Quantification of Ras membrane diffusion and multimer formation in live cells

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***Short Abstract* — Ras small GTPases are key regulators of cell signaling. Recent evidence suggests a critical role of Ras dimers and clusters in cell signaling, but the mechanisms of how these structures form and function in cells remain unclear. Here we describe nanoscopic sites on the cell membrane that can transiently trap and enrich Ras to potentially facilitate dimer and cluster formation, as revealed by single-molecule tracking, quantitative trajectory analysis, and simulations. Our results demonstrate the importance of membrane heterogeneity in regulating biological processes in cells.**

***Keywords* — single-molecule tracking, quantitative imaging, Ras, diffusion, dimer, membrane**

I. Purpose

Ras is predominantly monomeric in solution but readily forms dimers and clusters in cells where it is membrane-bound, suggesting a role of the membrane in promoting Ras-Ras interaction [1,2]. To understand how Ras forms dimers and clusters on the membrane, we used single particle tracking photoactivated localization microscopy (spt-PALM) to quantitatively measure the diffusion of Ras in living cells at the single-molecule and nanometer scales [3]. spt-PALM yields tens of thousands of single-molecule trajectories with 10-35 millisecond time resolution and 20-30 nm spatial precision, from which Ras membrane diffusion models could be inferred via statistical analysis. We quantified the diffusion, occupancy, and transition rates for each state, which were in simulations to test the hypothesis that *Ras dimers and clusters primarily form in specialized membrane compartments.*

II. Results

A. Ras exhibits three distinctive diffusion states

The large spt-PALM dataset enabled the use of statistical analysis such as variational Bayes SPT to derive the diffusion states, occupancy, and state-transition rates unavailable through bulk measurements or conventional SPT [4]. We observed three distinctive diffusive states of Ras on living U2OS cell membranes as listed below (D: diffusion coefficient, error: standard deviation):

	State 1	State 2	State 3
D ($\mu m^2/s$)	0.07 ± 0.01	0.26 ± 0.05	0.84 ± 0.07
Occupancy (%)	17.4 ± 6.8	38.9 ± 9.0	43.7 ± 13.5

Further, Ras switches between the fast diffusive state (state 3) and the immobile state (state 1) predominantly by going through the intermediary state (state 2).

B. Membrane nanodomains trap and enrich Ras

We identified membrane regions that are about 40 nm in radius and are correlated with the slowest diffusive state of Ras; these regions are termed Ras-associated nanodomains (RANDs). Trajectory analysis also indicated at least two populations of RANDs, a transient population lasting for 1-2 s on average and a stable population lasting up to 15 minutes. Importantly, each RAND can contain multiple Ras molecules, providing a mechanism for Ras to be locally enriched, which potentially facilitates Ras-Ras interactions.

C. Initial simulations show RANDs promote Ras multimers

To further determine the role of RANDs in facilitating Ras dimer and cluster formation, we have run computer simulations with experimentally derived Ras diffusion and RAND parameters to compare Ras interactions in fast and immobile (inside RANDs) diffusive states. Preliminary results suggest that in general, Ras molecules trapped in RANDs are more likely to yield dimers and clusters than those that are in the fast diffusive state. There is an optimal RAND to Ras ratio for maximal Ras dimer and cluster formation, with a low ratio yielding a low fraction of total Ras in RANDs and a high ratio yielding too few Ras per RAND. Additional simulations will be tested with the Ras diffusion parameters from section A.

III. Conclusion

Our work suggests that membrane nanodomains (RANDs) can increase the local concentration of Ras to potentially facilitate Ras dimer and cluster formation. Ras molecules can interact with multiple types of RANDs, indicative of parallel mechanisms that could regulate Ras dimer and cluster formation. Lastly, the majority of Ras molecules enter an intermediary state before interacting with RANDs, implying that RANDs are likely associated with specialized membrane compartments. The same paradigm may apply to other membrane molecules.

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