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 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 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 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.

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 (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.

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 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

is given to non-covalent interactions.

Generally speaking, but not necessarily, a ligand is smaller, preferably at least an order of magnitude smaller, than a macromolecule in terms of its molecular mass.

Ligands may be cognate ligands. The term “cognate ligand” designates a ligand which interacts under physiological or *in vivo* conditions with a macromolecule, the ligand generally being formed by the biosynthetic machinery of the given biological system. Ligands in accordance with the invention may be cognate ligands, but do not have to be so. For example, a xenobiotic compound, i.e. a compound which does not occur in nature, especially not in an *in vivo* setting, may be found to be a ligand of a macromolecule.

It is, however, expected that endogenous compounds, i.e. cognate ligands, have a number of advantages such as lower toxicology and higher specificity as they are the result of evolutionary selection within the biological system under study.

In terms of binding strength, it is preferred that the dissociation constant K_D of the ligand-macromolecule complex is in or below the two digit micromolar range, i.e. less than 100 µM. Preferably, K_D is less than 10 µM, less than 1 µM, less than 100 nM, less than 10 nM, less than 1 nM, less than 100 pM, or less than 10 pM.

The term “unbound ligand” refers to ligands which are not bound to macromolecules. The term comprises molecules which are not capable of binding to any macromolecule present in the sample subjected to the method in accordance with the first aspect. Furthermore, the term, in its broadest sense, extends to those molecules which are capable of binding a macromolecule, but do not occur in bound form, for example for thermodynamic reasons such as presence of the respective ligand in excess.

In a preferred embodiment, the term “unbound ligand” designates only those molecules which are not capable of binding any macromolecule present in the sample, regardless of the amounts of macromolecules and ligand. These are molecules, generally smaller than macromolecules (as noted above), which do not interact with macromolecules comprised in the sample.

A macromolecule in accordance with the invention is a molecule which is greater than the above defined ligand in terms of its molecule mass. In general, the macromolecule is significantly larger than the ligand, preferably by one, two or more orders of magnitude. The

term “order of magnitude” corresponds to a factor 10. Preference is given to biological macromolecules. Biological macromolecules are large molecules as they occur in biological systems. Biological macromolecules are generally polymers or polycondensates of smaller building blocks. A particularly preferred type of macromolecule is a polypeptide. Also preferred are nucleic acids. Nucleic acids include both DNA and RNA, RNA being preferred.

In a strict sense, the term “macromolecule” designates a single molecule. In a wider sense,

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 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 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).