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

Zamaleeva A., Collot M., Bahembera E., Tisseyre C., Rostaing P., Yakovlev A., Oheim M., De Waard M., Mallet J., Feltz A.
Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

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