# Molecular sensor development for the detection of *hepatitis C virus* (HCV) using engineered RNA

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We designed the new aptazyme that was composed of hammerhead ribozyme with self-cleavage catalytic acitivity and aptamer with binding affinity on biomolecules and was used as a sensor for detection of biomolecules. The mechanism aptazyme is that the target molecule attaches on aptamer and then activated aptamer induces a conformational change of the ribozyme that has the self-cleavage activity on the catalytic domain. The increase of catalytic activity by target molecule is measured by the FRET assay using quencher and fluorescent.

In this study, we designed aptazyme for sensing of *hepatitis C virus* (HCV) replicase and helicase. For generation of aptazyme, *hepatitis C virus* (HCV) replicase aptamer domain or helicase aptamer domain was fused with the stem-loop II region of a hammerhead ribozyme. As increasing concentration of target molecule, *hepatitis C virus* (HCV) replicase and helicase, the catalytic activity was changed and specificity of designed allosteric ribozyme was measured by the FRET assay. As a result, allosteric ribozymes of joining with the aptamer binding part and hammerhead ribozyme exhibited higher catalytic activity as increasing of target concentration. The cleavage rate of hammerhead ribozyme was increased by up to ~8-fold than self-cleavage rate of hammerhead ribozyme. The detection limit of this system was several nM.

For the design of higher sensitive aptazyme, the aptamer domain of aptazyme was inserted in stem I and stem III of ribozyme. Namely, this aptazyme, we called bi-aptazyme, had two aptamer domains and a different structure with previous designed stem II aptazyme. In consequence of this study, bi-aptazyme was also activated by replicase or helicase and had two times higher catalytic

activity than stem II aptazyme with one aptamer binding site. Moreover this aptazyme had no background signal by self-cleavage. So catalytic activity of bi-aptazyme showed a reliable change as increasing of target concentration. The cleavage rate of hammerhead ribozyme was increased by up to ~16-fold than nature ribozyme. Our result showed the potential ability as RNA sensor for diagnosis and therapy of *hepatitis C virus* (HCV) with high sensitivity and selectivity.

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## References

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