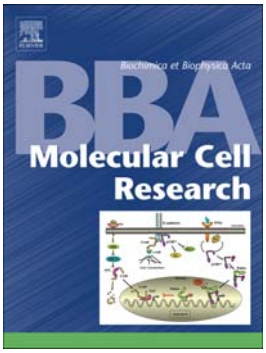

Tracking and Localization of Calmodulin in Live Cells

Carey K. Johnson, Gregory S. Harms

PII: S0167-4889(16)30121-5
DOI: doi: 10.1016/j.bbamcr.2016.04.021
Reference: BBAMCR 17864

To appear in: *BBA - Molecular Cell Research*

Received date: 11 February 2016
Revised date: 19 April 2016
Accepted date: 20 April 2016

Please cite this article as: Carey K. Johnson, Gregory S. Harms, Tracking and Localization of Calmodulin in Live Cells, *BBA - Molecular Cell Research* (2016), doi: 10.1016/j.bbamcr.2016.04.021

Tracking and Localization of Calmodulin in Live Cells

Carey K. Johnson,[a,*] Gregory S. Harms[b,c]

[a]Department of Chemistry, University of Kansas, Lawrence, Kansas

[b]Rudolf-Virchow-Zentrum, University of Würzburg, Würzburg, Germany

[c]Department of Biology and Health Sciences, Department of Electrical Engineering and Physics, Wilkes University, Wilkes-Barre, Pennsylvania

*Corresponding author. Email: ckjohnson@ku.edu

Abstract

The calcium signaling protein calmodulin (CaM) interacts with many target proteins inside the cell to regulate a wide range of biological signals. CaM's availability to propagate signals depends on its mobility, which may be regulated by interactions with multiple target proteins. We detected single molecules of CaM labeled with a fluorescent dye and injected into living HEK 293 cells, and we used high-speed, wide-field, single-molecule imaging to track single CaM molecules. Single-molecule trajectories were analyzed to characterize the motions of individual CaM molecules. Single-molecule localization resolved CaM positions with a position accuracy of < 100 nm, permitting sub-diffraction imaging of features with localized CaM that form in response to increased free Ca^{2+}. Single-molecule tracking demonstrated the presence of a wide range of mobilities of individual calmodulin molecules in a cell, with diffusion coefficients ranging from < 0.01 $\mu m^2\ s^{-1}$ to ~ 5 $\mu m^2\ s^{-1}$, whereas analysis by spatio-temporal image correlation spectroscopy revealed faster-moving components with diffusion coefficients of > 10 $\mu m^2\ s^{-1}$. For molecules confined to small regions of the cell, super-resolved images of presumed signaling complexes were recovered. Individual trajectories were classified as normal diffusion,