protein or protein complexes. Non-specific binding of proteins from cell lysate to the solid support results in high background that further complicates the identification of the cellular target using mass spectrometry (MS). Such methods often result in a large number of putative hits, making it necessary to run secondary screens to validate the potential targets. High-throughput validation assays for target molecule-protein interactions require further development and optimization making it resource intensive process.

SUMMARY

In some embodiments, the present ~~invention~~ disclosure provides compositions and systems (e.g., cells, reaction mixture, kit, container, etc.) comprising one or more (e.g., all) of: (a) a cellular target of a bioactive agent; (b) a fusion of a first capture protein and a second capture protein; (c) the bioactive agent tethered to a first capture ligand, wherein the first capture ligand forms a covalent bond with the first capture protein upon interaction thereof; and (d) a solid surface displaying a second capture ligand, wherein the second capture ligand forms a covalent bond with the second capture protein upon interaction thereof. In an aspect, the present disclosure provides a system comprising: (a) a cellular target of a bioactive agent; (b) a dimer of a first capture protein and a second capture protein; (c) the bioactive agent tethered to a first capture ligand, wherein the first capture ligand forms a covalent bond with the first capture protein upon interaction thereof; and (d) a solid surface displaying a second capture ligand, wherein the second capture ligand forms a covalent bond with the second capture protein upon interaction thereof. In some embodiments, (a) comprises a plurality of cellular targets of a bioactive compound. In some embodiments, the cellular target is expressed intracellularly as a fusion with a reporter. In some embodiments, the reporter is a bioluminescent reporter. In some embodiments, the reporter is a portion, component, or subunit of a bioluminescent protein. In some embodiments, the bioluminescent reporter comprises a polypeptide with at least 70% sequence identity with SEQ ID NO.: 3 (e.g., 75%, 80%, 85%, 90%, 95%, 98%, 99%). In some embodiments, the first and second capture proteins both comprise at least 70% sequence identity with SEQ ID NO.: 1 (e.g., 75%, 80%, 85%, 90%, 95%, 98%, 99%). In some embodiments, the fusion is a homodimer. In some embodiments, the bioactive agent is a small molecule. In some embodiments, the cellular target is a binding partner of the bioactive agent. In some embodiments, the cellular target comprises a combination of molecules, such as a complex of two or more proteins, or proteins and nucleic acids. In some embodiments, the first capture ligand and second capture ligand comprise the same molecular structure. In some embodiments, the solid surface is a selected from the list consisting of: well, tube, slide, plate, matrix, resin, micro fluidics channel, capillary, bead, particle (e.g., microparticle, nanoparticle, etc.), etc. In some embodiments, a solid surface is magnetic. In some embodiments, a solid surface is non-magnetic. In some embodiments, the cellular target is bound to the bioactive agent, the first capture protein is bound to the first capture ligand, and the second capture protein is bound to the second capture ligand on the solid surface.

In certain embodiments, the present ~~invention~~ disclosure provides methods of capturing the cellular target comprising the steps of: (a) administering a bioactive agent tethered to a first capture ligand to a cell comprising a cellular target of the bioactive agent under conditions such that the cellular target binds the bioactive agent; (b) lysing the cell to produce a cell lysate; (c) contacting the cell lysate with a fusion of a first capture protein and a second capture protein under conditions in which the first capture ligand forms a covalent bond with the first capture protein; (d) contacting the cell lysate with a solid surface displaying a second capture ligand under conditions in which the first capture ligand forms a covalent bond with the first capture protein; and (e) separating the solid surface from the cell lysate. In other embodiments, the present ~~invention~~ disclosure provides methods of capturing the cellular target comprising the steps of: (a) administering a bioactive agent tethered to a first capture ligand to a cell comprising a cellular target of the bioactive agent under conditions such that the cellular target binds the bioactive agent; (b) lysing the cell to produce a cell lysate; (c) contacting the cell lysate with a solid surface displaying a second capture ligand bound to a fusion of a first capture protein and a second capture protein, wherein the second capture protein and the second capture ligand are covalently bound; and (d) separating the solid surface from the cell lysate. In some embodiments, the cellular target is endogenous to a cell. In some embodiments, the cellular target is a fusion with a reporter. In an aspect, the present disclosure provides a method of capturing the cellular target comprising the steps of: (a) administering a bioactive agent tethered to a first capture ligand to a cell comprising a cellular target of the bioactive agent under conditions such that the cellular target binds the bioactive agent; (b) lysing the cell to produce a cell lysate; (c) contacting the cell lysate with a dimer of a first capture protein and a second capture protein under conditions in which the first capture ligand forms a covalent bond with the first capture protein; (d) contacting the cell lysate with a solid surface displaying a second capture ligand under conditions in which the first capture ligand forms a covalent bond with the first capture protein; and (e) separating the solid surface from the cell lysate. In another aspect, the present disclosure provides a method of capturing the cellular target comprising the steps of: (a) administering a bioactive agent tethered to a first capture ligand to a cell comprising a cellular target of the bioactive agent under conditions such that the cellular target binds the bioactive agent; (b) lysing the cell to produce a cell lysate; (c) contacting the cell lysate with a solid surface displaying a second capture ligand bound to a dimer of a first capture protein and a second capture protein, wherein the second capture protein and the second capture ligand are covalently bound; and (d) separating the solid surface from the cell lysate.

In certain embodiments, the present ~~invention~~ disclosure provides methods of capturing the cellular target comprising the steps of: (a) administering a bioactive agent tethered to a first capture ligand to a cell comprising a cellular target of the bioactive agent under conditions such that the cellular target binds the bioactive agent; (b) contacting the first capture ligand with a fusion of a first capture protein and a second capture protein under conditions in which the first capture ligand forms a covalent bond with the first capture protein; (c) contacting the capture fusion with a solid surface displaying a second capture ligand under conditions in which the first capture ligand forms a covalent bond with the

first capture protein; and (d) separating the solid surface from the cell lysate. In some embodiments, methods comprise a step of lysing the cell to form a lysate. In other embodiments, the present ~~invention~~ disclosure provides methods of capturing the cellular target comprising the steps of: (a) administering a bioactive agent tethered to a first capture ligand to a cell comprising a cellular target of the bioactive agent under conditions such that the cellular target binds the bioactive agent; (b) contacting the first capture ligand with a solid surface displaying a second capture ligand bound to a fusion of a first capture protein and a second capture protein, wherein the second capture protein and the second capture ligand are covalently bound; and (c) separating the solid

surface from the cell lysate. In some embodiments, methods comprise a step of lysing the cell to form a lysate. In some embodiments, the cellular target is a fusion with a reporter. In some embodiments, the product of steps (a) and/or (b) exits the cells (e.g., secreted, exocytosis, active removal) without cell lysis.

In some embodiments, the present ~~invention~~ disclosure provides cell lysate, cell component, or cell fraction, of a cell comprising: (a) a cellular target of a bioactive agent, and (b) a bioactive agent tethered to a first capture ligand; the cell lysate further comprising: (c) a fusion of a first capture protein and a second capture protein, wherein the first capture ligand forms a covalent bond with the first capture protein upon interaction thereof, and (d) a solid surface displaying a second capture ligand, wherein the second capture ligand forms a covalent bond with the second capture protein upon interaction thereof. In an aspect, the present disclosure provides a cell lysate, of a cell comprising: (a) a cellular target of a bioactive agent; and (b) a bioactive agent tethered to a first capture ligand; the cell lysate further comprising: (c) a dimer of a first capture protein and a second capture protein, wherein the first capture ligand forms a covalent bond with the first capture protein upon interaction thereof; and (d) a solid surface displaying a second capture ligand, wherein the second capture ligand forms a covalent bond with the second capture protein upon interaction thereof. In some embodiments, (a) comprises a plurality of cellular targets of a bioactive compound. In some embodiments, the cellular target is a fusion with a reporter. In some embodiments, the reporter is a bioluminescent reporter. In some embodiments, the reporter is a portion, component, or subunit of a bioluminescent protein. In some embodiments, the bioluminescent reporter comprises a polypeptide with at least 70% sequence identity with SEQ ID NO.: 3 (e.g., 75%, 80%, 85%, 90%, 95%, 98%, 99%). In some embodiments, the first and second capture proteins both comprise at least 70% sequence identity with SEQ ID NO.: 1 (e.g., 75%, 80%, 85%, 90%, 95%, 98%, 99%). In some embodiments, the fusion is a homodimer. In some embodiments, the bioactive agent is a small molecule. In some embodiments, the cellular target is a binding partner of the bioactive agent. In some embodiments, the cellular target comprises a combination of molecules, such as a complex of two or more proteins, or proteins and nucleic acids. In some embodiments, the first capture ligand and second capture ligand comprise the same molecular structure. In some embodiments, the solid surface is a selected from the list consisting of: well, tube, slide, plate, matrix, resin, micro fluidics channel, capillary, bead, particle (e.g., microparticle, nanoparticle, etc.), etc. In some embodiments, the cellular target is bound to the bioactive agent, the first capture protein is bound to the first capture ligand, and the second capture protein is bound to the second capture ligand on the solid surface.

In some embodiments, the present ~~invention~~ disclosure provides compositions and systems (e.g., cells, reaction mixture, kit, container, etc.) comprising one or more (e.g., all) of: (a) a cellular target of a bioactive agent; (b) the bioactive agent tethered to a capture ligand; and (c) a solid surface displaying a capture protein, wherein the capture protein forms a covalent bond with the capture ligand upon interaction thereof. In an aspect, the present disclosure provides a system comprising: (a) a cellular target of a bioactive agent; (b) the bioactive agent tethered to a capture ligand; and (c) a solid surface displaying a capture protein, wherein the capture protein forms a covalent bond with the capture ligand upon interaction thereof. In some embodiments, (a) comprises a plurality

of cellular targets of a bioactive compound. In some embodiments, the cellular target is endogenous to a cell. In some embodiments, the cellular target is expressed intracellularly as a fusion with a reporter. In some embodiments, the reporter is a bioluminescent reporter. In some embodiments, the reporter is a portion, component, or subunit of a bioluminescent protein. In some embodiments, the bioluminescent reporter comprises a polypeptide with at least 70% sequence identity with SEQ ID NO.: 3. In some embodiments, the capture protein comprises at least 70% sequence identity with SEQ ID NO.: 1. In some embodiments, the ~~invention~~ disclosure comprises an additional step (e) measuring bioluminescence bound to, or released from, the solid surface. In some embodiments, the bioactive agent is a small molecule. In some embodiments, the small molecule is a synthetic molecule. In some embodiments, the small molecule is an inhibitor of protein function, such as an inhibitor of an enzyme or a receptor. In some embodiments, the cellular target comprises a combination of molecules, such as a complex of two or more proteins, or proteins and nucleic acids. In some embodiments, the cellular target is a binding partner of the bioactive agent. In some embodiments, the cellular target comprises a combination of molecules, such as a complex of two or more proteins, or proteins and nucleic acids. In some embodiments, the cellular target binds non-covalently to the bioactive agent. In some embodiments, the solid surface is a selected from the list consisting of: well, tube, slide, plate, matrix, resin, micro fluidics channel, capillary, bead, particle (e.g., microparticle, nanoparticle, etc.), etc. . In some embodiments, the cellular target is bound to the bioactive agent, and the capture protein is bound to the capture ligand. In some embodiments, the cellular target is bound non-covalently to the bioactive agent, and the capture protein is bound covalently to the capture ligand. In some embodiments, the capture ligand is a chloroalkane. In some embodiments, the capture ligand comprises a linker. In some embodiments, the capture ligand comprises a carbamate linker. In some embodiments, the capture ligand comprises a cleavable linker. In certain embodiments, the capture ligand comprises a carbamate linker and a cleavable linker.

In some embodiments, the present ~~invention~~ disclosure provides compositions and systems (e.g., reaction mixture, kit, container, etc.) comprising: (a) a bioactive agent tethered to a capture ligand; and (b) a solid surface displaying a capture protein, wherein the capture protein forms a covalent bond with the capture ligand upon interaction thereof. In some embodiments, the bioactive agent binds non-covalently to a cellular target. In some embodiments, the cellular target is endogenous to a cell. In some embodiments, the cellular target is expressed intracellularly as a fusion with a reporter. In some embodiments, the reporter is a bioluminescent reporter. In some embodiments, the bioluminescent reporter comprises a

polypeptide with at least 70% sequence identity with SEQ ID NO.: 3. In some embodiments, the capture protein comprises at least 70% sequence identity with SEQ ID NO.: 1. In some embodiments, the bioactive agent is a small molecule. In some embodiments, the cellular target is a binding partner of the bioactive agent. In some embodiments, the cellular target binds non-covalently to the bioactive agent. In some embodiments, the solid surface is a selected from the list consisting of: well, tube, slide, plate, matrix, resin, micro fluidics channel, capillary, bead, particle (e.g., microparticle, nanoparticle, etc.), etc. In some embodiments, the cellular target is bound to the bioactive agent, and the capture protein is bound to the capture ligand. In some embodiments, the cellular target is bound non-covalently to the bioactive agent, and the capture protein is bound covalently to the capture ligand. In some embodiments, the capture ligand is a chloroalkane. In some embodiments, the capture ligand comprises a linker. In some embodiments, the capture ligand comprises a carbamate linker. In some embodiments, the capture ligand comprises a cleavable linker. In certain embodiments, the capture ligand comprises a carbamate linker and a cleavable linker.

In ~~some embodiments~~an aspect, the present ~~invention~~ disclosure provides methods of capturing a cellular target comprising the steps of: (a) administering a bioactive agent tethered to a capture ligand to a cell comprising a cellular target of the bioactive agent under conditions such that the cellular target binds the bioactive agent; (b) lysing the cell to produce a cell lysate; (c) contacting the cell lysate with a solid surface displaying a capture protein under conditions in which the capture protein forms a covalent bond with the capture ligand; and (d) separating the solid surface from the cell lysate. In some embodiments, the cellular target is endogenous to a cell. In some embodiments, the cellular target is expressed intracellularly as a fusion with a reporter. In some embodiments, the reporter is a bioluminescent reporter. In some embodiments, the bioluminescent reporter comprises a polypeptide with at least 70% sequence identity with SEQ ID NO.: 3. In some embodiments, the capture protein comprises at least 70% sequence identity with SEQ ID NO.: 1. In some embodiments, the bioactive agent is a small molecule. In some embodiments, the cellular target is a binding partner of the bioactive agent. In some embodiments, the cellular target binds non-covalently to the bioactive agent. In some embodiments, the solid surface is a selected from the list consisting of: well, tube, slide, plate, matrix, resin, micro fluidics channel, capillary, bead, particle (e.g., microparticle, nanoparticle, etc.), etc. In some embodiments, the cellular target is bound to the bioactive agent, and the capture protein is bound to the capture ligand. In some embodiments, the cellular target is bound non-covalently to the bioactive agent, and the

capture protein is bound covalently to the capture ligand. In some embodiments, the capture ligand is a chloroalkane. In some embodiments, the capture ligand comprises a linker. In some embodiments, the capture ligand comprises a carbamate linker. In some embodiments, the capture ligand comprises a cleavable linker. In certain embodiments, the capture ligand comprises a carbamate linker and a cleavable linker.

In some embodiments, the method further comprises: (e) detecting or analyzing the cellular target. In other embodiments, the method further comprises: (e) eluting the cellular target from the solid support and (f) detecting or analyzing the cellular target. In some embodiments, the cellular target is detected or analyzed using mass spectrometry (MS).

In some embodiments, the present ~~invention~~ disclosure provides a cell lysate of a cell comprising: (a) a cellular target of a bioactive agent; and (b) a bioactive agent tethered to a capture ligand. In some embodiments, the cell lysate further comprising: (c) a solid surface displaying a capture protein, wherein the capture protein forms a covalent bond with the capture ligand upon interaction thereof. In an aspect, the present disclosure provides a cell lysate of a cell comprising: (a) a cellular target of a bioactive agent; and (b) a bioactive agent tethered to a capture ligand; the cell lysate further comprising: (c) a solid surface displaying a capture protein, wherein the capture protein forms a covalent bond with the capture ligand upon interaction thereof. In some embodiments, (a) comprises a plurality of cellular targets of a bioactive compound. In some embodiments, the cellular target is endogenous to the cell. In some embodiments, the cellular target is a fusion with a reporter. In some embodiments, the reporter is a bioluminescent reporter. In some embodiments, the reporter is a portion, component, or subunit of a bioluminescent protein. In some embodiments, the bioluminescent reporter comprises a polypeptide with at least 70% sequence identity with SEQ ID NO.: 3. In some embodiments, the capture protein comprises at least 70% sequence identity with SEQ ID NO.: 1. In some embodiments, the bioactive agent is a small molecule. In some embodiments, the small molecule is a synthetic molecule. In some embodiments, the small molecule is an inhibitor of protein function, such as an inhibitor of an enzyme or a receptor. In some embodiments, the cellular target is a binding partner of the bioactive agent. In some embodiments, the cellular target comprises a combination of molecules, such as a complex of two or more proteins, or proteins and nucleic acids. In some embodiments, the cellular target binds non-covalently to the bioactive agent. In some embodiments, the solid surface is a selected from the list consisting of: well, tube, slide, plate, matrix, resin, micro fluidics channel, capillary, bead, particle (e.g., microparticle, nanoparticle, etc.), etc. In some embodiments, the cellular target is bound to the bioactive agent, and the capture protein is bound to the capture ligand. In some embodiments, the cellular target is bound non-covalently to the bioactive agent, and the capture protein is bound covalently to the capture ligand. In some embodiments, the capture ligand is a chloroalkane. In some embodiments, the capture ligand comprises a linker. In some

embodiments, the capture ligand comprises a carbamate linker. In an aspect, the present disclosure provides a capture ligand comprising a carbamate linker. In some embodiments, the capture ligand comprises a cleavable linker. In certain embodiments, the capture ligand comprises a carbamate linker and a cleavable linker.

DRAWINGS

Figure 1 shows a schematic representation of embodiments of the present invention for rapid and specific capture of bioactive agent tethered to a capture ligand (e.g., SM-HTL) complexed with protein or protein complexes using a capture protein (e.g. HALOTAG).

Figure 2 shows a schematic representation of embodiments of the present invention for rapid and specific elution of protein complexes using various methods.

Figure 3 shows graph depicting the effect of bead volume, and the presence or absence of SM-HTL on luminescence in various capture assays: (A) p38α and BIRB-chloroalkane, (B) HDAC6 and SAHA-chloroalkane; (C) BIRB-chloroalkane pull-down with overexpressed NANOLUC-p38α; and (D) SAHA-chloroalkane pull-down with overexpressed NANOLUC- HDAC6

Figure 4 shows Western/silver stain analysis using anti-p38 of lysates following a p38α and BIRB-chloroalkane capture assay.

Figure 5 shows a graph depicting the ability of embodiments of the present invention to capture target proteins that bind to drugs in a shor time span.

Figure 6 shows graphs depicting the use of a carbamate chloroalkane linker for pull-down of a target protein from cells using chloroalkane modified small molecules.

Figure 7 shows a graph depicting the effect of various release methods on signal-over-background.

Figure 8 shows (A) a Western blot demonstrating specific pull down of DHFR (21kDa) with Methotrexate-CA, and (B) mass spectrometry analysis of proteins pulled down by Methotrexate-CA from HEK293 cells.

Figure 9 shows (A) a Western blot demonstrating specific pull down of p38 (41kDa) with BIRB-CA, and (B) mass spectrometry analysis of proteins pulled down by BIRB-CA from HEK293 cells.

Figure 10 shows the minimal impact of the chloroalkane modification on permeability and potency of the tethered bioactive agent, SAHA.

Figure 11 shows that all known targets of SAHA, including low affinity target (HDAC8) and low abundance target (HDAC3), can be specifically pulled down from the cells using an embodiment of the present invention. (A) a western blot demonstrating