[0005] The present invention addresses this need by providing technologies for the efficient and systematic identification of the repertoire of receptor:ligand interactions relevant to human physiology, disease and medicine.

SUMMARY

[0005a] In one aspect of the present disclosure, there is provided a system comprising:

(i) a first plurality of suspension-adapted mammalian or insect cells, wherein cells of the plurality are transformed with a vector so as to:

(a) express on a cell-surface thereof a first heterologous protein; and

(b) express a first cytoplasmic-expressing fluorescent protein, such that the first plurality of suspension-adapted cells expresses at least two different types of first heterologous protein, and

(ii) a second plurality of suspension-adapted mammalian or insect cells transformed with a second vector so as to:

(a) express on a cell-surface thereof a second heterologous protein; and

(b) express a second cytoplasmic-expressing fluorescent protein, wherein the second plurality of suspension-adapted cells expresses a single type of second heterologous protein;

wherein each vector expressed by the first plurality of suspension-adapted cells comprises a predetermined 15 to 35 nucleotide sequence unique for the heterologous protein expressed; and

wherein co-localization of both the first and second fluorescent proteins is determined by FACS analysis.

[0005b] In another aspect of the present disclosure, there is provided a method for determining if a candidate ligand protein or peptide binds to second protein or peptide, the method comprising:

a) expressing the candidate ligand protein or peptide as a first heterologous protein of the first plurality of cells in the system disclosed herein; and

b) expressing the second protein or peptide as a second heterologous protein in the system disclosed herein under conditions permitting the first heterologous protein to bind to the second heterologous protein; and

optionally, washing to remove any unbound first heterologous protein, then recovering cells with co-localization of both the first and second heterologous protein; and

c) obtaining nucleic acid from the recovered cells and sequencing the nucleic acid to identify the unique 15-35 nucleotide sequence contained therein so as to identify the candidate ligand protein or peptide corresponding to the unique 15-35 nucleotide that has bound the second protein or peptide.

[0005c] In a further aspect of the present disclosure, there is provided a method for determining the effect of a predetermined amino acid residue of a first protein on binding of the first protein to a second protein, the method comprising expressing the proteins mutated with one or more point mutations relative to the first protein as the plurality of different types of heterologous proteins in the first plurality of suspension-adapted cells of the system disclosed herein, and contacting the plurality with the second protein in the form of the second heterologous protein of the second plurality of cells of the system disclosed herein transformed so as to express the second protein and the second fluorescent protein, and recovering cells that show co-localization of both the first and second fluorescent protein, obtaining nucleic acid from the recovered cells and sequencing the nucleic acid to identify the unique 15-35 nucleotide sequence contained therein so as to identify the first protein that has bound the second protein or peptide, and comparing the level of protein that has bound the second protein or peptide to a predetermined reference level, wherein a level of protein that has bound the second protein or peptide in excess of the predetermined reference level indicates that the residue or residues as mutated in the protein enhance first protein binding to the second protein, and wherein a level of protein that has bound the second protein or peptide below the predetermined reference level indicates that the residue or residues as mutated in the protein inhibit first protein binding to the second protein.

[0005d] In another aspect of the present disclosure, there is provided a mutant PD-L1 polypeptide that binds specifically to PD-1, wherein said PD-Ll polypeptide contains an amino acid residue point mutation of either Gly-119 or Gly-120, and wherein said point mutation comprises one of G119A, G119D, G119R, G120A or G120D, where Gly-119 and Gly-120 correspond to residues 104 and 105 respectively in the amino acid sequence set out in Figure 18.

[0006] A cell microarray is provided comprising:

(i) a first plurality of cells transformed so as to expres a first predetermined heterologous secreted protein, heterologous membrane protein or heterologous cell surface protein and a first fluorescent protein and (ii) at least a second plurality of cells transformed so as to express a second predetermined heterologous secreted protein or a second heterologous protein and a second fluorescent protein,

wherein the first and second plurality of cells are adhered to a solid surface of the microarray, and wherein the first and second plurality of cells are in spatially distinct locations on the solid surface.

[0007] A cell microarray is provided comprising:

(i) a first plurality of cells transformed so as to express (a) a first predetermined heterologous protein and (b) a first fluorescent protein and (ii) at least a second plurality of cells transformed so as to express (a) a second predetermined heterologous protein and (b) a second fluorescent protein,

wherein the first and second plurality of cells are adhered to a solid surface of the microarray, and wherein the first and second plurality of cells are in spatially distinct locations on the solid surface.

[0008] A process is provided for making a cell microarray as described herein, comprising affixing a first plurality of expression constructs encoding the first heterologous protein and the fluorescent protein on a solid surface of a microarray and affixing at least a second plurality of expression constructs encoding the second heterologous protein and the second fluorescent protein on the solid surface of the microarray on the solid surface in a spatially distinct location different from the affixed first plurality of expression constructs, and contacting the expression constructs with a plurality of cells under conditions comprising the presence of a transfection agent, so as to permit the cells to adhere to the solid surface and for transfection to occur of at least a portion of the cells in each spatially distinct location with the respective expression constructs.

What is claimed is:

1. A system comprising:

(i) a first plurality of suspension-adapted mammalian or insect cells, wherein cells of the plurality are transformed with a vector so as to:

(a) express on a cell-surface thereof a first heterologous protein; and

(b) express a first cytoplasmic-expressing fluorescent protein, such that the first plurality of suspension-adapted cells expresses at least two different types of first heterologous protein, and

(ii) a second plurality of suspension-adapted mammalian or insect cells transformed with a second vector so as to:

(a) express on a cell-surface thereof a second heterologous protein; and

(b) express a second cytoplasmic-expressing fluorescent protein, wherein the second plurality of suspension-adapted cells expresses a single type of second heterologous protein;

wherein each vector expressed by the first plurality of suspension-adapted cells comprises a predetermined 15 to 35 nucleotide sequence unique for the heterologous protein expressed; and

wherein co-localization of both the first and second fluorescent proteins is determined by FACS analysis.

2. The system of claim 1, wherein the second heterologous protein is a ligand for the at least two different types of first heterologous protein.

3. The system of claim 1 or 2, wherein any individual cell of the first plurality of cells expresses only one first heterologous protein on the cell surface thereof.

4. The system of claim 1, 2 or 3, wherein different types of first heterologous proteins of the plurality are each mutants of a predetermined wildtype protein.

5. The system of any one of claims 1 to 4, wherein the second heterologous protein is a wildtype protein.

6. The system of any one of claims 1 to 5, wherein each type of heterologous protein of the first plurality of cells differs from each other type of heterologous protein of the plurality by 1, 2, 3, 4 or 5 amino acid residue point mutations.

7. The system of any one of claims 1 to 6, wherein the first or second heterologous protein is an immunoglobulin superfamily protein, a TNF receptor protein, a cytokine, a chemokine, a type 1 transmembrane receptor protein, a type 2 transmembrane receptor protein, an ion channel protein, a membrane transporter protein, a toll-like receptor, a G-protein coupled receptor, a growth factor receptor, a nectin, an interleukin, or an interleukin receptor.

8. The system of any one of claims 1 to 7, wherein the first or second heterologous protein is a PD-1 polypeptide, a PD-L1 polypeptide, a PD-L2 polypeptide, a B7-1 polypeptide, a B7-2 polypeptide, a CD28 polypeptide, a CTLA-4 polypeptide, an ICOS polypeptide, or an ICOS-L polypeptide.

9. The system of any one of claims 1 to 8, wherein the unique sequence is capable of being primed by one or more universal primer(s).

10. The system of any one of claims 1 to 9, wherein the first and second fluorescent proteins are different colours.

11. The system of any one of claims 1 to 10, wherein the mammalian cells are human cells.

12. A method for determining if a candidate ligand protein or peptide binds to second protein or peptide, the method comprising:

a) expressing the candidate ligand protein or peptide as a first heterologous protein of the first plurality of cells in the system of any one of claims 1 to 11; and

b) expressing the second protein or peptide as a second heterologous protein in the system of any one of claims 1 to 11 under conditions permitting the first heterologous protein to bind to the second heterologous protein; and
optionally, washing to remove any unbound first heterologous protein, then recovering cells with co-localization of both the first and second heterologous protein; and

c) obtaining nucleic acid from the recovered cells and sequencing the nucleic acid to identify the unique 15-35 nucleotide sequence contained therein so as to identify the candidate ligand protein or peptide corresponding to the unique 15-35 nucleotide that has bound the second protein or peptide.

13. A method for determining the effect of a predetermined amino acid residue of a first protein on binding of the first protein to a second protein, the method comprising expressing the proteins mutated with one or more point mutations relative to the first protein as the plurality of different types of heterologous proteins in the first plurality of suspension-adapted cells of the system of any one of claims 1 to 11, and contacting the plurality with the second protein in the form of the second heterologous protein of the second plurality of cells of the system of any one of claims 1 to 11 transformed so as to express the second protein and the second fluorescent protein, and recovering cells that show co-localization of both the first and second fluorescent protein, obtaining nucleic acid from the recovered cells and sequencing the nucleic acid to identify the unique 15-35 nucleotide sequence contained therein so as to identify the first protein that has bound the second protein or peptide, and comparing the level of protein that has bound the second protein or peptide to a predetermined reference level,

wherein a level of protein that has bound the second protein or peptide in excess of the predetermined reference level indicates that the residue or residues as mutated in the protein enhance first protein binding to the second protein, and wherein a level of protein that has bound the second protein or peptide below the predetermined reference level indicates that the residue or residues as mutated in the protein inhibit first protein binding to the second protein.

14. The method of claim 13, wherein the predetermined level is a control, optionally wherein the predetermined level is obtained by assaying the level of un-mutated first protein binding to the second protein.

15. The method of any one of claims 12 to 14, wherein cells that show co-localization of both the first and second fluorescent protein are recovered through FACS analysis.

16. A mutant PD-L1 polypeptide that binds specifically to PD-1, wherein said PD-L1 polypeptide contains an amino acid residue point mutation of either Gly-119 or Gly-120, and wherein said point mutation comprises one of G119A, G119D, G119R, G120A or G120D, where Gly-119 and Gly-120 correspond to residues 104 and 105 respectively in the amino acid sequence set out in Figure 18.