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Cell-penetrating nanobiosensors for pointillistic intracellular Ca 2+-transient detection

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Abstract

Small-molecule chemical calcium (Ca2+) indicators are invaluable tools for studying intracellular signaling pathways but have severe shortcomings for detecting local Ca2+ entry. Nanobiosensors incorporating functionalized quantum dots (QDs) have emerged as promising alternatives but their intracellular use remains a major challenge. We designed cell-penetrating FRET-based Ca2+ nanobiosensors for the detection of local Ca2+ concentration transients, using commercially available CANdot565QD as a donor and CaRuby, a custom red-emitting Ca2+ indicator, as an acceptor. With Ca2+-binding affinities covering the range of 3-20 μM, our CaRubies allow building sensors with a scalable affinity for detecting intracellular Ca2+ transients at various concentrations. To facilitate their cytoplasmic delivery, QDs were further functionalized with a small cell-penetrating peptide (CPP) derived from hadrucalcin (HadUF1-11: H11), a ryanodine receptor-directed scorpion toxin identified within the venom of Hadrurus gertschi. Efficient internalization of QDs doubly functionalized with PEG5-CaRuby and H11 (in a molar ratio of 1:10:10, respectively) is demonstrated. In BHK cells expressing a N-methyl-d-aspartate receptor (NMDAR) construct, these nanobiosensors report rapid intracellular near-membrane Ca2+ transients following agonist application when imaged by TIRF microscopy. Our work presents the elaboration of cell-penetrating FRET-based nanobiosensors and validates their function for detection of intracellular Ca2+ transients.

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Keywords

cell penetrating peptide, FRET-based calcium probes, intracellular calcium fluorimetry, nanoparticle surface chemistry, Quantum dot biosensors, red-emitting calcium indicator