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The re-design of a theophylline riboswitch for DNT sensing[1]
YAROSLAV CHUSHAK, Biotechnology HPC Software Applications Institute, USAMRMC, NANCY KELLEY-LOUGHNANE, SVETLANA HARBAUGH, MORLEY STONE, Air Force Research Laboratory, Wright-Patterson AFB — Riboswitches are noncoding elements of mRNA that recognize and bind to small molecules and regulate the translation process of downstream genes. As an initial study, we used a theophylline riboswitch that regulates the expression of the Tobacco etch virus (TEV) protease placed downstream of the switch as a controlling element. Upon expression of TEV protease, an optical reporter is cleaved producing change in fluorescence resonance energy transfer (FRET) between BFP and eGFP. We altered the sensing domain of the original construct to create a synthetic riboswitch that responds to the presence of 2,4-dinitrotoluene (DNT) molecules. Computational analysis using Autodock4 and AMBER9 software packages showed that U24A mutant has a significantly higher binding affinity for DNT molecule compared to the original theophylline. Cells expressing the re-designed riboswitch showed a marked optical difference in fluorescence emission in the presence of DNT molecules, leading to the potential of using this construct in biosentinel applications of highly nitrated compounds.

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Yaroslav Chushak
Biotechnology HPC Software Applications Institute, USAMRMC

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