

Description of Techniques and Explanation of Concepts

Monolipidated Substrates for S-Palmitoylation Rapidly and Randomly Partition Over All Membranes

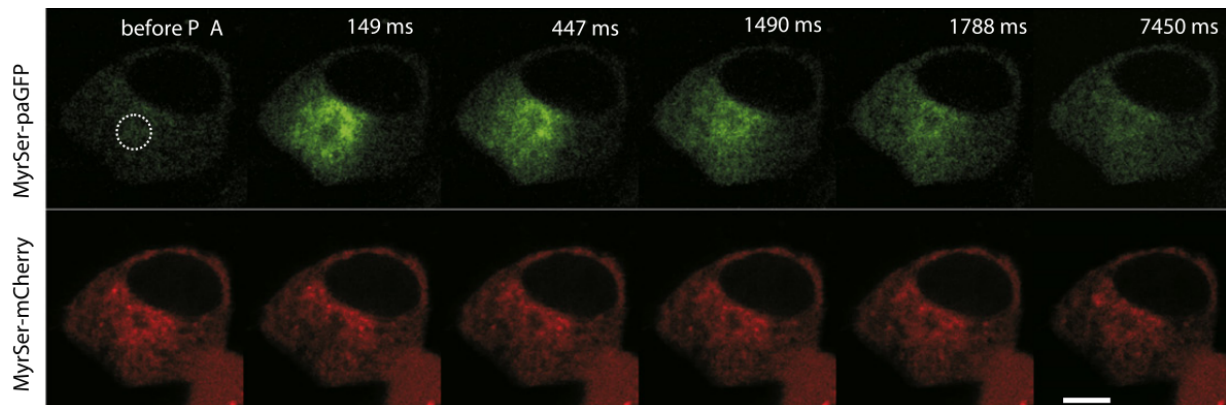
- “Palmitoylated peripheral membrane proteins typically contain an **irreversibly attached prenyl or myristoyl moiety** in proximity to the palmitoylation site.”
 - **Prenylation (lipidation)**: the addition of hydrophobic molecules to a protein or chemical compound.
 - Prenyl groups (are often assumed to) facilitate attachment to cell membranes, similar to lipid anchors like the GPI anchor.
 - **Myristoylation**: a lipidation modification where a myristoyl group (a common saturated fatty acid derived from myristic acid) is covalently attached by an amide bond to the alpha-amino group of an N-terminal glycine residue.
 - Myristoylation allows for weak protein–protein and protein–lipid interactions and plays an essential role in membrane targeting, protein–protein interactions, and functions widely in a variety of signal transduction pathways.
 - I.e., these **irreversible modifications** that help facilitate protein-protein and protein-lipid interactions may help **identify spatial organization** of palmitoylated peripheral membrane proteins, as **palmitoylation is a reversible lipid modification and thus hard to localize**.



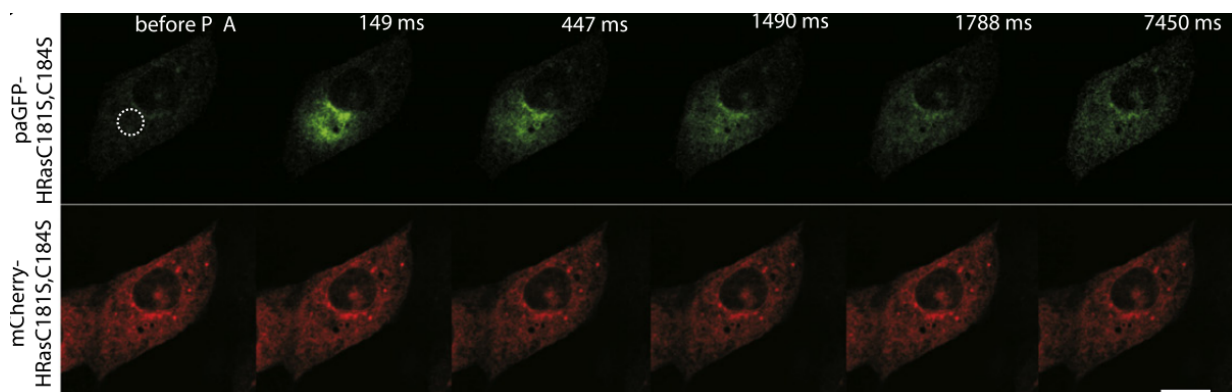
(A) Steady-State Localization

- “We first **questioned whether singly lipidated proteins** exhibit a **specific membrane distribution to facilitate their subsequent S-palmitoylation**. Mutant solely farnesylated HRasC181,184S and solely myristoylated MyrSer that cannot get palmitoylated were **equipped with mCherry and mCitrine, respectively**, to observe their **steady-state localization**. In both cases, the fluorescence distribution did not show a preference for any membrane compartment, but merely reflected membrane densities (Figure A).”
 - **Steady-state localization**: steady state refers to the maintenance of constant internal concentrations of molecules and ions in the cells and organs of living systems, basically homeostasis at a cellular level.

- I'm uncertain on what the "localization" exactly is specifying, but I'm assuming that it means where proteins (cellular organelles, lipids, and possibly others components) actually end up when steady-state is maintained.
- The use of **mCherry** and **mCitrine** (stains) allowed for **visualization of the localization via fluorescence** of HRasC181,184S and MySer (singly lipidated proteins); these proteins were **mutated in order to control for any preemptive palmitoylation**, allowing researchers to **test for any prior distribution** that effects later palmitoylation—"results reflected membrane densities (Figure A)."

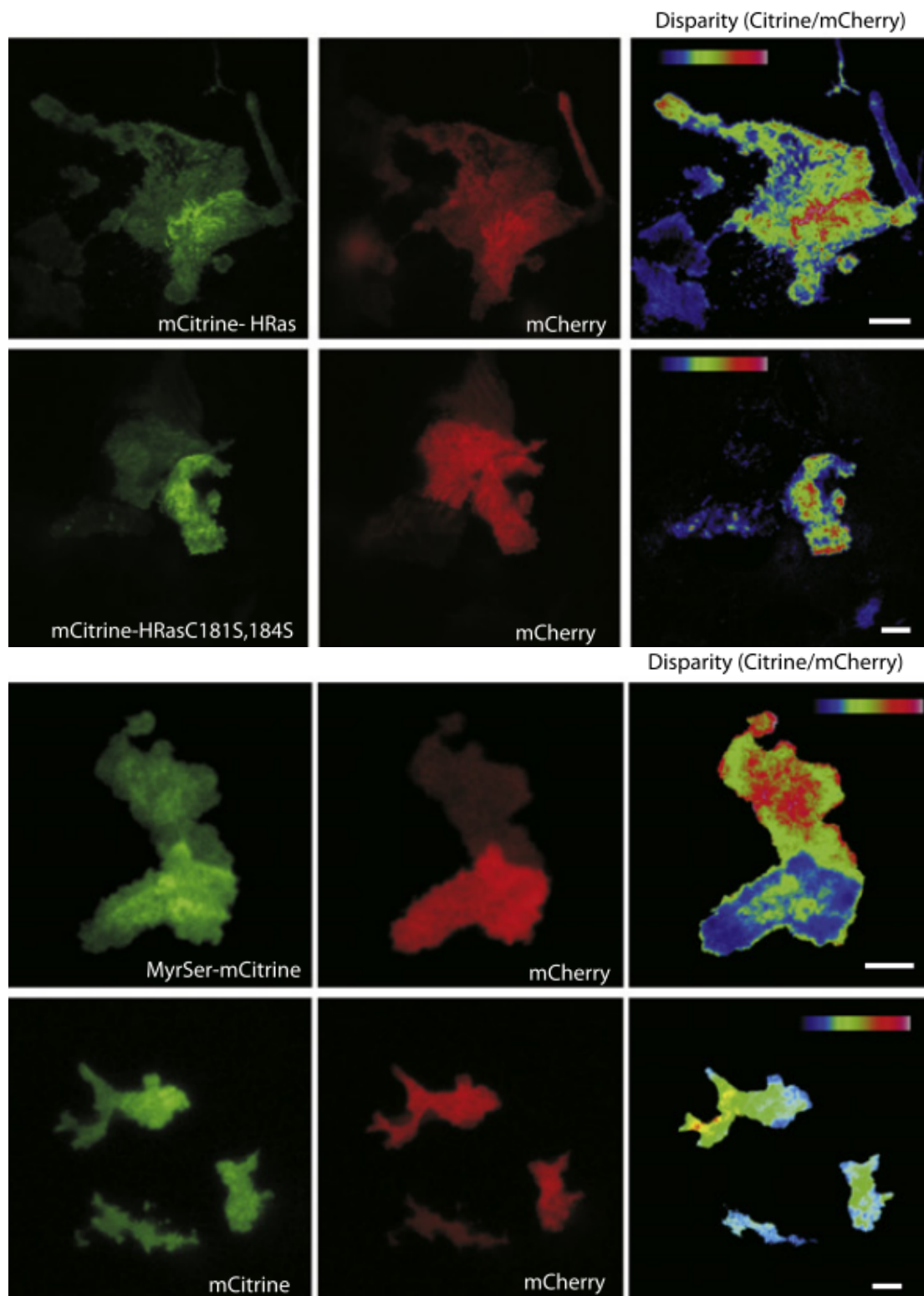


(B) Fluorescence Loss of Photoactivated MySer-paGFP



(C) Fluorescence Loss of Photoactivated paGFP-HRasC181S,C184S

- "Photoactivatable GFP-fused" versions of both proteins rapidly redistributed over all membranes, reaching steady-state within the first seconds after **photo-activation** (Figures B/C)".
- **Photo-activation localization**: fluorescence microscopy imaging methods that allow obtaining images with a resolution beyond the diffraction limit targeted biophysical imaging method was largely prompted by the discovery of new species and the engineering of mutants of fluorescent proteins displaying a controllable **photochromism** (a reversible change of color upon exposure to light, using the photo-activatable GFP).
- Essentially, the **use of photoactivation** allowed for the researchers to **determine the rate of steady-state localization**.



(D) Total Internal Reflection Fluorescence

- “In order to confirm that the monolipidated proteins also had access to the PM, **TIRF microscopy was performed** on the wild-type and monolipidated mutant proteins. As expected, the fully lipidated wild-type proteins clearly showed an **enrichment at the PM** (Figure D).”

- **Total internal reflection fluorescence (TIRF)**: a fluorescence microscope technique that allows for a thin region of a specimen, usually less than 200 nanometers to be

observed.

- The fluorescence signals from the lipidated proteins were **normalized to soluble mCherry** to show the **disparity in contrast reflecting their PM localization**.
- Free mCitrine/ Free mCitrine/mCherry images are shown as controls, showing disparity arising due to differences in optical parameters of the TIRF field.
- Scale bars represent 10 mM. Color bar indicates normalized range of pixel ratios from minimum (blue) to maximum (red).
- **Enrichment at the plasma membrane (PM)**: depalmitoylation was shown to occur at least at the plasma membrane (El-Hus-seini et al., 2002; Rocks et al., 2005)—this is why it was expected.
- “However, both **monolipidated mutants also exhibited clear PM localization**, establishing that **they have access to this membrane**. These **experiments are inconsistent with** the presence of receptors for monolipidated proteins on specific membrane compartments (Choy et al., 1999). Instead, **proteins with only one attached lipid** rapidly and randomly sample all membranes until they are **trapped because of an increase in their affinity for membranes** by the acquisition of additional lipid anchors at the site of palmitoylation. This **kinetic trapping (Shahinian and Silvius, 1995)** is an essential aspect of the spatial organization of palmitoylated peripheral membrane proteins that can be **exploited to detect the subcellular site of palmitoylation**.”
 - **Kinetic traps**: folding kinetics may trap a protein in a high-energy conformation, i.e., a high-energy intermediate conformation blocks access to the lowest-energy conformation.
 - The authors go on to use this phenomenon, stating that “kinetic tapping is apparent from a local probe accumulation caused by a decrease in effective diffusion.”
 - Essentially, what I’m gathering is that **exploitation of the higher energy state** may lead to different more observable functions, such as decreased effective diffusion, which is the technique used to identify the subcellular sites that are currently (previously?) not well known.