

CLAIMS

1. A method of detecting or measuring a protein that is bound to a ligand in a sample, the method comprising:
 - a) contacting a sample comprising one or more intact or live cells with a cell-permeable denaturant to promote intracellular unfolding of the protein in the sample, wherein the cell-permeable denaturant is a cell-permeable denaturant that is capable of permeating intact or live cells to promote intracellular unfolding of the protein;
 - b) lysing the sample; and
 - c) detecting or measuring the level of non-aggregated protein or aggregated protein, wherein a difference in the level of non-aggregated protein or aggregated protein as compared to a reference indicates the presence or level of protein that is bound to the ligand in the sample, wherein the reference is a sample comprising one or more intact or live cells wherein the ligand is not present.
2. The method of claim 1, wherein the sample is a cell or tissue sample.
3. The method of claim 1, wherein the cell-permeable denaturant is urea or a derivative thereof.
4. The method of any one of claims 1 to 3, wherein step b) comprises lysing and/or diluting the sample to promote aggregation of the protein.
5. The method of any one of claims 1 to 4, wherein the method comprises removing aggregated and/or unfolded protein prior to step c).
6. The method of any one of claims 1 to 5, wherein the method further comprises detecting or measuring binding of the ligand to the protein at different concentrations of denaturant.
7. The method of any one of claims 1 to 6, wherein the method is performed at physiological temperature of an animal.

8. The method of any of claims 1 to 7, wherein the protein is coupled to a label.
9. The method of any of claims 1 to 8, wherein the protein is detected by mass spectrometry or by a recognition molecule.
10. A kit when used in a method according to any of claims 1 to 9.
11. A method of identifying a candidate ligand that is capable of binding to a protein, the method comprising:
 - a) contacting a sample comprising one or more intact or live cells with the candidate ligand;
 - b) contacting the sample with a cell-permeable denaturant to promote intracellular unfolding of the protein, wherein the cell-permeable denaturant is a cell-permeable denaturant that is capable of permeating the intact or live cells to promote intracellular unfolding of the protein;
 - c) lysing the sample; and
 - d) detecting or measuring the level of the non-aggregated protein or aggregated protein, wherein a difference in the level of non-aggregated protein or aggregated protein as compared to a reference indicates that the candidate ligand is capable of binding to the protein, wherein the reference is a sample comprising one or more intact or live cells wherein the ligand is not present.
12. A method of predicting the efficacy of a drug in a subject, the method comprising
 - a) contacting a sample comprising one or more intact or live cells from a subject who has been treated with the drug with a cell-permeable denaturant to promote intracellular unfolding of a protein, wherein the cell-permeable denaturant is a cell-permeable denaturant that is capable of permeating intact or live cells to promote intracellular unfolding of the protein;
 - b) lysing the sample;
 - c) detecting or measuring the level of non-aggregated protein or aggregated protein, wherein a difference in the level of the non-aggregated protein or aggregated protein as compared to a reference indicates binding of the drug to the protein, therefore predicting efficacy of the drug in the subject,

wherein the reference is a sample comprising one or more intact or live cells wherein the ligand is not present.

13. A method of identifying a protein that is bound to a drug in a subject, the method comprising:

- a) contacting a sample comprising one or more intact or live cells from a subject who has been treated with the drug with a cell-permeable denaturant to promote intracellular unfolding of the protein, wherein the cell-permeable denaturant is a cell-permeable denaturant that is capable of permeating the intact or live cells to promote intracellular unfolding of the protein;
- b) lysing the sample; and
- c) detecting or measuring the level of non-aggregated protein or aggregated protein, wherein a difference in the level of the non-aggregated protein or aggregated protein as compared to a reference indicates binding of the drug to the protein, wherein the reference is a sample comprising one or more intact or live cells wherein the ligand is not present.