GAVIN PUBLISHERS

Biosensors and Bioelectronics Open Access

Short Commentary

Eroglu E, et al. Biosens Bioelectron Open Acc: BBOA-104.

DOI: 10.29011/BBOA-104. 100004

# Looking at Cell Signaling: Years of Valuable Insights with Genetically Encoded Fluorescent Probes What Comes Next?

**Emrah Eroglu*, Markus Waldeck Weiermair, Wolfgang F Graier, Roland Malli**

Institute of Molecular Biology and Biochemistry, Medical University of Graz , Neue Stiftingtalstrasse, Graz, Austria

***Corresponding author:** Emrah Eroglu, Postdoctoral Researcher, Institute of Molecular Biology and Biochemistry, Medical University of Graz , Neue Stiftingtalstrasse 6/6, 8010 Graz, Austria. Tel: +4331638571958, Fax: +4331638579615; Email: emrah.eroglu@medunigraz.at

**Citation:** Eroglu E, Weiermair MW, Graier WF, Malli R (2017) Looking at Cell Signaling: Years of Valuable Insights with Genetically Encoded Fluorescent Probes What Comes Next?. Biosens Bioelectron Open Acc: BBOA-104. DOI: 10.29011/BBOA-104. 100004

**Received Date:** 29 September, 2017; **Accepted Date:** 12 October, 2017; **Published Date:** 20 October, 2017

## Summary

Fluorescent protein-based techniques have revolutionized our understanding of cellular processes. Almost any biological process can be investigated in detail with high spatial and temporal resolution using differently colored fluorescent protein variants. However, often the development and usage of these sophisticated probes is restricted to a limited number of specialists. Further developments that aim to generate high performance FP-based probes, high efficient gen transfer methods and easy-to-use powerful imaging systems are required to gain the usability of this enlightening technique. When these issues are solved multiple fluorescent protein-based tools will evolve from promises to fully mature probes that will more and more be used as matter of routine.

## Introduction

One of the most ambitious approaches to understand the miracles of life is to visualize the structure and functional processes in intact living cells: the basic units of all living organisms. Cells consist of thousands of different molecules with distinct structure, characteristics and function. Different cell types have different morphologies and fulfill different essential functions in multicellular organisms. Cells are able to grow, replicate, differentiate, sense, respond, communicate, mature, age and, ultimately, die. Occasionally, cells become dysfunctional causing all kind of organ malfunctions as basis of disease. Understanding of how cells actually function, how they respond to changes in their microenvironment, and how cell functions and dysfunctions can be manipulated and normalized has been occupying scientists from different fields all over the world since decades.

Since approximately 20 years Fluorescent Protein (FP) -based tools allow looking at processes within cells with high spatial and temporal resolution[1-3]. Mainly based on the pioneering work of Osamu Shimomura, who identified and characterized the Green Fluorescent Protein (GFP) from the jellyfish *Aequorea victoria*, Martin Chalfie, who was the first expressing GFP successfully in bacteria and worms, and Roger Y. Tsien, who developed several colorful GFP variants and fluorescent probes, a new bright era of real time imaging of cell signaling events has been launched (reviewed in [3]). FPs equipped with distinct targeting sequences has become powerful tools to label cellular organelles and structures. Expression of organelle targeted FPs allows imaging of organelle dynamics [4,5], morphological changes of organelles and organelle-organelle interactions [5-7] in real time.

FPs fused to proteins of interest are frequently used to visualize subcellular distribution of proteins [8,9], protein translocations [8,10] and protein-protein interactions [8,11] in lifetime on the single cell level. Using such simple FP-based constructs numerous cellular phenomena such as the fission and fusion events of mitochondria [5,7], the assembly and disassembly of elements of the cytoskeleton [12], the $Ca^{2+}$-induced oligomerization and translocation of distinct proteins [10,13] and many other cellular spectacles could be discovered, visualized, quantified and investigated with high precision and on the molecular level.

## The Power of Genetically Encoded Fluorescent Probes (GEFPs)

A further sophisticated approach to use FPs is their implementation as fundamental components of so called Genetically

Encoded Fluorescent Probes (GEFPs) [14,15]. Usually, GEFPs are carefully designed chimeric constructs that are composed of a naturally-specific sensor domain fused to one or two FPs. Binding of the natural mediator (e.g. ion, protein, lipid or small molecules like ATP) to the respective specific sensor (binding) domain or the modification of the sensor domain by a biochemical process within the cell (e.g. (de-)phosphorylation, cleavage) affects the spectral properties of the attached FPs, which can be measured in real time using fluorescence spectroscopy or microscopy. Accordingly, changes of fluorescence intensities of FPs in GEFPs report intracellular changes of the concentration, kinetics and/or the activity of the analyte, which can be an ion [16-19], a metabolite [20-22], a substrate [23], or an enzyme [24-26], and eventually the activity status of certain cell signaling pathways. As the molecular processes of functioning of all different GEFPs is plagiarized from nature, every cell signaling event can be principally visualized with respective GEFPs. Hundreds of different GEFPs have been developed in order to answer specific questions and to discover complex phenomena in cell biology, biochemistry and medicine.

GEFPs composed of FPs with different spectral properties can be combined [27], or combined with small chemical fluorescent sensors [28] in order to record different signaling events simultaneously in single individual cells. Such simultaneous recordings of cellular process by distinct sensors with high resolution in time and location allow multidimensional acquisitions of cell signaling mechanisms. Accordingly, the usage of GEFPs undoubtedly enables to overcome previous limits and to explore new frontiers in biology research from different fields. Moreover, such experiments highly motivate researchers to ask new questions, design novel informative experiments, and develop further original GEFPs.

## Improving the usability of GEFPs

However, the appropriate design, construction and effective usage of GEFPs are not so trivial [29]. Despite the enormous potential of GEFPs, there are only a limited number of specialist research groups that continuously develop and improve GEFPs. In addition, the usage of many sophisticated GEFPs is often restricted to those scientists who actually have invented the sensor. What are the main reasons that most of the already available and novel GEFPs are not used as a matter of routine in most laboratories by many scientists that perform research related to cell biology? What is actually required to increase the usability and distribution of GEFPs? GEFPs, as the name implies, are encoded by DNA and the respective genetic information has to be transferred effectively and without injury into the cells of interest. While there are several transfection procedures and transgenic technologies available that basically allow the insertion of DNA coding for GEFPs, most of these procedures are optimized for a limited number of cell lines, tissues and whole organisms.

Often scientists consider GEFPs as unusable tools as they have experienced huge difficulties in transfecting their cells of interest. Though the viral transfer has been found to overcome limitations of the gene transfer, in whole animals but also sensitive cells, the application of viral infection to achieve transfection with the sensor DNA/RNA is limited. An extension of the spectrum of effective transfection methods and transgenic technologies will certainly increase the applicability of GEFPs in future. The coding DNA for many GEFPs is under the control of a strong viral promotor so that high levels of GEFPs are produced within several hours after incorporation of the genetic information. However, high expression rates can induce cell stress and dysfunctions such as the unfolded protein response [30] and, hence, can limit the usability of GEFPs.

The availability of GEFPs under the control of promotors with different activities and inducible promotors would help in optimizing the actual concentration of sensors for different cell types. GEFPs for $Ca^{2+}$ imaging has been continuously improved mainly by exchanging classical GFP variants with novel brighter, more photostable, and less pH-sensitive FPs [2,31,32]. Such developments results in more robust GEFPs with different spectral properties ranging from blue to red and considerably better signal to noise ratios. However, often the dynamic range of GEFPs is rather poor with only a maximal change in the fluorescence signal of 5-10%. Using such sensors appropriately requires trained users and optimized imaging setups. In order to expand utilization of these sophisticated tools to laboratories that are not specialists in fluorescence microscopy but highly benefit from using GEFPs in their research, affordable and easy-to-use devices with improved hardware and software components for automatic imaging and analyzes that are optimized for live cell imaging with GEFPs are urgently required.

Light emitting protein-based sensors have become indispensable tools in modern cell biology. They enable visualization of cell signaling with (almost) unlimited spatial and temporal resolution. More than all other methods GEFPs have taught us so much about the precision and beauty of cellular processes that maintain life. Future developments in the design and usage of GEFPs, along with achievements in molecular biology, physics, chemistry, microscopic techniques, and computer science, these probes will serve among researchers' best tools in exploring life's mysteries.

## References

1. Olenych SG, Claxton NS, Ottenberg GK, Davidson MW (2007) The fluorescent protein color palette. In: Curr Protoc Cell Biol Chapter 21(2007) Unit 21.5.

2. Depry C, Mehta S, Zhang (2013) Multiplexed visualization of dynamic signaling networks using genetically encoded fluorescent protein-based biosensors. Pflugers Arch 465: 373-381.

3. Miyawaki A (2008) Green fluorescent protein glows gold. Cell 135: 987-990.

4. Graier WF, Frieden M, Malli R (2007) Mitochondria and $Ca^{2+}$ signaling: old guests, new functions. Pflugers Arch 455: 375-396.

5. Liu X, Weaver D, Shirihai O, Hajnóczky G (2009) Mitochondrial 'kiss-and-run': interplay between mitochondrial motility and fusion-fission dynamics. EMBO J 28: 3074-3089.

6. V Eisner, G Csordás, and G. Hajnóczky (2013) Interactions between sarco-endoplasmic reticulum and mitochondria in cardiac and skeletal muscle - pivotal roles in $Ca^{2+}$ and reactive oxygen species signaling. J Cell Sci 126: 2965-2978.

7. Karbowski M, Cleland MM, Roelofs BA (2014) Photoactivatable green fluorescent protein-based visualization and quantification of mitochondrial fusion and mitochondrial network complexity in living cells. Methods Enzymol 547: 57-73.

8. Snapp E (2005) Design and use of fluorescent fusion proteins in cell biology. Curr Protoc Cell Biol Chapter 21(2005) Unit 21.4.

9. Wu B, Piatkevich KD, Lionnet T, Singer RH, Verkhusha VV (2011) Modern fluorescent proteins and imaging technologies to study gene expression, nuclear localization, and dynamics. Curr Opin Cell Biol 23: 310-317.

10. Malli R, Naghdi S, Romanin C, Graier WF (2008) Cytosolic $Ca^{2+}$ prevents the subplasmalemmal clustering of STIM1: an intrinsic mechanism to avoid $Ca^{2+}$ overload. J Cell Sci 121: 3133-3139.

11. VanEngelenburg SB and Palmer AE (2008) Fluorescent biosensors of protein function. Curr Opin Chem Biol 12: 60-65.

12. Kemper AG, Weissmann C, Reyher HJ, Brandt R (2012) Monitoring cytoskeletal dynamics in living neurons using fluorescence photoactivation. Methods Enzymol 505: 3-21.

13. Deak AT, Groschner LN, Alam MR, Seles E, Bondarenko AI, et al. (2013) The endocannabinoid N-arachidonoyl glycine (NAGly) inhibits store-operated $Ca^{2+}$ entry by preventing STIM1-Orai1 interaction. J Cell Sci 126: 879-888.

14. Mehta S and Zhang J (2011) Reporting from the field: genetically encoded fluorescent reporters uncover signaling dynamics in living biological systems. Annu Rev Biochem 80: 375-401.

15. Morris MC (2010) Fluorescent biosensors of intracellular targets from genetically encoded reporters to modular polypeptide probes. Cell Biochem Biophys 56: 19-37.

16. Domingo JS, Giacomello M, Poburko D, Scorrano L, Demaurex N (2013) OPA1 promotes pH flashes that spread betw`een contiguous mitochondria without matrix protein exchange. EMBO J 32: 1927-1940.

17. Miyawaki A, Llopis J, Heim A, McCaffery JM, Adams JA, et al. (1997) Fluorescent indicators for $Ca^{2+}$ based on green fluorescent proteins and calmodulin. Nature 388: 882-887.

18. Nagai T, Sawano A, Park ES, M iyawaki A (2001) Circularly permuted green fluorescent proteins engineered to sense $Ca^{2+}$. Proc Natl Acad Sci U S A 98: 3197-3202.

19. Quartier CJ, Bondarenko AI, Alam MR, Trenker M, Weiermair MW, et al. (2012) Studying mitochondrial $Ca^{2+}$ uptake - a revisit. Mol Cell Endocrinol 353: 114-127.

20. Vishnu N, Khan MJ, Karsten F, Lmfs NG, Weiermair W, et al. (2014) ATP increases within the lumen of the endoplasmic reticulum upon intracellular $Ca^{2+}$ release. Mol Biol Cell 25: 368-379.

21. Imamura H, Nhat KPH, Togawa H, Saito K, Iino R, et al. (2009) Visualization of ATP levels inside single living cells with fluorescence resonance energy transfer-based genetically encoded indicators. Proc Natl Acad Sci U S A 106: 15651-15656.

22. Martín AS, Ceballo S, Lehnert FB, Lerchundi R, Valdebenito R et al. (2014) Imaging Mitochondrial Flux in Single Cells with a FRET Sensor for Pyruvate. PloS one 9: e85780.

23. Veetil JV, Jin S, Ye K (2012) Fluorescence lifetime imaging microscopy of intracellular glucose dynamics. Journal of diabetes science and technology 6: 1276-1285.

24. Luo KQ, Yu VC, Pu Y, Chang DC (2003) Measuring dynamics of caspase-8 activation in a single living HeLa cell during TNFalpha-induced apoptosis. Biochem Bophys Res Commun 304: 217-222.

25. Tsou P, Zheng B, Hsu CH, Sasaki AT, Cantley LC (2011) A fluorescent reporter of AMPK activity and cellular energy stress. Cell Metab 13: 476-486.

26. Kunkel MT and Newton AC (2014) Imaging kinase activity at protein scaffolds. Methods Mol Biol 1071: 129-137.

27. Zhao Y, Araki S, Wu J, Teramoto T, Chang YF (2011) An expanded palette of genetically encoded $Ca^{2+}$ indicators. Science 333: 1888-1891.

28. Weiermair MW, Alam MR, Khan MJ, Deak AT, Vishnu N (2012) Spatiotemporal Correlations between Cytosolic and Mitochondrial $Ca^{2+}$ Signals Using a Novel Red-Shifted Mitochondrial Targeted Cameleon. PloS one 7: e45917.

29. Lindenburg L and Merkx M (2014) Engineering genetically encoded FRET sensors. Sensors (Basel, Switzerland) 14:

30. 11691-11713.

31. Jørgensen MM, Bross P, Gregersen N (2003) Protein quality control in the endoplasmic reticulum. APMIS Suppl 109: 86-91.

32. Rose T, Goltstein PM, Portugues R, Griesbeck O (2014) Putting a finishing touch on GECIs. Frontiers in molecular neuroscience 7: 88.

33. Miyawaki A, Griesbeck O, Heim R, Tsien RY (1999) Dynamic and quantitative $Ca^{2+}$ measurements using improved cameleons. Proc Natl Acad Sci U S A 96: 2135-2140.