Novel Biosensors to Monitor Cellular Events in Live Cells

REVIEW

Review of Fan, F. *et al.* (2008) Novel genetically encoded biosensors using firefly luciferase. *ACS Chem. Biol.* **3**, 346–51.

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Real-time signals of cellular events such as modulation by GPCRs can be obtained in minutes following treatment using this technology.

INTRODUCTION

Drug discovery and life science researchers desire to collect meaningful data that advance scientific knowledge. To accomplish this, often the data need to be gathered in a biologically relevant context. As the technologies available to scientists have advanced, the demand for cell-based assays, especially live-cell assays, has increased.

Recently Promega scientists published the first peer-reviewed article on a new technology that incorporates genetically modified forms of firefly luciferase used to detect molecular events within living cells (1). The paper demonstrates multiple approaches to constructing biosensors using circularly permuted or nonpermuted forms of luciferases. In brief, the scientists targeted the hinge region of the luciferase molecule using three design strategies, covalent, noncovalent and allosteric, to modulate enzyme activity (Figure 1).

To create the cAMP biosensor, for example, the scientists constructed a circularly permuted luciferase by placing new N- and C-termini in the middle of the protein. They then connected the native N- and C-termini with a cAMP binding moiety of protein kinase A (RIIβB). The resulting mutant form of luciferase emits increasing amounts of light in response to increasing concentrations of cAMP.

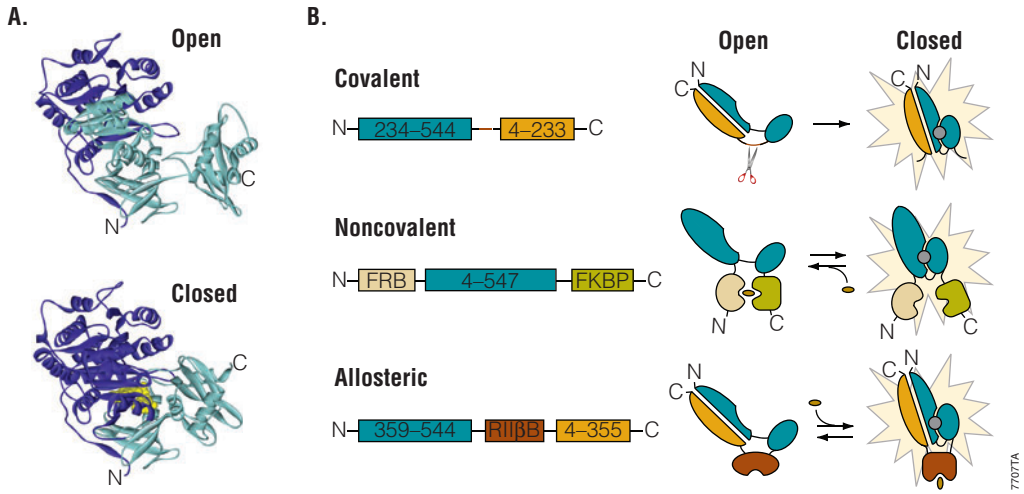

Figure 1. Design strategies for biosensors using firefly luciferase. A. Molecular models of firefly luciferase in the absence of substrates (open conformation) or bound to an analog of the luciferyl-adenylate reaction intermediate (closed conformation, analog colored yellow) generated using PDB files 1LCI and 2D1S, respectively. The smaller C-terminal domain of luciferase (residues 441–544) is predicted to rotate and translocate toward the larger N-terminal domain during the catalytic cycle. Residues 4–233 and 234–544 are colored blue and gray, respectively, for the 'open' conformation. Residues 4–355 and 359–544 are colored blue and gray, respectively, for the 'closed' conformation. Nonvisible residues at the N- and C-termini of PDB file 1LCI were typically excluded from the various biosensor design strategies. **B.** Schematic representation of the three design strategies used to generate luciferase biosensors. **Covalent.** Fusion of the wild-type N- and C-termini with a polypeptide containing a protease cleavage site inhibits formation of the closed conformation. Cleavage by protease relieves this constraint, allowing increased luminescence. **Noncovalent**. Association of polypeptides FRB and FKBP12 in the presence of rapamycin inhibits formation of the closed conformation, causing decreased luminescence. **Allosteric**. The conformational change of an analyte binding domain modulates luminescence, e.g., cAMP binding to RIIβB promotes increased luminescence. Reprinted with permission from Fan, F. *et al.* (2008) *ACS Chem. Biol.* **3**, 346–51.

BIOSENSOR ADVANTAGES

Intracellular biosensor technologies remain an area of keen interest that until recently was dominated mostly by fluorescent approaches. The most common examples are FRET-based biosensors using variants of GFP. However, FRET-based biosensors suffer from low dynamic range of response due to characteristics inherent in the detection modality (2). The results are FRET ratios of 30–100% or 2-fold response at best, which can be a significant drawback. In contrast, the genetically encoded firefly luciferase biosensor described by Fan *et al.* (1) exhibits 20-fold and greater response. This response is 10 times that of FRET. In addition, the detection of cAMP-mediated cellular events by the luciferase biosensor is reversible. Figure 2 illustrates the large change in light output and reversibility of the luminescent signal when the biosensor is used to interrogate endogenous β2-adrenergic receptor activity in HEK293 cells.

The key advantages of genetically encoded luciferase biosensors are better dynamic range and assay simplicity. Following stable or transient transfection of the biosensor, the cells are pre-equilibrated with the substrate and then treated with compounds that are known or suspected to modulate intracellular cAMP. There are no additional steps, and no specialized instrumentation or software is required. A standard luminometer with injectors is sufficient to detect signal readout and obtain real-time kinetic data, all without any loss in the information that can be collected. As evident in Figure 2, signal kinetics of reversible agonists and antagonists can be obtained in minutes following treatment. Important to HTS applications, these assays can be performed at room temperature with little difference in the range of response and more stable kinetics.

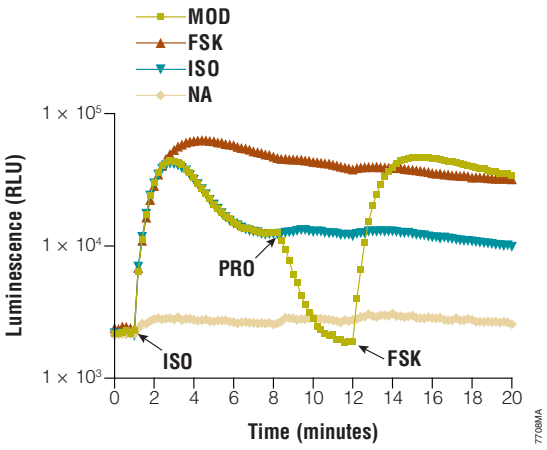

Figure 2. Allosteric cAMP biosensor. Signal kinetics and reversibility in living cells at 37 °C. HEK293 cells transiently expressing CP359-*Luc*/RIIβB were treated with 10 mM isoproterenol (ISO) or 10 mM forskolin (FSK) alone. Modulated cells were treated sequentially with 10 µM ISO, 10 µM propranolol (PRO), and 10 µM FSK (n = 3). Reprinted with permission from Fan, F. *et al.* (2008) *ACS Chem. Biol.* **3**, 346–51.

CONCLUSION

Louis Hodgson wrote in his review of the biosensor article in *ACS Chemical Biology*, "Making these genetically encoded chemiluminescence sensors is no easy task"(2). The GloSensor™ cAMP Assay[a], developed using Promega's expertise in bioluminescence, greatly simplifies this task for researchers. We encourage those interested in better understanding this new technology to view the original paper for a detailed description of how the various biosensors were developed and how they function.

REFERENCES

1. Fan, F. *et al.* (2008) *ACS Chem. Biol.* **3**, 346–51.
2. Hodgson, L. (2008) *ACS Chem. Biol.* **3**, 335–7.

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