O-18.5 Short talk

Long-term single molecule localization microscopy uncovers dynamic co-assembly of LRP6 and ROR2 into Wnt-signalosomes

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The conserved Wnt signaling has been classified as two categories of canonical and noncanonical Wnt signaling. With a high promiscuity of Wnt signaling, how receptors from the two distinct pathways re-arrange in multi-protein signalosomes remains elusive. We here developed single-molecule tracking and localization microscopy based on labeling with reversibly binding nanobodies (rbTALM) for imaging receptor dynamics in the plasma membrane for extended time periods. To this end, we engineered nanobody-tag pairs with fine-tuned binding stabilities ensuring single-molecule tracking with high fidelity, yet continuous exchange of photobleached labels. Multicolor rbTALM imaging enabled simultaneous tracking and super-resolution imaging of three different Wnt co-receptors in the same cell for more than one hour at video rate. Time-lapse correlation analyses uncovered cooperative association of canonical and noncanonical Wnt co-receptors into a common, hybrid Wnt signalosome, demonstrating the exciting possibilities of rbTALM imaging for exploring nanoscale dynamics across millisecond to hour timescales.