AgriCos e-Newsletter
ISSN: 2582-7049
Volume : 01 Issue : 04 August 2020 Article No: 47

Genetically Encoded Biosensors: A New Method of Detecting Molecular Interactions

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SUMMARY

With the development of the new detection methods and the function of fluorescent molecule, researchers hope to further explore the internal mechanisms of organisms, observe changes in the intracellular microenvironment, and dynamic processes of molecular interactions in cells. For this purpose the exact location and nature of the interactions between specific molecular species in living cells is of major interest, but investigations are often hampered by the limited resolution of the instruments employed to examine these phenomena. In order to understand the physical interactions between proteins partners involved in a typical biomolecular process, the relative proximity of the molecules must be determined more accurately than diffraction-limited traditional optical imaging methods permit. This technique of fluorescence resonance energy transfer, permits determination of the approach between two molecules within several nanometers, a distance sufficiently close for molecular interactions to occur. FRET biosensors provide a robust tool for visualizing signaling molecules in live cells with high spatiotemporal resolution.

INTRODUCTION

In recent years, in vivo luminescence/ fluorescence biosensing has gained more attention as a means to non-invasively probe living animals under physiological conditions with subcellular resolution. The resulting images give insight into complex processes such as interaction between specific molecule in living cell and change in intracellular microenvironment etc via in situ monitoring. Thus for in vivo biosensing diverse array of biosensors has been developed of which the mostly and recently used is genetically encoded biosensor.

What is genetically Encoded Biosensor?

Genetically encoded biosensors consist of a sensory module coupled with fluorescent proteins (FPs) which can detected by fluorescence microscopy (Martiniere, 2013)

Specific Characters of genetically encoded biosensors

- Highly selective for a specific analyte
- Enables a quantitative readout
- Does not disturb the biological process

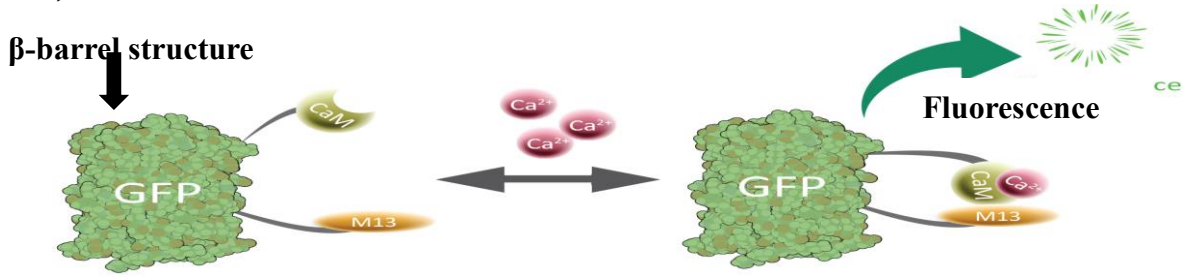
Types of genetically encoded biosensors

Single Fluorescent Protein (Single-FP) sensors and Fluorescence Resonance Energy Transfer (FRET) sensors

Single Fluorescent Protein (Single-FP) sensors

Single-FP sensors take advantage of the fact that the chromophore of FPs, usually well protected by the b-barrel structure around it which is sensitive to the change in oxidative status and the hydrogen bond network within its environment. Due to this fact, either an FP or FP–peptide chimera that responds to a ligand by changing the structure of the b-barrel (and hence the microenvironment of the chromophore) change its fluorescent property drastically, and therefore function as a sensor for the ligand.

E.g. pHluorins, DII-VENUS auxin sensor



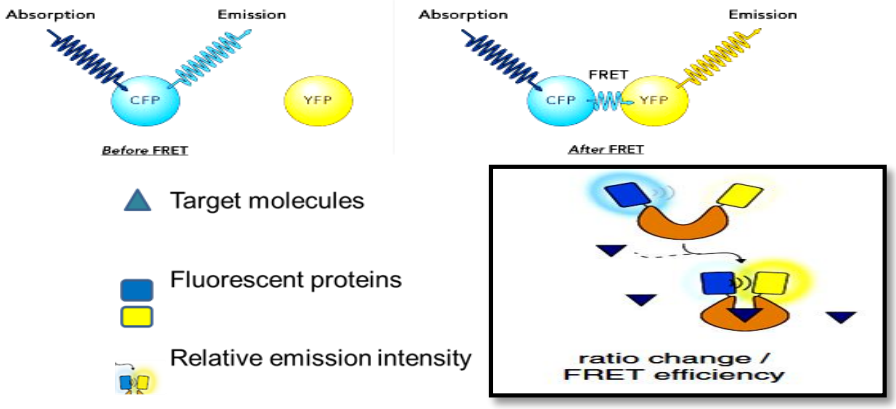
(β-barrel structure act as sensory molecule for ligand) (Okumoto, 2012)

Fluorescence Resonance Energy Transfer sensors (FRET)

Fluorescence Resonance Energy Transfer (FRET) is a physical phenomenon used in biology to observe interaction between specific molecule in living cell and change in intracellular microenvironment etc. First described over 50 years ago, that is being recently used more and more in biomedical research and drug discovery today. FRET relies on the distance-dependent transfer of energy from a donor molecule to an acceptor molecule. Due to its sensitivity to distance, FRET has been used to explore molecular interactions. FRET is the radiationless transmission of energy from a donor molecule to an acceptor molecule. (Morales-Narvaez and Merkoci, 2012)

Mechanism of FRET based biosensor

FRET sensors consist of a FRET donor–acceptor pair and a ligand-binding domain. The conformational change in the ligand-binding domain as soon as analyte binds to it, is revealed by the change of FRET efficiency between the attached donor and acceptor molecules



(Uslu, 2016)

Different applications of FRET in different areas of Agriculture

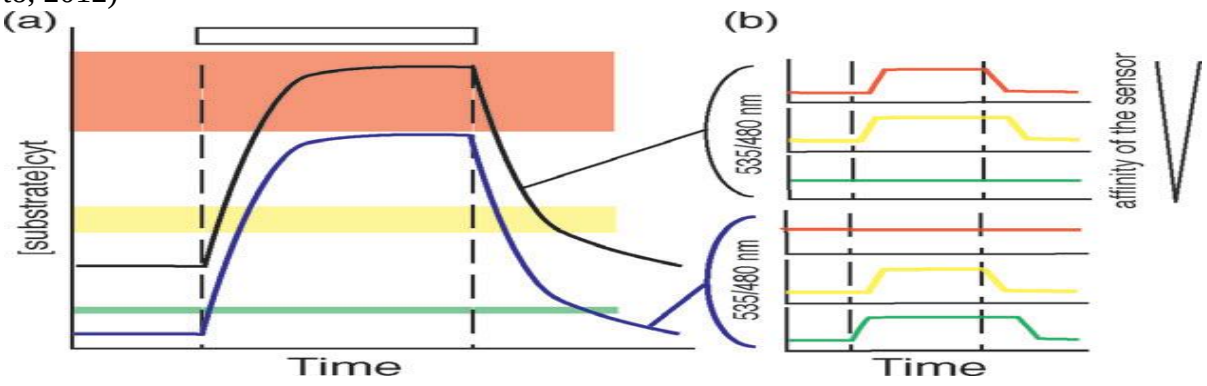
- Metabolite imaging
- Imaging of reactive oxygen species (ROS)
- Structural studies
- Conformational analysis
- pH analysis
- Interaction between molecules (Ex-Protein-Protein interaction)

1. Metabolite Imaging

Measuring metabolites at the cellular or subcellular level is a difficult task. Many central metabolites are synthesized, catabolized, and transported through different pathways, making it almost impossible to capture the concentration under a certain condition without introducing an artifact. Moreover, metabolites are highly compartmentalized, making it challenging to isolate an individual compartment. For these reason, a large number of genetically encoded FRET sensors for metabolites has been developed.

Ex- FRET glucose and sucrose sensor shown that cytosolic levels of sugar are fluctuated by extracellular concentration of these substrate.

(Okumoto, 2012)

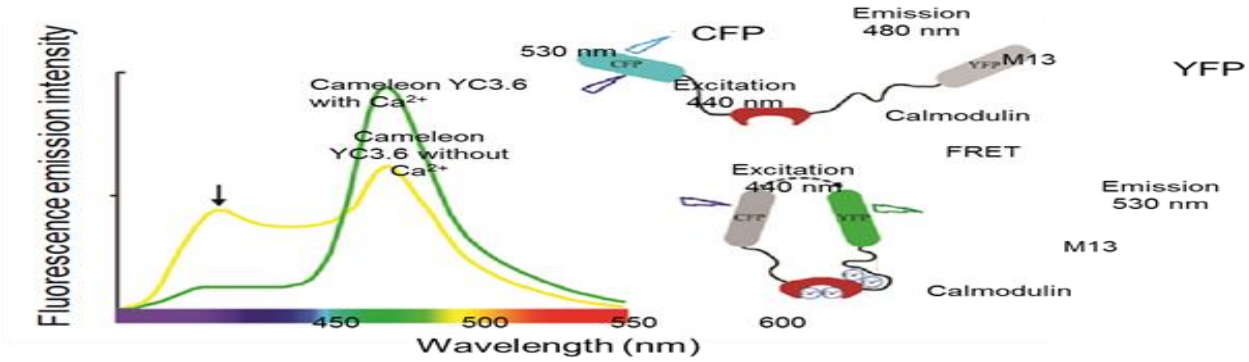


A schematic representation of two model cases where the steady state levels of a substrate are altered, and the response of sensors in each scenario.
(a) The changes in cytosolic concentration of a substrate. Two cell types with different steady-state levels of the substrate are represented as black and blue traces. The box above the trace indicates the time period when the substrate was externally supplied. Three shaded areas represent the working ranges of sensors with different affinities. Note that the sensors with higher affinity have the smaller absolute working range.
(b) The response of cytosolic sensors at higher (upper panel) and lower (lower panel) steady-state substrate concentration.

2. Imaging of Reactive Oxygen Species

Reactive oxygen species in plant cells constitute an important component in the transduction of a number of signals such as biotic and abiotic stress (Moller et al., 2007) Traditionally, various dyes such as dihydrodichlorofluorescein diacetate (H2DCF-DA, for H2O2) and nitroblue tetrazolium (NBT, for the superoxide radical) have been used to visualize different species of ROS in plant cells (reviewed in Swanson et al., 2011). However many of these chemicals need to be loaded into the cells, making the measurement difficult, and/or have some problems with photo-oxidation. Therefore, genetically encoded sensors that eliminate the loading process have gained importance amongst plant scientists in recent years.
EX- Hyper (OxyR) an genetically encoded sensor with cpYFP - H2O2 specific sensor which has been used to monitor H2O2 levels in cytosol and peroxisome of tabacoo and Arabidopsis (Costa *et al*., 2010)

3. Calcium Imaging



(Kanchiswamy *et al*., 2014)

In absence of free Ca2+, the donor protein (CFP) releases the absorbed energy as fluorescence at 480 nm. In the presence of Ca2+, the calmodulin and M13 domains bind the free Ca2+. The conformational change of chimeric protein allows FRET to occur between the donor fluorescent protein CFP and the acceptor fluorescent protein YFP with light emission at 530 nm.

Commercial Products

	Sensor	Description	Advantages
1	HyPer	Detection of H_2O_2	cificity sitivity
2	Case 12	Detection of Ca^{2+}	cificity amic range sitivity
3	Casper-3-BG	FRET Based Sensor	sitivity
4	Casper-3-GR	FRET Based Sensor	sitivity

CONCLUSION

Scientific evidence have successfully shown that Genetically encoded biosensors have high efficiency in in-vivo biosensing and FRET based biosensors provide a robust tool for Intracellular biomolecules analysis which also helps in monitoring the biological, chemical and molecular events.

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