Target-triggered hairpin assembly for the sensitive enzyme activity assay

이창열, 장효원, 박현규†
한국과학기술원
(hgpark@kaist.ac.kr†)

We herein describe an enzyme-free signal amplification strategy for the sensitive assay of ribonuclease H (RNase H) activity, which relies on the target-triggered catalytic hairpin assembly (CHA) with a G-quadruplex specific fluorescent binder, N-methyl mesoporphyrin IX (NMM). In the absence of RNase H, RNA/DNA duplex serving as a substrate for RNase H cannot initiate CHA that produces G-quadruplexes, so NMM shows the weak fluorescence. But, the presence of RNase H that degrades RNA in RNA/DNA duplex releases DNA designed to function as the catalyst for CHA. This consequently promotes the CHA to generate a large number of G-quadruplexes with the significantly enhanced fluorescence from NMM. Based on this strategy, we successfully determined the RNase H activity down to 0.037 U/mL and screened potential RNase H inhibitors. These results suggest that this system serve as a promising platform for the cost-effective, sensitive enzyme activity assay and inhibitor screening.