Development of sensitive RNase H activity assay using catalytic hairpin assembly

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We herein describe a label-free and enzyme-free signal amplification strategy for the sensitive determination of ribonuclease H (RNase H) activity, which relies on the target-triggered catalytic hairpin assembly (CHA) in conjunction with a G-quadruplex specific fluorescent binder, N-methyl mesoporphyrin IX (NMM). In the absence of RNase H, the RNA/DNA duplex serving as a substrate for RNase H cannot initiate the execution of CHA that produces G-quadruplexes; so NMM shows a low fluorescence signal. In contrast, the presence of RNase H that degrades RNA in the RNA/DNA duplex releases DNA designed to function as the catalyst for CHA. This consequently promotes the efficient CHA and generates a large number of G-quadruplexes with a significantly enhanced fluorescence signal from NMM. Based on this label-free and enzyme-free signal amplification strategy, we successfully determined the RNase H activity with a detection limit of 0.037 U mL−1 and screened potential RNase H inhibitors. Our results suggest that the developed system is a promising platform for a cost-effective, sensitive enzyme activity assay and inhibitor screening.