VISUALIZATION OF β-SECRETASE CLEAVAGE IN LIVING CELLS USING A GENETICALLY ENCODED SURFACE-DISPLAYED FRET PROBE

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Alzheimer's disease (AD) is characterized pathologically by senile plaques containing the amyloid β-peptide (Aβ), a product derived from the sequential cleavage of amyloid precursor protein (APP) by β-secretase and γ-secretase [1]. The β-secretase, also named β-site APP cleaving enzyme (BACE), plays a key role in the generation of pathogenic Aβ in Alzheimer’s disease and has been identified as an ideal target for therapy.

Previous studies reported the monitoring of BACE activity in vitro utilizing chemical synthesized sensors [2]. Here we describe the first genetically encoded FRET probe (dYβC) that can detect BACE activity in vivo. The FRET probe was constructed with the BACE substrate site (BSS) and two mutated green fluorescent proteins. In living cell, the FRET probe was directed to the secretary pathway and anchored on the cell surface to measure BACE enzymatic activity. The results show that the FRET probe can be cleaved by BACE effectively in vivo (Figure 1), suggesting that the probe can be used for real-time monitoring of BACE activity. This assay provides a novel platform for BACE inhibitor screening in vivo.

Figure 1: Confocal imaging of surface-displayed fluorescence probes and spectral FRET imaging of the probes. (A) dYβC expressing HeLa cells. (B) HeLa cells co-expressing dYβC and BACE-mCherry. Only the CFP signal was prominent on the plasma membrane. (C) The control probe dYcC expressing HeLa cells. (D) HeLa cells co-expressing dYcC and BACE-mCherry. (E) The emission spectra of ROI 1 in A and ROI 2 in B with excitation at 458nm. (F) The emission spectra of ROI 3 in C and ROI 4 in D. The scale bar represents 10 μm.