Tracking individual Hedgehog-pathway proteins in live cells by single-molecule microscopy

- Conventional microscopy obscures the heterogeneity of protein movements and dynamics in live cells.
- We used single-molecule imaging and tracking to analyze the dynamics of the Hedgehog-pathway protein Smoothened in primary cilia, micrometer scale cellular antennae.

Trajectories of individual Smoothened proteins uncovered complex movement which was frequently interrupted by binding at the base or tip of cilia.

The measured dissociation constant for binding at the base depended on the activation state of the Hedgehog pathway.

Our findings quantify activation-dependent changes of Smoothened dynamics in cilia and highlight a previously unknown step of Hedgehog-pathway activation.