Presentation title:

A new quantum dot luminiscent probe for caspase 3/7 imaging inside cells based on Förster resonance energy transfer.

Abstract

Nowadays, luminescent semiconductor quantum dots (QDs) are widely applied in different areas due to their unique optical properties. Modern bioanalytical technologies and instrumentations of laser-induced fluorescence or bioluminescence offer the possibility to study to analyze and understand biological phenomena at a cellular or even molecular level.

Our research is focused on the fluorescence microscopy analyses of biologically active molecules, such as proteolytic active caspases, which play important roles in cell signaling regulations in normal and diseased states. Consequently, they are attractive targets for clinical diagnosis and medical therapy.

In this work, synthesis and testing of a novel quantum dot luminescent probe is presented. The caspase enzyme reaction is based on the specific cleavage of the DEVD peptide sequence. Thus, the BHQ-2 quencher is released, the Förster resonance transfer between quantum dot and quencher is interrupted, and consequently the red light luminescence of the quantum dot is emitted (**Figure 1**).

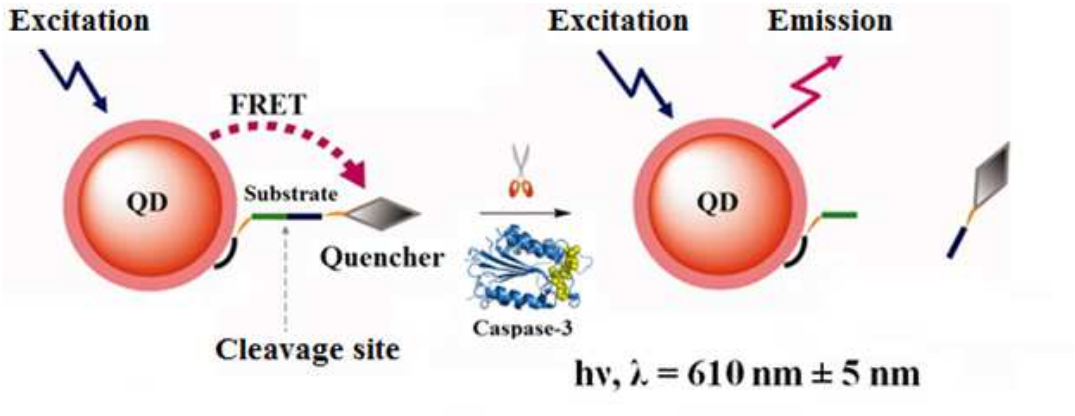

Figure 1 Scheme of the quantum dot FRET-based luminescent probe reaction with cleaving enzyme.

Implementation of molecular probes based on Förster resonance energy transfer (FRET) between a highly luminescent quantum dot (QD) as a donor and a fluorophore or fluorescence quencher as an acceptor linked by a specific peptide bring several advantages. Thus, QD luminescence, effectively dissipated in the probe, is switched on after the cleavage of the peptide and the release of the quencher. According to this principle, we proposed a novel synthesis strategy of a probe. Our two steps synthesis consists of: (i) Conjugation of CdTe QDs functionalized by -COOH groups of succinic acid on the nanoparticle surface with the designed specific peptide (GTADVEDTSC) using a ligand-exchange approach; (ii) A fast, high-yield reaction of amine-reactive succinimidyl group on the BHQ-2 quencher with N-terminal of the peptide. This way, any crosslinking between individual nanoparticles and any nonspecific

conjugation bonds are excluded. The analysis of the product after the first step proved a high reaction yield and nearly no occurrence of unreacted QDs, a prerequisite of the specificity of our luminescent probe. The synthesized luminescent probe was tested by a model reaction with active human recombinant caspase-3 protein in quartz cuvette of a fluorimeter. The parameters of enzymatic kinetics were evaluated as Michaelis-Menten description.

The ultimate goal of the presented work was to synthesize a new QD luminescent probe for a long-time quantitative monitoring of active caspase-3/7 distribution in apoptotic osteoblastic MC3T3-E1 cells treated with camptothecin (**Figure 2**).

As a result of comparison, under fluorescence microscope, our synthetized luminescent probe, provides much longer observation of active caspases in living cells than commercial products. The probe proved the stability of the luminescence signal inside cells for more than 14 days.

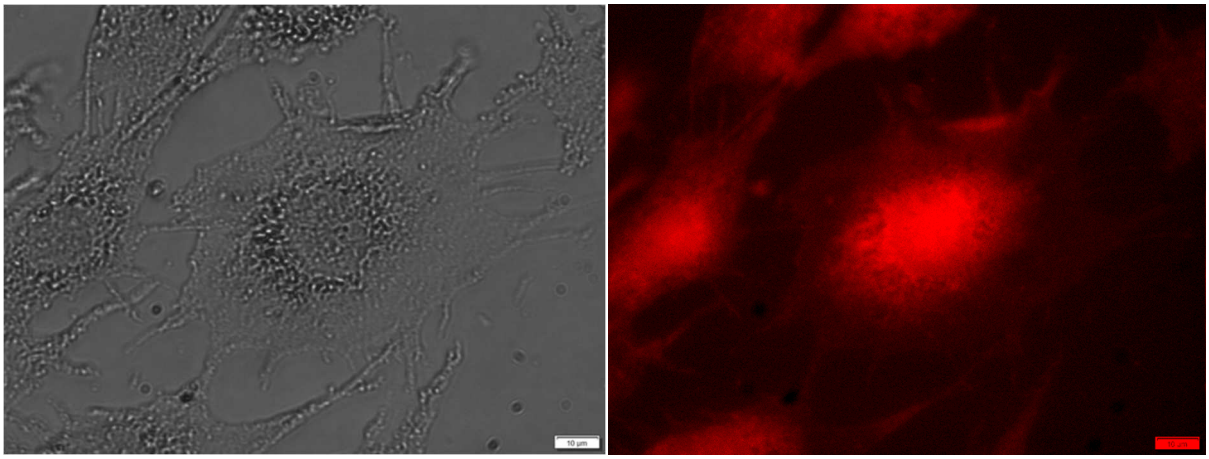

Figure 2 Caspase-3/7 activation in apoptotic MC3T3-E1 cells treated with camptothecin for 24 hours after incubation with the QD luminescent probe. Phase contrast in white light of respective sample (left side) and luminescence of QD luminescent probe (right side). Scale bar = 20 μm.

Acknowledgement

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