CLAIMS

1. A method for screening of an *in vitro* display library for binding within a cell of a small-molecule chemical compound binding entity of the library to a protein or RNA target of interest in order to identify at least one individual chemical compound binding entity of the library that is capable of binding within the cell to the protein or RNA target of interest and wherein the method comprises the steps of:

(i): expressing at least one target T_n, wherein n = 1 or more, in a cell and wherein the at least one target is a protein or RNA - the structure of the target is herein termed T-structure;

(ii): introducing an *in vitro* display library into the cell of (i), wherein:

(a): the library is a library of at least 1000 different binding entities B_n, wherein n = 1000 or more, wherein each binding entity is attached to a nucleic acid molecule and the nucleic acid molecule comprises specific nucleic acid sequence information allowing to identify the binding entity – wherein once one knows the specific nucleic acid sequence information of the nucleic acid molecule one directly knows the structure of the specific binding entity attached to the nucleic acid molecule and wherein the binding entities of the library are chemical compounds with an average molecular weight MW below 10000 dalton - the structure of the binding entity attached to the nucleic acid molecule is herein termed B-structure; and

(b): the library is introduced into the cell under binding conditions, which are conditions where a B-structure containing a binding entity capable of binding to a target molecule, binds more efficiently to the corresponding T-structure, than a B-structure containing a binding entity not capable of binding to the same target do and wherein one within the cell gets binding of at least one of the binding entities to at least one target thereby creating within the cell a complex comprising a B-structure bound to a T-structure, which is termed $B_{BoundTo}$T-structure;

(iii):- making lysis of the cell of (ii) to breaking down the membrane of cells in order to:

(A): get the $B_{BoundTo}$T-structures of (ii) out of the cell and thereby obtain a solution comprising $B_{BoundTo}$T-structures**,** wherein the $B_{BoundTo}$T-structures are not within the cells; or

(B): get B-structures out of the cells that had bound to target in step (ii) and have been marked before step (iii) in a way that makes it possible to distinguish the marked B-structures from a B-structure that has not bound to target in step (ii) and thereby obtain a solution comprising binder marked B-structures, wherein the binder marked B-structures are not within the cells; and

(iv): identifying, via use of the solution of (iii) and the nucleic acid molecules that comprises the specific nucleic acid sequence information allowing to identify the binding entity of the B-structures, at least one individual binding entity that binds within the cell of (ii) to at least one target of interest.

2. The method of claim 1, wherein the target T is a protein and wherein the cell is a eukaryotic cell.

3. The method of any of the preceding claims, wherein the cell is a *Xenopus* oocyte.

4. The method of claim 3, wherein the cell is a *Xenopus laevis* oocyte.

5. The method of any of claims 3 to 4, wherein the introducing of the *in vitro* display library into the cell of step (ii) of claim 1 is done via injection of the *in vitro* display library into the Xenopus oocyte; and

wherein the *in vitro* display library of step (ii) of claim 1 is a library of at least 10^6 different binding entities B_n, wherein n = 10^6.

6. The method of any of the preceding claims, wherein the concentration of T-structures in the binding step

step(ii)(b) of claim 1 is at least 10^{-10} M.

7. The method of any of the preceding claims, wherein the step (iii) of claim 1 is step (iii)(A).

8. The method of claim 7, wherein step (iv) of claim 1 is done by:

- binding $B_{BoundTo}T$-structures of the solution (iii)(A) of to a solid support via binding of target of the $B_{BoundTo}T$-structures to the solid support and remove B-structures not bound to target and

thereby not bound to the solid support; and

- identifying, via use of the specific nucleic acid sequence information allowing to identify the binding entity of the $B_{BoundTo}T$-structures bound to the solid support, at least one individual binding entity that binds within the cell of (ii) to at least one target of interest.

9. The method of claim 7, wherein ~~the method is a method wherein:~~:

- the T-structure of (i) of claim 1 comprises a prey (e.g. a target-prey fusion protein) and there in

step (ii) of claim 1 is also introduced a bait attached to a nucleic acid molecule (which e.g. has complementary cohesive ends to the nucleic acid molecule of the B-structure) and the nucleic

acid molecule comprises specific nucleic acid sequence information allowing to identify the specific target and wherein the bait binds to the prey of the target in step (ii) of claim 1 and

thereby creates $B_{BoundTo}T$-structures with the bait bound to the prey of target;

- after lysis step (iii)(A) - fusing (e.g. by ligating via use of a ligase) the nucleic acid molecules of the $B_{BoundTo}T$-structures with the bait bound to the prey of target, wherein the fused nucleic

acid molecules comprise the sequence information allowing to identify binding entity and target; and

- identifying in step (iv) of claim 1 via use of the specific nucleic acid sequence information allowing to identify the binding entity and target of the fused (e.g. ligated) nucleic acid

molecules of the preceding step at least one individual binding entity that binds within the cell of (ii) to at least one target of interest.

10. The method of claim 9, wherein the cell is a Xenopus oocyte, preferably wherein the cell is a Xenopus laevis oocyte.

11. The method of ~~any of~~ claims 9 to 10, wherein ~~there~~ before the "fusing (e.g. by ligating via use of a ligase) the nucleic acid molecules of the $B_{BoundTo}T$-structures with the bait bound to the prey of target" step is made there is a dilution step of the solution (e.g. supernatant) obtained from cell lysis step (iii) and wherein the dilution is a diluting the solution of at least 10^2 fold; and

wherein there is only one target (n = 1) in step (i) of claim 1; and

wherein the "identifying" step (iv) comprises a step, wherein the fused nucleic acid molecules that comprise the sequence information for binding entity and for target are amplified, preferably by use of polymerase chain reaction (PCR); and

wherein the "prey-bait" system is:

- prey: tetrameric streptavidin and bait: biotin;

- prey: monomeric streptavidin (e.g. mSA2) and bait: biotin;

- prey: His-tag and bait: NTA;

- prey: SNAP-tag® and bait: benzyl guanin (BG);

- prey: Halo-Tag® and bait: reactive chloroalkane linker bound to a functional group;

- prey: carbonic anhydrase IX (CAIX) and bait: VD11-4-2; or

- prey: maltose binding protein (MBP) and bait: maltose or maltotriose; and

wherein the nucleic acid molecule attached to the bait is DNA and the nucleic acid molecule of the B-structure is DNA.

12. The method ~~of any~~ of claims 9 to 11, wherein the nucleic acid molecule attached to the bait is a nucleic acid molecule which has complementary cohesive ends to the nucleic acid molecule of the B-structure; and

wherein there are phosphorothioate bonds installed in the phosphate backbone at the complementary cohesive ends of the nucleic acid molecule of the B-structure and the nucleic

acid molecule attached to the bait; and

the number of phosphorothioate bonds is for each nucleic acid molecule at least 2 phosphorothioate bonds.

13. The method of any of claims 1 to 6, wherein the step (iii) of claim 1 is step (iii)(B); and wherein method is a method, wherein

- the T-structure of (i) of claim 1 comprises an enzyme fused to the target and this enzyme is due

to the proximity created by the binding of the binding entity and target of the $B_{BoundTo}T$-structures of (ii) of claim 1 capable of making an reaction on the B-structure of the $B_{BoundTo}T$-structure that marks B-structure and thereby makes it possible to distinguish the B-structure of the $B_{BoundTo}T$-structure from a B-structure that not have bound to target; and

wherein the enzyme fused to the target is a ligase and wherein there in step (ii) of first aspect is also introduced nucleic acid molecules that have complementary cohesive ends to the nucleic acid molecule of the B-structure and wherein the ligase due to the proximity ligates the introduced nucleic acid molecules to the nucleic acid molecule of the B-structure and thereby marks the B-structure.

14. The method of any of the preceding claims, wherein the "identifying" step (iv) of claim 1 comprises a step, wherein the nucleic acid molecules that comprises the specific nucleic acid sequence information allowing to identify the binding entity of the B-structures are amplified, preferably the nucleic acid molecules are amplified by use of polymerase chain reaction (PCR).

15. The method of any of the preceding claims, wherein there in the identifying step (iv) of claim 1 are identified at least 6 different individual binding entities that binds within the cell of (ii) to at least one target of interest; and

wherein there is only one target (n = 1) in step (i) of claim 1.