some embodiments, (a) is added extracellularly and enters the cell. In some embodiments, (a) is present both within a cell and in the medium surrounding the cell. In some embodiments, (a) is present both bound to a cell and in the medium surrounding the cell. In some embodiments, the amount of (a) present in the surrounding medium is significantly greater than the amount in the cell or bound to the cell, e.g., greater by at least 2 fold, at least 5 fold, 10 fold, 30 fold, or 100 fold. In some embodiments, the cellular target is a binding partner of the bioactive agent. In some embodiments, the emission spectrum of the bioluminescent reporter overlaps with the absorption spectrum of a fluorophore. In some embodiments, upon binding of the bioactive agent to the cellular target, conversion of the substrate to a reaction product by the bioluminescent reporter results in excitation of a fluorophore by BRET and light emission from a fluorophore. In some embodiments, (a) is one of a library of agents or compounds tethered to a chromophore. In some embodiments, (a) is one of a library of agents or compounds tethered to fluorophores. In some embodiments, (b) is one of a plurality of potential cellular targets fused to a bioluminescent reporter. In some embodiments, (b) is a library of cells expressing one of a plurality of potential cellular targets fused to a bioluminescent reporter.

In some embodiments, the present disclosure provides methods of detecting, analyzing, characterizing, etc. the binding of a bioactive agent to a cellular target. In an embodiment, the present disclosure provides a method of detecting the binding of a bioactive agent to a cellular target comprising the system as disclosed herein. In some embodiments, the cellular target may be the primary drug target. In some embodiments, the cellular target is an off-target liability that may cause undesirable side effects *in vivo*. In some embodiments, binding by the bioactive agent to the cellular target may have no discernible biological effect. In some embodiments, the present disclosure provides cells comprising one or more of (e.g., each of) (a) a bioactive agent tethered to a chromophore (e.g., fluorophore); (b) a cellular target fused to a bioluminescent reporter; and (c) a substrate for the bioluminescent reporter. In an embodiment, the present disclosure provides a cell comprising the system as disclosed herein. In some embodiments, the binding of a bioactive agent to a cellular target is non-covalent. In some embodiments, the chromophore is a fluorophore. In an aspect, the present disclosure provides methods for detection of an interaction between a small molecule bioactive agent and a cellular target comprising: (a) expressing in a cell a fusion of said cellular target and a bioluminescent reporter, the bioluminescent reporter having a first emission spectrum; (b) contacting said cell extracellularly with a conjugate of said small molecule bioactive agent tethered to a small molecule fluorophore, said small molecule fluorophore having an excitation spectrum that overlaps said first emission spectrum and having a second emission spectrum that is distinct from said first emission spectrum, wherein the conjugate is cell permeable, and wherein the small molecule bioactive agent is capable of binding non-covalently to the cellular target upon interaction therewith; (c) contacting said cell with a substrate for said bioluminescent reporter, wherein the substrate is cell permeable; (d) detecting fluorescence at within said second emission spectrum, wherein the intensity of said fluorescence within the second emission spectrum correlates with binding between said small molecule bioactive agent with said cellular target.

CLAIMS

1. An assay system comprising:

(a) a small molecule bioactive agent tethered to a small molecule fluorophore;

(b) a cell that expresses a protein fusion of: a cellular target and a bioluminescent reporter, wherein the small molecule bioactive agent is capable of binding non-covalently to the cellular target upon interaction therewith, wherein the emission spectrum of the bioluminescent reporter overlaps with the absorption spectrum of the fluorophore; and

(c) a substrate for the bioluminescent reporter.

2. The system of claim 1, wherein the fluorophore is a carboxy rhodamine analog.

3. The system of claim 1, wherein the bioluminescent reporter comprises a polypeptide with at least 70% sequence identity with SEQ ID NO.: 1.

4. The system of claim 1, wherein upon binding of the small molecule bioactive agent to the cellular target, conversion of the substrate to a reaction product by the bioluminescent reporter results in excitation of the small molecule fluorophore by BRET and fluorescence emission from the fluorophore.

5. The system of claim 1, wherein the small molecule bioactive agent is one of a library of small molecule bioactive agents tethered to fluorophores.

6. The system of claim 1, wherein the cellular target is one of a plurality of cellular targets fused to bioluminescent reporters.

7. A method for detection of an interaction between a small molecule bioactive agent and a cellular target comprising:

(a) expressing in a cell a fusion of said cellular target and a bioluminescent reporter, the bioluminescent reporter having a first emission spectrum ;

(b) contacting said cell extracellularly with a conjugate of said small molecule bioactive agent tethered to a small molecule fluorophore, said small molecule fluorophore having an excitation spectrum that overlaps said first emission spectrum and having a second emission spectrum that is distinct from said first emission spectrum, wherein the conjugate is cell permeable, and wherein the small molecule bioactive agent is capable of binding non-covalently to the cellular target upon interaction therewith;

(c) contacting said cell with a substrate for said bioluminescent reporter, wherein the substrate is cell permeable;

(d) detecting fluorescence at within said second emission spectrum, wherein the intensity of said fluorescence within the second emission spectrum correlates with binding between said small molecule bioactive agent with said cellular target.