

consuming and as a rule done by testing one compound after the other in discrete assays. Only recently, novel approaches have been introduced which allow the testing of mixtures of compounds. An exemplary procedure, which relies on the addition of DNA tags to the chemical compound (see, e.g. Mullard, Nature 530, 367-369 (2016), proves to be of major advantage however it suffers from two inherent problems: (i) it is only applicable to chemically synthesized compound libraries; and (ii) the addition of the DNA tag leads to a change in the structure of the compound thus leading to false negatives and false positives during the screening. With respect to the first limitation, this means that natural compounds which have the highest hit rate in screenings are excluded.

In view of the shortcomings of the prior art, the technical problem underlying the present invention can be seen in the provision of improved or alternative means and methods for elucidating intermolecular interactions in biological systems or identifying modulators of biological macromolecules.

Summary

In one aspect, the present disclosure refers to a method of determining ligands of macromolecules in a sample, wherein said sample is a cell-free cell extract, said method comprising or consisting of

- (a) subjecting said sample comprising (i) complexes formed by said macromolecules and said ligands and (ii) unbound ligands to a method which separates said complexes from said unbound ligands, wherein said method yields fractions and wherein said method is size exclusion chromatography (SEC);
- (b) releasing ligands from complexes obtained in step (a); and
- (c) subjecting the released ligands obtained in step (b) to a chemical analysis method, thereby determining said ligands of said macromolecules;
- (d) determining one or more macromolecules which were bound to ligands by a chemical analysis;

wherein said method further comprises the step of

- (e) determining which ligand binds which macromolecule by determining the amount of a given ligand in each of said fractions, thereby obtaining an elution profile of said given ligand, determining the amount of a given macromolecule in each of said fractions, thereby obtaining an elution profile of said macromolecule and comparing obtained elution profiles of ligands with such obtained elution profiles of macromolecules.

In another aspect, the present disclosure refers to a method of identifying, out of a plurality of

test compounds, (a) ligand(s) of a plurality of macromolecules, said method comprising or consisting of:

- (a) bringing said macromolecules into contact with said plurality of test compounds;
- (b) subjecting the mixture obtained in step (a) to a method which separates
5 complexes, if any, formed between said macromolecules and (a) ligand(s) from unbound test compounds, wherein said method yields fractions and wherein said method is size exclusion chromatography (SEC);
- (c) dissociating complexes, thereby releasing bound ligands, if any, from macromolecules;
- (d) subjecting the released ligand(s) obtained in step (c), if any, to a chemical
10 analysis method, thereby identifying a ligand(s) of said macromolecules;
- (e) determining one or more macromolecules which were bound to ligands by a chemical analysis;

wherein said method further comprises the step of

- (f) determining which ligand binds which macromolecule by determining the
15 amount of a given ligand in each of said fractions, thereby obtaining an elution profile of said given ligand, determining the amount of a given macromolecule in each of said fractions, thereby obtaining an elution profile of said macromolecule and comparing obtained elution profiles of ligands with such
20 obtained elution profiles of macromolecules.

Detailed Description

The present invention, in a first aspect, relates to a method of determining ligands of macromolecules, said method comprising or consisting of (a) subjecting a sample comprising
25 (i) complexes formed by said macromolecules and said ligands and (ii) unbound ligands to a method which separates said complexes from said unbound ligands; (b) releasing ligands from complexes obtained in step (a); and (c) subjecting the released ligands obtained in step (b) to a chemical analysis method, thereby determining said ligands of said macromolecules.

30 The term "ligand" in accordance with the invention designates a molecule which is capable of binding to a macromolecule of the invention, macromolecules being defined further below. Binding of ligands to macromolecules is direct in the sense that a chemical and/or physico-chemical interaction between ligand and macromolecule occurs. Said chemical and physico-chemical interactions are preferably selected from dipole-dipole interactions, dipole-charge
35 interactions, charge-charge interactions, van-der-Waals interactions, hydrophobic interactions, stacking interactions and covalent interactions. Generally speaking, the interaction between ligand and macromolecule may be covalent or non-covalent. Preference

Claims

1. A method of determining ligands of macromolecules in a sample, wherein said sample is a cell-free cell extract, said method comprising or consisting of
 - (a) subjecting said sample comprising (i) complexes formed by said macromolecules and said ligands and (ii) unbound ligands to a method which separates said complexes from said unbound ligands, wherein said method yields fractions and wherein said method is size exclusion chromatography (SEC);
 - (b) releasing ligands from complexes obtained in step (a); and
 - (c) subjecting the released ligands obtained in step (b) to a chemical analysis method, thereby determining said ligands of said macromolecules;
 - (d) determining one or more macromolecules which were bound to ligands by a chemical analysis;wherein said method further comprises the step of
 - (e) determining which ligand binds which macromolecule by determining the amount of a given ligand in each of said fractions, thereby obtaining an elution profile of said given ligand, determining the amount of a given macromolecule in each of said fractions, thereby obtaining an elution profile of said macromolecule and comparing obtained elution profiles of ligands with such obtained elution profiles of macromolecules.
2. The method of claim 1, wherein said cell extract is a cell lysate.
3. The method of any one of the preceding claims, wherein
 - (i) said fractions cover a molecular mass range from 10 kDa to 10000 kDa; and/or
 - (ii) 2, 3, 4, 5, 6, 7, 8, 9, 10 or more fractions are collected.
4. The method of any one of the preceding claims which is for identifying druggable macromolecules and in which a macromolecule which binds a ligand is identified as being a druggable macromolecule.
5. The method of any one of the preceding claims, wherein said chemical analysis method is mass spectrometry (MS), nuclear magnetic resonance (NMR), sequencing and/or detection by antibodies.
6. The method of any one of the preceding claims, wherein said ligand is a non-covalent ligand.

7. The method of claim 6, wherein said releasing in step (b) is effected by denaturation of said complexes.
8. The method of any one of the preceding claims, wherein said method further comprises one, more or all of the following further steps:
 - (aa) prior to step (a), breaking up cells comprising said macromolecules and optionally said ligands, followed by removal of insoluble material;
 - (ab) after step (a) and prior to step (b), washing;
 - (ca) after step (b) and prior to step (c), removing macromolecules, extracting ligands and/or performing liquid chromatography (LC) or gas chromatography (GC) of the ligands, or, if applicable, of the extracted ligands, wherein preferably said LC or GC, to the extent it is performed, is effected in an online LC/MS device, an online LC/NMR device, and online GC/MS device or an online GC/NMR device;
 - (da) after step (b) and prior to step (d), extracting macromolecules and optionally performing LC of the extracted macromolecules, wherein preferably LC is effected in an online LC/MS device or online LC/NMR device.
9. A method of identifying, out of a plurality of test compounds, (a) ligand(s) of a plurality of macromolecules, said method comprising or consisting of:
 - (a) bringing said macromolecules into contact with said plurality of test compounds;
 - (b) subjecting the mixture obtained in step (a) to a method which separates complexes, if any, formed between said macromolecules and (a) ligand(s) from unbound test compounds, wherein said method yields fractions and wherein said method is size exclusion chromatography (SEC);
 - (c) dissociating complexes, thereby releasing bound ligands, if any, from macromolecules;
 - (d) subjecting the released ligand(s) obtained in step (c), if any, to a chemical analysis method, thereby identifying a ligand(s) of said macromolecules;
 - (e) determining one or more macromolecules which were bound to ligands by a chemical analysis;wherein said method further comprises the step of
 - (f) determining which ligand binds which macromolecule by determining the amount of a given ligand in each of said fractions, thereby obtaining an elution profile of said given ligand, determining the amount of a given macromolecule in each of said fractions, thereby obtaining an elution profile of said macromolecule and comparing obtained elution profiles of ligands with such obtained elution profiles of macromolecules.

10. The method of claim 9, wherein
 - (i) said macromolecule(s) is/are (a) protein(s) or (a) nucleic acid(s), preferably RNAs; and/or
 - (ii) said plurality of macromolecules are 2, 3, 4, 5, 6, 7, 8, 9 or 10 macromolecules.
11. The method of claim 9 or 10, wherein
said chemical analysis method of step (d) or (e) is mass spectrometry (MS) or nuclear magnetic resonance (NMR).