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CENTER FOR ADVANCED BIOENERGY AND BIOPRODUCTS INNOVATION

Genetically Encoded Fluorogenic DNA Aptamers for Imaging Metabolite in Living Cells

Background/Objective

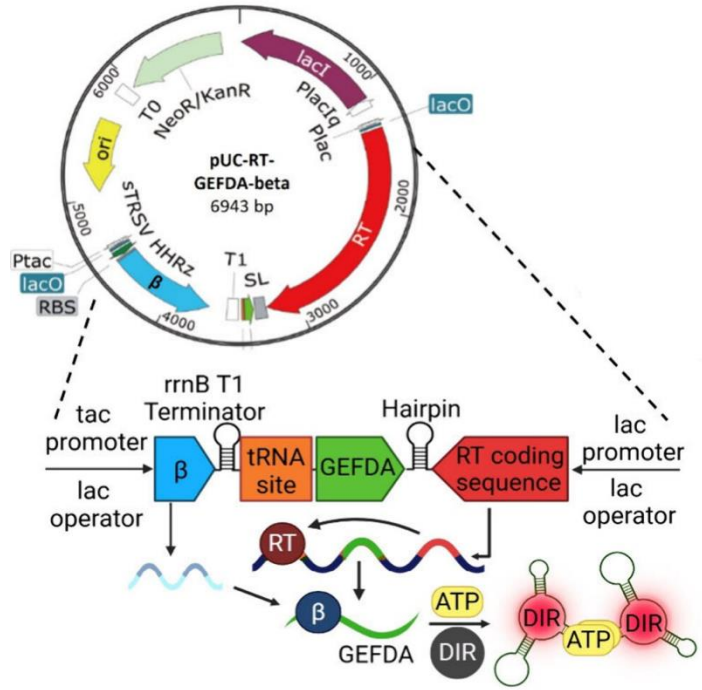
Genetically encoded fluorescent protein and fluorogenic RNA sensors are indispensable tools for imaging biomolecules in cells. To expand the toolboxes and improve the generalizability and stability of this type of sensor, we report herein a genetically encoded fluorogenic DNA aptamer (GEFDA) sensor that links a fluorogenic DNA aptamer for dimethylindole red (DIR) with an ATP aptamer.

Approach

We identified fluorogenic Lettuce aptamer plus DIR-specific Fluorogenic Aptamer (DIRFA) to increase in red fluorescence at 650 nm. Then, GEFDA was created by coupling DIRFA with an ATP aptamer and then dimerizing it to improve the signal-to-noise ratio and stability in living cells. GEFDA was integrated into a plasmid to image ATP in cells, and its effectiveness was validated.

Results

GEFDA enhanced red fluorescence by 4-fold at 650 nm in the presence of ATP, and the signal-to-noise ratio was improved by 2−3 folds after dimerization. The plasmid sensor showed enhanced stability over fluorogenic proteins and RNAs for real-time monitoring of a broader range of small molecular metabolites in biological systems.



Major elements of the plasmid that generates GEFDA sensor in bacterial cells. The reverse transcriptase (RT) binds to the tRNA binding site of the mRNA to initiate the reverse transcription of the GEFDA.

Significance/Impacts

This study provides a novel tool to monitor real-time dynamics of metabolites and other small molecules in biological systems.

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