LABEL-FREE, HOMOGENOUS DETECTION OF HCV HELICASE AND REPLICASE USING MOULAR ALOSTERIC ATAMER SENSOR (MAAS)

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Introduction. We report a new label-free, homogenous detection of HCV helicase and replicase using rationally-designed Modular Allosteric Aptamer Sensor (MAAS), which is composed of three parts. The first part, the malachite green (MG) binding aptamer as signaling domain was fused to the second part, each HCV helicase or replicase binding aptamer as recognition domain and the third part, inducing arm was added to make a conformational change of MAAS. Only three energy states, corresponding to each free aptamer sensor, target bound sensor, and both target and dye bound sensor were starting point for our rational design, and the energy difference between each state was the main driving force for sensor activation. Constructed MAAS could selectively detect HCV helicase and replicase, and activation of sensor was proportional to the amount of target protein present [1, 2]

Methods.

Modular allosteric aptamer sensor (MAAS)
Connection of two aptamers, HCV protein aptamer and MG aptamer. We added 'inducing arm' to the 5´ end of MG aptamer to complete our MAAS construction. Target protein binding makes conformational change of MAAS to release the inhibitory effect of inducing arm, which blocks MG binding to MAAS before target binding. Malachite green dye emits enhanced fluorescence (2,600 folds) upon binding to its target aptamer due to the reduced vibrational de-excitation, which makes 'label free' and 'homogeneous' detection of target protein.

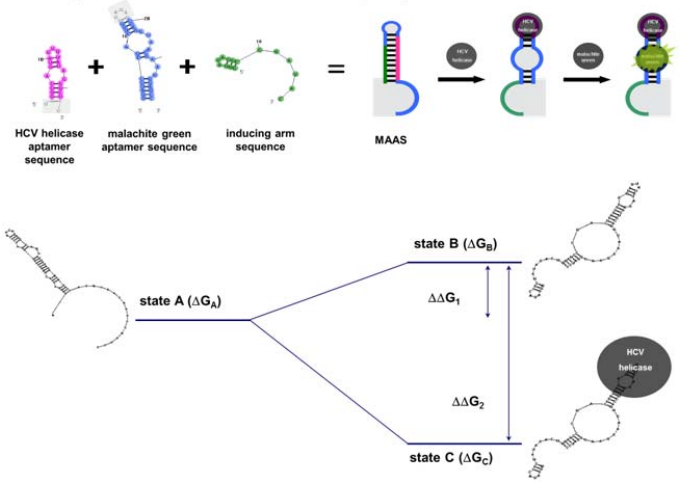

Fig.1 Design and allosteric regulation of modular allosteric aptamer sensor (MAAS)

Results.
Measurement of HCV helicase concentration.

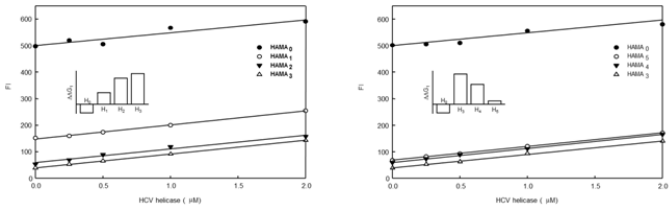

Fig.2 Measurement of HCV helicase concentration. (Left: Variation in module size, Right: Variation in inducing arm size).

1. We focused on the fluorescence intensity at zero helicase concentration, FI_0.
2. HAMA0, which has inverted energy state with $\Delta\Delta G_1$ of –1.5 kcal/mol, showed the highest background emission, as was expected due to the easy binding of MG to MAAS.
3. HAMA3, which has the most stable state of StateA ($\Delta\Delta G_1$ = 5.9 kcal/mol), showed the lowest background fluorescence emission.
4. $HAMA_2$ ($\Delta\Delta G_1$ = 4.9 kcal/mol) and HAMA3 ($\Delta\Delta G_1$ = 2.2 kcal/mol) showed gradual increase in background emission according to their relative instability of StateA.

Conclusions. ' Label-free' and 'homogeneous' protein detection system can eliminate many time and labor consuming experimental step such as immobilization, washing, separation of any component. And it gave additional cost saving in nucleic acid sensor preparation without covalent attachment of any fluorophores or any functional group. For this purpose, allosteric behavior of nucleic acid sensor was considered. Target binding induces allosteric conformational change of MAAS, to which structure signaling fluorescent dye, malachite green (MG), can bind. These sensors were modulated by recognition of target protein and showed good and sufficient responses for sensor application in simple, sensitive and selective modes.

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