2D cell cultures and in organs in mice. We found that Tpm3.1 spends more time bound to stress fibres than to cortical actin filaments in cultured mouse embryo fibroblasts. In salivary glands and pancreas Tpm3.1 filaments are organised in distinct micro-assemblies. Lastly, we observed differences in the binding duration and displacement of Tpm isoforms and myosin motors *in vivo* that reveal distinct interactions with actin filaments. This is the first demonstration of SMD and tracking in the organs of live mice yielding quantitative data. This novel imaging modality will help us understand how cytoskeletal proteins interact within heterogeneous micro-assemblies and distinct subcellular structures within a physiological environment.

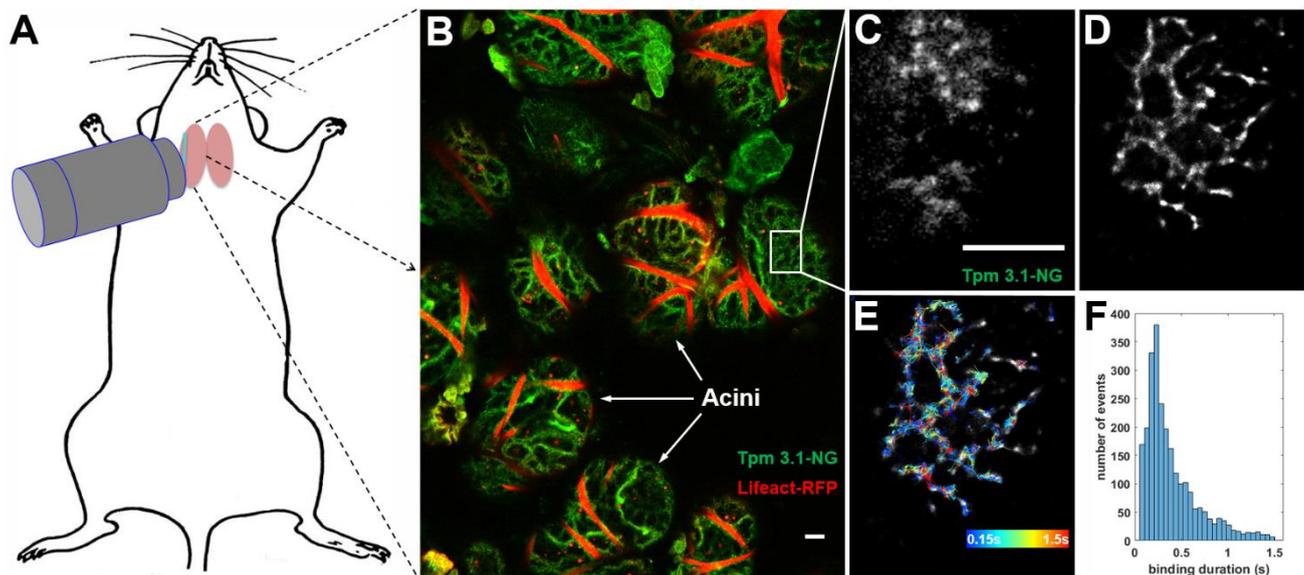


Figure 1: SMD and tracking analysis of Tpm-3.1 in salivary glands: Transgenic mouse expressing Tpm3.1-NG and Lifeact-RFP (A). Acini in a confocal scan (B), unaltered signal of HILO microscopy (C), super-resolution molecular density distribution of single molecules (D), superimposed molecule tracks (E) and histogram of their binding duration (F). Scalebar 5 microns.