Orthogonal FRET reporters for the real-time sensing of lysosomal proteases

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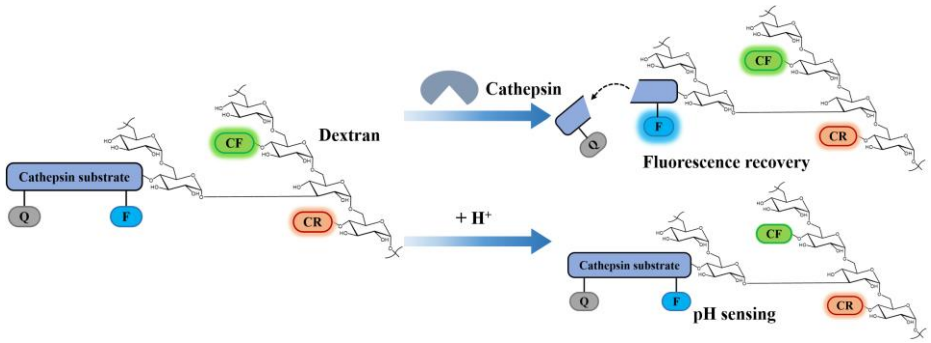
Cysteine proteases, one of the essential protease families with 11 members, are responsible for the non-specific, bulk proteolysis in the acidic environment of the endosomal or lysosomal compartment for degrading intracellular and extracellular proteins.[1] Increased expression and proteolytic activity of lysosomal cysteine proteases have often been correlated with poor prognosis for patients with a variety of malignancies.[2] Cathepsin B (CtB) is known to be overexpressed in various cancers, particularly in aggressive cancers, making it an attractive target for tumor-specific prodrug design. [3] In the acidic environment of lysosomes, procathepsin B can undergo autocatalytic activation, leading to formation of active CtB.[3] Alternatively, CtB can be activated by cathepsin D (CtD), which is an aspartic endo-protease universally found in lysosomes.[4] Therefore, a reporter for acidic environment or CtD would be a robust evidence for characterizing the activated CtB *in vivo*.

In this study, we report a dual-reporter approach to reporting activated CtB in cellular organelle. Peptidic substrates for CtB and CtD were synthesized and modified with FRET pairs for directly sensing the activity of CtB and CtD. These CtB and CtD sensors were loaded on dextran, a cell-permeable vehicle.[5] Separately from that, a ratiometric fluorescent pH probe was prepared by assembling two types of fluorophores on dextran for reporting the pH around the microenvironment of detected protease. Upon characterizing each sensor, the cathepsin sensor and the pH probe would be modified on dextran as orthogonal reporters.



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