

RESEARCH HIGHLIGHT

Using the newly developed nanoluciferase as an ultrasensitive bioluminescent probe for ligand-receptor interaction studies

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Nanoluciferase (NanoLuc) is a newly developed small monomeric luciferase reporter with the brightest bioluminescence reported to date. Recently, we have used NanoLuc as a novel ultrasensitive bioluminescent probe for ligand-receptor interaction studies. In the present highlight, I discuss its general application in ligand-receptor interactions and other binding studies. This novel ultrasensitive bioluminescent probe will facilitate a wide range of biological studies as its new applications are continuously developed.

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Nanoluciferase (NanoLuc) is the so far brightest bioluminescent reporter developed by the Promega Corporation in 2012 [1]. The new reporter produces a long half-life glow-type bioluminescence using furimazine and molecular oxygen as substrates, in an adenosine triphosphate-independent manner. In addition to high specific activity, NanoLuc has several other advantages, such as small size (171 amino acids, 19 kDa), high physical stability and lack of posttranslational modifications. Thus, this novel ultrasensitive reporter will have broad applications in biological studies. In the present highlight, I focus on its application in ligand-receptor interaction studies. Receptors are involved in the regulation of various biological functions. In general, a receptor receives a message from a specific molecule, termed a ligand, and transforms the message into a

downstream signal. The NanoLuc reporter can be used to monitor several aspects of ligand-receptor interactions, including ligand-receptor binding, ligand-induced receptor internalization and downstream signaling. Moreover, the NanoLuc reporter can also be applied to other binding studies, such as antigen-antibody binding, lectin-carbohydrate binding and protein-nucleic acid binding.

Monitoring ligand-receptor binding using the NanoLuc reporter

A receptor generally mediates the signal of a specific ligand. To receive the message from the ligand, a receptor needs to first capture the ligand. In vivo, the ligand concentration is very low and various ligands are present in a mixture, thus a receptor needs to bind its ligand with

high affinity and high specificity to efficiently capture the right ligand. Thus, ligand-receptor binding is the first step of a receptor-mediated signaling pathway. For ligand-receptor binding studies, receptor-binding assay is a widely used technique that can measure the binding potency of a ligand with its receptor, using a receptor source such as living cultured cells or crude membrane fractions with endogenous or overexpressed receptors. In this assay, an appropriately labeled ligand, termed a tracer, is used to monitor the ligand-receptor binding as the bound tracer can be sensitively and selectively quantified after the binding assay. Conventionally, radioactive tracers have been used for receptor-binding assays. For small chemical ligands, radioactive isotopes such as ^3H , ^{14}C , ^{35}S and ^{32}P are conventionally used to replace the corresponding stable isotopes; for protein/peptide ligands, radioactive isotopes such as ^{125}I and ^{32}P are conventionally used to label the ligand by chemical modification. However, the use of radionuclides has drawbacks, such as radioactive hazards and the short-half lives of the radioactive tracers. In recent years, lanthanide ions, such as Eu^{3+} , Tb^{3+} and Sm^{3+} , are used to label some ligands, especially protein and peptide ligands, as they can be sensitively and selectively quantified based on their long half-life fluorescence [2]. In a recent paper, we reported that the NanoLuc reporter could be covalently attached to a peptide ligand as a novel bioluminescent probe to monitor ligand-receptor binding in receptor-binding assays [3]. The NanoLuc probe is much more sensitive than conventional radionuclides and lanthanides. Thus, it represents an ultrasensitive probe for bioluminescent ligand-receptor binding assays.

To prepare the bioluminescent tracer, there are two approaches for covalent attachment of the NanoLuc reporter to the protein or peptide ligand: chemical conjugation and genetic fusion. The NanoLuc reporter can be efficiently overexpressed in *Escherichia coli* and easily purified to homogeneity. Using an appropriate chemical linker, the purified NanoLuc probe can be conjugated with the ligand. In our recent paper [3], we designed a 6×His-Cys-NanoLuc protein with a single exposed cysteine residue at its N-terminus for efficient chemical conjugation. Other engineered NanoLuc proteins carrying a unique reactive moiety for efficient coupling could also be designed and prepared in future work. This chemical conjugation approach can be used for covalent attachment of the NanoLuc reporter to proteins, peptides and other biomolecules, such as carbohydrates and nucleic acids. An alternative approach for NanoLuc attachment is genetic fusion for protein or peptide ligands. Using recombinant

DNA technology, NanoLuc can be fused at either the N-terminus or the C-terminus of the target protein or peptide, and the resultant fusion protein is then overexpressed in suitable host cells and purified as a bioluminescent tracer.

The NanoLuc reporter is much larger than radionuclides and lanthanides; therefore, its attachment could occasionally disturb the receptor-binding ability of the ligand. Thus, the tagging site of the NanoLuc reporter on the ligand should be carefully selected, and the receptor-binding ability of the bioluminescent tracer should be tested by a saturation receptor-binding assay. Once the NanoLuc-tagged ligand is confirmed to retain considerable receptor binding affinity, it could be used as an ultrasensitive tracer for bioluminescent competition receptor-binding assays to quantitatively measure the receptor-binding ability of various ligands.

Monitoring receptor internalization using the NanoLuc reporter

Besides attachment to a ligand to monitor ligand-receptor binding, the NanoLuc reporter can also be attached to a membrane receptor to monitor ligand-induced receptor internalization [4]. Using this approach, we could quantitatively measure the internalization of the only known iron efflux transporter, ferroportin, induced by the liver-secreted peptide hormone hepcidin, in transfected cells. Using this novel functional assay, we could quantify the potencies of various hepcidin analogs or screen novel agonists or antagonists of ferroportin. This novel quantitative receptor-internalization assay is based on the pH sensitivity of the NanoLuc reporter: it has a much lower enzymatic activity in acidic solution than in neutral solution. Once the NanoLuc-fused receptor was internalized from the cell membrane, it was ultimately sorted into lysosomes with an acidic environment (pH4–5). Thus, the measured NanoLuc activity would decrease significantly if intact living cells rather than cell lysate were used for the NanoLuc activity assay. We have demonstrated that the substrate furimazine can permeate the cell membrane and enter intracellular compartments efficiently [4]. In general, most of the membrane receptors will undergo internalization after binding with their ligands, thus this NanoLuc-based receptor internalization assay could be applied to other membrane receptors in which the NanoLuc reporter can be conveniently fused at the C-terminus or the N-terminus of the receptor using recombinant DNA technology. As the monomeric NanoLuc reporter is smaller than the widely used fluorescent protein tags, its attachment generally has little detrimental effect on the receptor's function and

intracellular trafficking.

Monitoring downstream signaling pathway using the NanoLuc reporter

After binding with a ligand, the receptor initiates downstream intracellular signaling. The NanoLuc reporter is very suitable for monitoring such intracellular signaling. For example, activation of a G protein-coupled receptor will usually change the intracellular cyclic adenosine monophosphate (cAMP) levels, which can be monitored sensitively by a cAMP-response element (CRE)-controlled NanoLuc reporter. The Promega Corporation has developed NanoLuc extensively as an intracellular reporter and interested readers can visit their web site for more information.

Monitoring other bindings using the NanoLuc reporter

In addition to ligand-receptor binding, there are various other biological binding processes, such as antigen-antibody binding, lectin-carbohydrate binding, protein-protein interactions and protein-nucleic acid interactions. The novel NanoLuc reporter could also be applied to these binding studies. For example, NanoLuc could be conjugated to antibodies for various bioluminescent immunoassays. Horseradish peroxidase (HRP) is conventionally used for antibody conjugation for enhanced chemiluminescent assays^[5]. HRP is a large carbohydrate protein purified from the roots of horseradish. We thought that NanoLuc-conjugation would have several advantages over the conventional HRP-conjugation. First, NanoLuc would have high biocompatibility and low background, because the endogenous luciferase activity is absent in common biosystems and the NanoLuc substrate is not spontaneously degraded. Second, NanoLuc has high detection sensitivity and low cost because of its higher specific activity and ease of preparation by recombinant expression in *E. coli*. Thus, we conceive that the novel

NanoLuc conjugation could replace the conventional HRP conjugation for antibody labeling in the future, especially for certain specialized assays, such as cell-ELISA, in which the endogenous peroxidase activity is a major barrier^[6]. Additionally, the novel NanoLuc-conjugation could be adapted to other biomolecules, including DNA, RNA and carbohydrates, for various ultrasensitive bioluminescent assays in the future. As novel applications of the NanoLuc reporter are continuously developed, this ultrasensitive reporter will greatly facilitate biological research.

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Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Hall MP, Unch J, Binkowski BF, Valley MP, Butler BL, Wood MG, *et al.* Engineered luciferase reporter from a deep sea shrimp utilizing a novel imidazopyrazinone substrate. *ACS Chem Biol* 2012; 7: 1848-1857.
2. Selvin PR. Principles and biophysical applications of lanthanide-based probes. *Annu Rev Biophys Biomol Struct* 2002; 31: 275-302.
3. Zhang L, Song G, Xu T, Wu QP, Shao XX, Liu YL, *et al.* A novel ultrasensitive bioluminescent receptor-binding assay of INSL3 through chemical conjugation with nanoluciferase. *Biochimie* 2013; 95: 2454-2459.
4. Song G, Jiang Q, Xu T, Liu YL, Xu ZG, Guo ZY. A convenient luminescence assay of ferroportin internalization to study its interaction with hepcidin. *FEBS J* 2013; 280: 1773-1781.
5. Marquette CA, Blum LJ. Chemiluminescent enzyme immunoassays: a review of bioanalytical applications. *Bioanalysis* 2009; 1: 1259-1269.
6. Liu Z, Gurlo T, von Grafenstein H. Cell-ELISA using β -galactosidase conjugated antibodies. *J Immunol Methods* 2000; 234: 153-167.