CLAIMS

WHAT IS CLAIMED IS:

1. A multiplexed method for quantitating the binding of a test compound to a predetermined target molecule and off-target target molecules, comprising the steps of:

(a) obtaining a mixture of target molecules from a crude extract from at least one of (i) healthy or non-healthy human or non-human tissue, and (ii) a synthetic protein preparation, wherein said target molecules are present in a mixture of the target molecule that do not exist in nature in the same tissue preparation;

(b) incubating said mixture of the target molecules in a plurality of mixtures of ligands and test compounds, wherein said target molecules bind to different ligands;

(c) removing unbound ligands from said plurality of mixtures of the target molecules;

(d) isolating ligands bound to the target molecules in said mixture of the target molecules;

(e) determining a quantity of the ligand bound by the target molecule, by measuring ligands obtained in step (d) at defined time points in a reaction mixture using mass spectrometry and a calibration curve;

(f) determining affinity of the test compound for the target molecules in said mixture of the target molecules using data obtained in step (e);

(g) measuring the binding of said test compound to the predetermined target molecule and comparing said binding to the binding of said test compound to off-target molecules; and

(h) calculating K_{on} or K_{off} of the test compound for the target molecule using data obtained in step (e).

2. The method of claim 1, wherein said mixture of target molecules further comprises a heterologous mixture of target molecules, the heterologous mixture of the target molecules is either

(a) a mixture of different target molecules not found in nature in a single tissue preparation; or

(b) a mixture of different target molecules present in a single tissue wherein each target molecule has a different biological function than the other target molecule present in the mixture.

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3. The method of claim 1 or claim 2, wherein said mixture of target molecules comprises targets that are human target molecules.

4. The method of any one of claims 1 to 3, wherein said target molecules are selected from at least one group from the group consisting of: Na+ channels, alpha1 beta-adenoreceptors, alpha 2 beta-adrenoceptors, A1 (adenosine receptor), M1 (muscarinic receptor), 5-HT_{2A} (serotonin receptor), Alpha 1ns (adrenergic receptor), Alpha 2ns (adrenergic), D1 (dopamine receptor), and 5HTtrans (serotonin receptor).

5. The method of any one of claims 1 to 4, wherein the ligands within the plurality of mixture of ligands are at least one ligand selected from the group consisting of: CPX, pirenzepine, prazosine, RX821002, SCH233900. 8-OH-DPAT, EMD281014, paroxetine, D600, MK801, and naloxone.

6. The method of any one of claim 1 to 5, wherein the method further comprises quantitating the binding affinity of at least two different test compounds (test compound C1 -C_n) to at least two different receptor target molecules (receptor

RT1 for C1, RT_n for C_n.), based on competitive binding between the test compounds and known binders for RT1 and RT2 (known binder B1- $B_{n)}$, comprising:

(a) providing a mixture from a crude extract comprising (i) test compounds C1- C_n; (ii) known binders B1- B_n and (iii) receptor target molecules RT1-$RT_{n,}$ wherein RT1 to RTn do not exist in nature in the same tissue preparation;

(b) allowing complexes to form in said mixture between the test compounds C1-C_n, RT1-RT_n, and B1- $B_{n;}$

(c) separating the test compounds which do not form complexes with their receptor target molecules from said complexes;

(d) isolating known binders from complexes obtained in step (c) and passing isolated binders through a mass spectrometer to measure binding of the test compounds using mass spectroscopy; and

(e) determining the relative affinities of compounds C1-C_n for RT1- RT_n, respectively, wherein C_n, B_n, and RT_n represent a series of members wherein n is between 2 and 40.

7. The method of claim 6, wherein said receptor target molecules are obtained from ex vivo membranes of at least two cell membrane preparation of cortex membrane preparations, cerebellum membrane preparations, ventricular membrane preparations, and hepatic membrane preparations.

8. The method of any one of claims 7, wherein said step of providing receptor target molecules RT1-RT_n comprises providing human receptor target molecules.

9. The method of any one of claims 1 to 8, further comprising the step of determining a K_{on} and K_{off} of the test compound to the target molecule, optionally

(a) wherein K_{off} is determined by a displacement method, or

(b) wherein K_{off} is determined by a dilution method.

10. A multiplexed method for quantitating the binding affinity of a test compound to a target molecule, comprising the steps of:

(a) obtaining at least three target molecules from a crude extract as set forth in the chart below

Target molecule
Adenosine receptor A1
Muscarinic acetylcholine receptor
5-HT_{2A} (serotonin)
Alpha-1A adrenergic receptor
Alpha-2A adrenergic receptor
Dopamine receptor D1
5HT transporter
5HT1a receptor
5HT2a receptor
Cave Ca channel
PCP receptor
Opioid receptor

(b) incubating said target molecules in a plurality of mixture of ligands and the test compounds wherein said target molecules bind to different ligands;

(c) removing unbound ligands from the mixtures;

(d) isolating ligands bound to the target molecules after incubating;

(e) determining the quantity of each ligand present on the target molecules by measuring ligands that were obtained in step (d) at defined time points in a reaction mixture using mass spectrometry and a calibration curve prepared with known concentrations of ligand; and

(f) calculating affinity of the test compound for the target molecule from the data obtained in step (e); and

(g) calculating K_{on} or K_{off} of the test compound for the target molecule using data obtained in step (e).

11. The method of claim 10, wherein the same test compound is used with each target molecule.

12. The method of claim 10, comprising the use of the following target molecules and ligands:

Target molecule	Ligand
Adenosine receptor A1	CPX
Muscarinic acetylcholine receptor	pyrenzepine
5-HT_{2A} (serotonin)	EMD281014
Alpha-1A adrenergic receptor	prazosine
Alpha-2A adrenergic receptor	RX82102
Dopamine receptor D1	SCH23390
5HT transporter	paroxetine
5HT1a receptor	8-OH-DPAT
5HT2a receptor	EMD281014
Cave Ca channel	D600
PCP receptor	MK801
Opioid receptor	naloxone

13. A multiplexed method for determining K_{on} and/or K_{off} values of a test compound to a target molecule, comprising the steps of:

(a) obtaining a mixture of target molecules from a crude extract from at least one of (i) healthy or non-healthy human or non-human tissue, and (ii) a synthetic protein preparation wherein said target molecules are present in a mixture of the target molecules that do not exist in nature in the same tissue preparation;

(b) incubating said target molecules in a plurality of mixtures of ligands and the test compounds, wherein said target molecules bind to different ligands;

(c) removing unbound ligands from the mixtures of the target molecules;

(d) isolating ligands bound to the target molecules;

(e) determining quantity of ligand bound by the target molecule, by measuring ligands that were obtained in step (d) at defined time points in a reaction mixture, using mass spectrometry and a calibration curve; and

(f) calculating K_{on} or K_{off} of the test compound for the target molecule using data obtained in step (e).

14. The method of claim 13, wherein K_{on} and K_{off} are determined in cell membrane mixtures comprising different ex vivo membranes with at least two cell membrane preparation of: cortex membrane preparations, cerebellum membrane preparations, ventricular membrane preparations, and hepatic membrane preparations.

15. The method of claim 13 or claim 14, wherein cell membrane mixtures comprise at least two of receptor A1, A2A (h), A3 (h), M1, M2 (h), Alpha1ns, Alpha2ns, D1, D2S (h), 5HT1a, 5HT2a, 5HTtrans, Cave, PCP, Opioid ns, AT2 (h), B2 (h), CB1 (h), CCK1 (CCKA), H4 (h), and CysLT1 (LTD4) (h).

16. The method of claim 15, wherein the cell membrane mixtures comprise all of the listed receptors.

17. The method of one of claims 13 to 16, wherein K_{off} is determined by a displacement method.

18. The method of one of claims 13 to 16, wherein K_{off} is determined by a dilution method.