BIOCHEMISTRY AND MOLECULAR BIOLOGY SEMINAR SERIES

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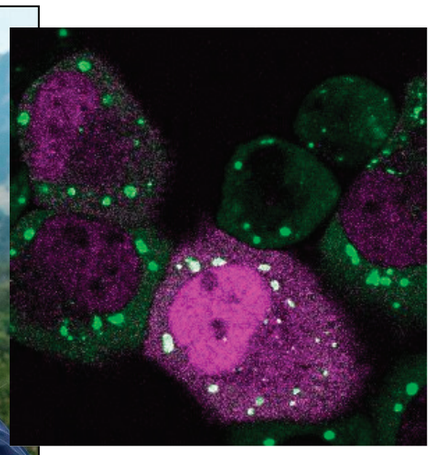

Protein (green) and RNA (pink) labeling in live mammalian cells. Braselmann et al, Nat. Chem. Bio., 2018

Visualizing RNA Dynamics in Live Cells by Fluorescence Lifetime Imaging Microscopy

Central roles for ribonucleic acids (RNAs) continue to be discovered across all domains of life. Subcellular RNA localizations in space and time are closely linked to their function, motivating the need to robustly visualize RNAs and their dynamics in live cells. In contrast to the protein world, no genetically encoded fluorescent RNAs were found in nature, leading to intense tool development efforts. We are redesigning a bacterial RNA riboswitch as a fluorescence sensor, called *Riboglow*[1]. The riboswitch RNA can be genetically fused as a tag to an RNA of interest. The natural riboswitch ligand Cobalamin (Cbl, vitamin B12) serves as a small molecule probe that includes a synthetic fluorophore. Binding of the probe to the RNA tag induces a change in probe fluorescence intensity and fluorescence lifetime. We exploit fluorescence lifetime changes of the probe upon binding the RNA tag to demonstrate that fluorescence lifetime imaging microscopy (FLIM) is an advantageous modality of *Riboglow* for RNA sensing in live cells.[2] First, we demonstrate that cellular contrast is superior for lifetime imaging compared with intensity imaging. Second, the intensity independence of FLIM makes it unnecessary to multiplex the RNA tag to achieve intensity-contrast, a common strategy for traditional RNA fluorescent tags. Third, versatility of *Riboglow* probes allows for using far-red fluorophores, enabling visualizing RNA dynamics in multicellular, complex environments.[3,4] Finally, we exploit the phylogenetic diversity of the riboswitch sequence and show that different RNA tag sequences bind the ligand while changing fluorescence lifetime to different extends. This feature leads us to explore *Riboglow*FLIM as an RNA multiplexing sensor to detect different RNAs simultaneously in the same cell. Together, we demonstrate that FLIM is an advantageous approach for RNA sensing that addresses many current challenges in the field of RNA imaging tool development.

1 Braselmann, E. *et al. Nat. Chem. Biol.* 14, 964-971 (2018)
2 Sarfraz, N., *et al. Nat. Comm.*, 14(1):867 (2023)
3 Sarfraz, N., *et al. Biophys. Rep.*, 3(4):100132 (2023)
4 Sarfraz, N., *et al. RSC Chem. Bio.*, 5(2):109-116 (2024)

Tuesday, March 26, 2024 • 4:00 PM
Science Center Room 200
Light snacks and drinks will be provided

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