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Talk Title: **Cholesterol accessibility at the ciliary membrane regulates Hedgehog signaling**

Abstract:
The Hedgehog pathway is a paracrine cell-cell communication system that regulates the development of most tissues in our bodies (and the bodies of many metazoan organisms). Mutations in Hedgehog genes lead to a wide spectrum of birth defects, cancers of the skin and brain and degenerative conditions. A long-standing mystery in vertebrate Hedgehog signaling is how Patched 1 (PTCH1), the receptor for Hedgehog ligands, inhibits the activity of Smoothened (SMO), the protein that transmits the signal across the membrane. Using a combination of CRISPR screens, toxin-based lipid sensors and imaging-based assays, we find that SMO activation is driven by a biochemically-defined, minor fraction of membrane cholesterol, termed accessible cholesterol. Hedgehog ligands, which bind and inactivate PTCH1, selectively increase cholesterol accessibility in the outer leaflet of the ciliary membrane (without changing cholesterol in the bulk plasma membrane). Novel transporter assays in live cells show that PTCH1 depletes outer leaflet accessible cholesterol in a manner that depends on the transmembrane potassium gradient (but not the sodium gradient). We propose that PTCH1 inhibits SMO by using accessible cholesterol as a second messenger, thus solving a 30-year mystery in how this iconic pathway transmits signals to the cytoplasm. More generally, our work shows that the organization of cholesterol in the ciliary membrane can be modified by extracellular ligands to control the activity of cilia-localized signaling proteins.