Development of Light-Responsive Metal Chelators and Fluorescent Indicators to Probe Calcium Oscillation

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Calcium (Ca^{2+}) is one of the most important metal ions necessary for normal brain function. Specifically, it is an integral component of signal transduction, where it acts as a second messenger to exert control over membrane excitability, synaptogenesis, and neurotransmission. To achieve control over such a diverse range of cellular functions, the concentration of cytosolic Ca^{2+} is tightly regulated to maintain a resting-state concentration 20,000-fold more dilute than extracellular Ca^{2+} concentrations. However, upon stimulation, extracellular Ca^{2+}, as well as Ca^{2+} in the mitochondrial and endoplasmic reticulum stores are rapidly released down this gradient into the cytoplasm, elevating levels by up to 100-fold. This change triggers downstream signaling events such as activation of the Ca^{2+}-dependent transcription factor, NFAT, to initiate transcription. While this bulk phenomena has been appreciated for decades, the relative contributions of calcium oscillation frequency, amplitude, and duty cycle to transcriptional activity remain unclear. In order to understand this fundamental biological process, we are developing light-responsive Ca^{2+} chelators and fluorescent reporters based on a tetra-acetate motif to mimic the Ca^{2+} waveforms using light and to visualize the consequent Ca^{2+} oscillation via confocal imaging. An important consideration in the design of these chemical tools is that the binding affinity of the light-responsive chelator must be greater than the fluorescent indicator to maximize the linear dynamic range. Additionally, the binding affinity must be altered upon irradiation with light to ensure rapid release the bound metal ion. Progress toward both of these aims are reported.