molecule libraries)) by correlating such interactions to the formation of a bioluminescent complex of otherwise non-luminescent (e.g., substantially non-luminescent) amino acid chains.

In one aspect, the present disclosure provides a polypeptide comprising an amino acid sequence having less than 100% and greater than 90% sequence identity with SEQ ID NO: 440, wherein a bioluminescent signal produced in the presence of a peptide consisting of SEQ ID NO: 2 and a furmiazine substrate is enhanced relative to the polypeptide and the furmiazine substrate in the absence of the peptide consisting of SEQ ID NO: 2.

In another aspect, the present disclosure provides a nucleic acid comprising a sequence coding for a polypeptide as disclosed herein.

In a further aspect, the present disclosure provides a fusion polypeptide comprising the polypeptide as disclosed herein.

In another aspect, the present disclosure provides a bioluminescent complex comprising:

(a) the fusion polypeptide as disclosed herein; and

(b) a second fusion polypeptide comprising a complement peptide comprising an amino acid sequence having less than 100% and greater than 40% sequence identity with SEQ ID NO: 2; wherein a detectable bioluminescent signal is produced by the bioluminescent complex in the presence of a furimazine substrate.

In some embodiments, the present disclosure provides a bioluminescent complex comprising:

(a) a peptide comprising a peptide amino acid sequence having less than 100% and greater than 40% sequence identity with SEQ ID NO: 2; and

(b) a polypeptide comprising a polypeptide amino acid sequence having less than 100% and greater than 40% sequence identity with SEQ ID NO: 440, wherein the bioluminescent complex exhibits detectable luminescence.

In some embodiments, the present disclosure provides a bioluminescent complex comprising:

(a) a first amino acid sequence that is not a fragment of a preexisting protein; and

(b) a second amino acid sequence that is not a fragment of a preexisting protein, wherein the bioluminescent complex exhibits detectable luminescence, wherein the first amino acid sequence and the second amino acid sequence are associated, and wherein the bioluminescent complex emits a detectable bioluminescent signal in the presence of a substrate when the first amino acid sequence and the second amino acid sequence are associated.

In some embodiments, the present disclosure provides a bioluminescent complex comprising:

(a) a non-luminescent pair, wherein each non-luminescent element of the non-luminescent pair is not a fragment of a preexisting protein;

(b) an interaction pair, wherein each interaction element of the interaction pair is covalently attached to one of the non-luminescent elements of the non-luminescent pair.

In some embodiments, the present disclosure provides a method of detecting a stable interaction between a first amino acid sequence and a second amino acid sequence comprising:

(a) attaching the first amino acid sequence to a third amino acid sequence, and attaching the second amino acid sequence to a fourth amino acid sequence, wherein the third and fourth amino acid sequences are not fragments of a preexisting protein, wherein a stable complex of the third and fourth amino acid sequences emits a detectable bioluminescent signal in the presence of a substrate, wherein the non-covalent interactions between the third and fourth amino acid sequences are insufficient to form a complex of the third and fourth amino acid sequences in the absence of additional stabilizing and/or aggregating forces, and wherein a stable interaction between the first amino acid sequence and the second amino acid sequence provides the additional stabilizing and/or aggregating forces to produce a stable complex of the third and fourth amino acid sequences;

(b) placing the first, second, third, and fourth amino acid sequences of step (a) in conditions to allow for stable interactions between the first amino acid sequence and the second amino acid sequence to occur; and

(c) detecting the bioluminescent signal emitted, in the presence of a substrate, by the stable complex of the third and fourth amino acid sequences in the presence of a substrate, wherein detection of the bioluminescent signal indicates a stable interaction between the first amino acid sequence and the second amino acid sequence.

In some embodiments, the present disclosure provides a method of optimizing a non-luminescent pair comprising:

(a) aligning the sequences of three or more related proteins;

(b) determining a consensus sequence for the related proteins;

(c) providing first and second fragments of a protein related to three or more proteins, wherein the fragments are individually substantially non-luminescent but exhibit luminescence upon stable interaction of the fragments;

(d) mutating the first and second fragments at one or more positions each, wherein said mutations alter the sequences of the fragments to be more similar to a corresponding portion of the consensus sequence, wherein the mutating results in a non-luminescent pair that are not fragments of a preexisting protein,

(e) testing the non-luminescent pair for the absence of luminescence when unassociated and luminescence upon stable association of the non-luminescent pair.

In some embodiments, the present disclosure provides a detection reagent comprising: (a) a polypeptide comprising an amino acid sequence having less than 100% and greater than 40% sequence identity with SEQ ID NO: 440, wherein a detectable bioluminescent signal is produced, in the presence of a substrate, when the polypeptide contacts a peptide consisting of SEQ ID NO: 2, and (b) a substrate for a bioluminescent complex produced by said polypeptide and a peptide consisting of SEQ ID NO: 2.

In some embodiments, the assembled pair catalyzes a chemical reaction of an appropriate substrate into a high energy state, and light is emitted. In some embodiments, a bioluminescent complex exhibits luminescence in the presence of substrate (e.g., coelenterazine, furimazine, etc.).

Although the embodiments described herein primarily describe and refer to complementary, non-luminescent amino acid chains that form bioluminescent complexes, it is noted that the present technology can equally be applied to other detectable attributes (e.g., other enzymatic activities, generation of a fluorophore, generation of a chromophore, etc.). The embodiments described herein relating to luminescence should be viewed as applying to complementary, substantially non-enzymatically active amino acid chains (e.g., peptides and/or polypeptides that are not fragments of a preexisting protein) that separately lack a specified detectable activity (e.g., enzymatic activity) or substantially non-enzymatically active subunits of a polypeptide, complexes thereof, and methods of generating the detectable activity (e.g., an enzymatic activity) upon association of the complementary, substantially non-enzymatically active amino acid chains (e.g., peptides and/or polypeptides). Further, embodiments described herein that refer to non-luminescent peptides and/or polypeptides are applied, in some embodiments, to substantially non-luminescent peptides and/or polypeptides.

The present disclosure is further directed to assays for the detection of molecular interactions between molecules of interest by linking the interaction of a pair of non-luminescent peptides/polypeptides to the interaction molecules of interest (e.g., transient association, stable association, complex formation, etc.). In such embodiments, a pair of a non-luminescent elements are tethered (e.g., fused) to molecules of interest and assembly of the bioluminescent complex is operated by the molecular interaction of the molecules of interest. If the molecules of interest engage in a sufficiently stable interaction, the bioluminescent complex forms, and a bioluminescent signal is generated. If the molecules of interest fail to engage in a sufficiently stable interaction, the bioluminescent complex will not form or only form weakly, and a bioluminescent signal is not detectable or is substantially reduced (e.g., substantially

undetectable, essentially not detectable, etc.). In some embodiments, the magnitude of the detectable bioluminescent signal is proportional (e.g., directly proportional) to the amount, strength, favorability, and/or stability of the molecular interactions between the molecules of interest.

In some embodiments, the present disclosure provides peptides comprising an amino acid sequence having less than 100% (e.g., 20%... 30%... 40%... 50%... 60%... 70%... 80%, or more)

CLAIMS

1. A polypeptide comprising an amino acid sequence having less than 100% and greater than 90% sequence identity with SEQ ID NO: 440, wherein a bioluminescent signal produced in the presence of a peptide consisting of SEQ ID NO: 2 and a furmiazine substrate is enhanced relative to the polypeptide and the furmiazine substrate in the absence of the peptide consisting of SEQ ID NO: 2.

2. The polypeptide of claim 1, wherein the amino acid sequence is synthetic, contains non-natural amino acids or is a peptide mimic.

3. A nucleic acid comprising a sequence coding for a polypeptide of claim 1.

4. A fusion polypeptide comprising the polypeptide of claim 1.

5. A bioluminescent complex comprising:

(a) the fusion polypeptide of claim 4; and

(b) a second fusion polypeptide comprising a complement peptide comprising an amino acid sequence having less than 100% and greater than 40% sequence identity with SEQ ID NO: 2; wherein a detectable bioluminescent signal is produced by the bioluminescent complex in the presence of a furimazine substrate.

ACTIVATION OF BIOLUMINESCENCE BY STRUCTURAL COMPLEMENTATION

Abstract

Provided herein are compositions and methods for the assembly of a bioluminescent complex from two or more non-luminescent (e.g., substantially non-luminescent) peptide and/or polypeptide units. In particular, bioluminescent activity is conferred upon a non-luminescent polypeptide via structural complementation with another, complementary non-luminescent peptide. Biological processes rely on covalent and non-covalent interactions between molecules, macromolecules and molecular complexes. In order to understand such processes, and to develop techniques and compounds to manipulate them for research, clinical and other practical applications, it is necessary to have tools available to detect and monitor these interactions.

(No suitable figure)