CLAIMS

WHAT IS CLAIMED IS:

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

(a) obtaining a mixture of target molecules from at least one of (i) healthy or non-healthy human or non-human tissue, and (ii) a synthetic protein preparation, wherein the obtaining the mixture of target molecule comprises obtaining target molecules from a crude extract;

(b) incubating said target molecules in a plurality of mixtures of ligands and test compounds, wherein said target molecules ~~and~~ are incubated with different ligands;

(c) removing unbound ligands from said plurality of mixtures of ligands and test compounds;

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

(e) determining a quantity of ligand that was bound to~~by~~ a target molecule, by measuring ligands that were obtained in step (d), using mass spectrometry and a calibration curve;

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

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

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

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 step (c) comprises removing unbound ligands from said plurality of mixtures using a glass filter.~~

~~5. The method of claim 1, wherein step (a) comprises obtaining target molecule from a crude extract.~~

~~6. The method of any one of claims 1 to 5, wherein step (d) comprises eluting the bound ligands from the filter using a solvent, then concentrating samples from the filter.~~

~~7. The method of any one of claims 1 to 6, wherein step (e) using mass spectroscopy comprises using liquid chromatography/electrospray ionization tandem mass spectroscopy.~~

~~8. The method of any one of claims 1 to 7, further comprising the step of determining a K_{on} and K_{off} of the test compound to a target molecule.~~

~~9. The method of claim 8, wherein K_{off} is determined by a displacement method.~~

~~10. The method of claim 8, wherein K_{off} is determined by a dilution method.~~

4~~11~~. The method of any one of claims 1 to 3~~10~~, wherein said target molecules are formed in a mixture of receptor target molecules that does not exist in nature in a single mixture.

5~~12~~. The method of any one of claims 1 to 4~~11~~, 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).

6~~13~~. The method of any one of claims 1 to 5~~12~~, 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.

7~~14~~. The method of any one of claims 1 to 6, wherein the method further comprises ~~A multiplexed method for~~ quantitating 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 comprising (i) test compounds C1- C_n; (ii) known binders B1- B_n and (iii) receptor target molecules RT1-RT_n, wherein step (a) comprises obtaining receptor target molecules from a crude extract;

(b) allowing complexes to form in said mixture between the test compounds C1-C_n, RT1-RT_n, and B1- B_n;

(c) separating 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 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.

8~~15~~. The method of claim 7~~14~~, wherein the receptor target molecules RT1-RT_n are in a mixture not found in nature in the same tissue.

9~~16~~. The method of claim 7~~14 or claim 15~~, wherein ~~step (a) comprises obtaining receptor target molecules from a crude extract and~~ said receptor target molecules are obtained from ex vivo membranes of at least two of cortex, cerebellum, ventricular and hepatic membrane preparations.

10~~17~~. The method of ~~any one of~~ claim~~s~~ 7 to 9 ~~or 9814 to 16~~, wherein said step of providing receptor target molecules RT1-RT_n comprises providing human receptor target molecules.

~~18. The method of any one of claims 14 to 17, wherein step (c) comprises separating using a glass filter and washing.~~

~~19. The method of any one of claims 14 to 18, wherein step (d) comprises eluting the bound ligand from the filter using a solvent, then concentrating samples from the filter.~~

~~20. The method of any one of claims 14 to 19, wherein said mass spectroscopy comprises using liquid chromatography/electrospray ionization tandem mass spectroscopy.~~

1121. The method of any one of claims 1 to 10~~14 to 20,~~ 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.

1222. A multiplexed method for quantitating binding affinity of a test compound to a target molecule, comprising the steps of:

(a) obtaining at least three target molecules, the at least three target molecules obtained 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 test molecules;

(c) removing unbound ligands from the mixtures;

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

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

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

1323. The method of claim 1222, wherein the same test compound is used with each target molecule.

1424. The method of claim 1222, 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

1525. A multiplexed method for determining K_{on} and/or K_{off} values of a of a test compound to a target molecule, comprising the steps of:

(a) obtaining a mixture of target molecules from at least one of (i) healthy or non-healthy human or non-human tissue, and (ii) a synthetic protein preparation;

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

(c) removing unbound ligands from the mixtures;

(d) isolating bound ligands that were bound to the target molecules;

(e) determining a quantity of ligand that was bound by a 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).

1626. The method of claim 1525, wherein K_{on} and K_{off} are determined in mixtures of different ex vivo membranes comprised of at least two of: cortex, cerebellum, ventricular and hepatic membrane preparations.

1727. The method of claim 1525 or claim1626, wherein 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).

1828. The method of claim 1627, wherein the membrane mixtures comprise all of the listed receptors.

1929. The method of one of claims 15425 to 18728, wherein K_{off} is determined by a displacement method.

2030. The method of one of claims 15425 to 18729, wherein K_{off} is determined by a dilution method.