

What is claimed is:

1. A method of determining the effect of non-specific interactions on binding of a target molecule to a capture molecule in simulated *in vivo* conditions, comprising:

(a) selecting a biologically relevant molecular crowding agent, wherein said crowding agent does not specifically bind to a target molecule;

(b) contacting a solution comprising ~~saida~~ biologically relevant molecular crowding agent and ~~a-said~~ target molecule with a biosensor, wherein the surface of the biosensor comprises a capture molecule that specifically binds the target molecule;

(c) allowing the target molecule to bind to the capture molecule;

(d) determining an amount of the target molecule bound to the capture molecule using biolayer interferometry; and

(e) comparing the amount of the target molecule bound to the capture molecule to a control threshold to determine an effect of non-specific interactions on binding of the target molecule to the capture molecule,

wherein the control threshold is a normalized response to an ideal, dilute, or semi-dilute solution.

~~2. The method of claim 1, further comprising:~~

~~comparing the amount of the target biomolecule bound to the capture molecule to a control threshold that distinguishes between a target biomolecule having an attractive non-specific interaction with the molecular crowding agent and those having a repulsive non-specific interaction with the molecular crowding agent.~~

~~3. The method of claim 2, wherein the threshold is a normalized response to an ideal, dilute, or semi-dilute solution.~~

24. The method of ~~any one of claims 1-3~~, wherein the biologically relevant molecular crowding agent comprises human serum albumin (HSA), IgG, transferrin, fibrinogen, IgA, α 2-macroglobulin, IgM, α 1-antitrypsin, haptoglobin, α 1-acid glycoprotein, apolipoprotein A-1, apolipoprotein A-11, or any other protein components found in blood or serum samples.

35. The method of claim 24, wherein the molecular crowding agent is present at a physiologically relevant concentration.

46. The method of claim 24, wherein the molecular crowding agent is present at a

concentration of between about 1 g/L and about 100g/L.

57. The method of any one of claims 1-46, further comprising, determining an amount of binding at two or more concentrations of the biologically relevant molecular crowding agent.

68. The method of any one of claims 1-57, wherein the biologically relevant molecular crowding agent comprises serum and/or plasma.

79. The method of any one of claims 1-68, further comprising, determining an amount of binding at two or more pHs to determine a pH dependence of binding.

810. The method of any one of claims 1-79, further comprising, determining an amount of binding at two or more salt concentrations to determine a salt dependence of binding.

911. The method of any one of claims 1-810, wherein the target molecule comprises an antibody and the capture molecule comprises an antigen that specifically binds to the antibody.

1012. The method of any one of claims 1-810, wherein the target molecule comprises an antigen and the capture molecule comprises an antibody that specifically binds to the antigen.

1113. The method of any one of claims 1-810, wherein the target molecule comprises a receptor or ligand binding fragment thereof and the capture molecule comprises a ligand that specifically binds to the receptor or ligand binding fragment thereof.

1214. The method of any one of claims 1-810, wherein the target molecule comprises a ligand and the capture molecule comprises a receptor or ligand binding fragment thereof that specifically binds to the ligand.

1315. The method of any one of claims 1-1214, wherein the capture molecule is coupled to the surface of the sensor with a linker.

1416. The method of claim 1315, wherein the linker comprises biotin and streptavidin or avidin.

1517. The method of ~~any one of~~ claims 13-10, wherein the ~~linker~~target molecule ~~comprises an antibody and the capture molecule~~ comprises anti-IgG Fc.

1618. The method of claims 157 , wherein the anti-IgG Fc is anti-human IgG Fc.

1719. A method of selecting a biomolecule under simulated *in vivo* conditions, comprising:

(a) contacting a first solution simulating *in vivo* conditions with a biosensor, wherein a surface of the biosensor comprises a capture molecule that specifically binds target biomolecules from a set of two or more target biomolecules of interest, wherein the first solution further comprises a biologically relevant molecular crowding agent that does not specifically bind to the two or more target biomolecules of interest and a first target biomolecule selected from the set of two or more target biomolecules of interest;

(b) allowing the first target biomolecule to bind to the capture molecule; and

(c) determining an amount of the first target biomolecule bound to the capture molecule using biolayer interferometry;

(d) contacting a second solution simulating *in vivo* conditions with the biosensor, wherein the second solution further comprises the biologically relevant molecular crowding agent and a second target biomolecule selected from the set of two or more biomolecules of interest;

(e) allowing the second target biomolecule to bind to the capture molecule;

(f) determining an amount of the second target biomolecule bound to the capture molecule using biolayer interferometry; and

(g) comparing the determined amounts to identify whether the first or second target biomolecule has a greater amount of binding.

~~20. The method of claim 19, further comprising contacting a second solution simulating *in vivo* conditions with the biosensor, wherein the second solution further comprises the biologically relevant molecular crowding agent and a second target biomolecule selected from the set of two or more biomolecules of interest;~~

~~allowing the second target biomolecule to bind to the capture molecule;~~

~~determining an amount of the second target molecule bound to the capture molecule using biolayer interferometry; and~~

~~comparing the amount of the first biomolecule bound to the capture molecule and the amount of the second biomolecule bound to the capture molecule to identify which of the first biomolecule and the second biomolecule has a greater amount of binding.~~

1821. The method of claim 1719 or 20, further comprising:

comparing the amount of the first and/or second target biomolecule bound to the capture molecule to a control threshold that distinguishes between a target biomolecule having an attractive

non-specific interaction with the molecular crowding agent and those having a repulsive non-specific interaction with the molecular crowding agent.

[1922](#). The method of claim [1821](#), wherein the threshold is a normalized response to an ideal, dilute, or semi-dilute solution.

[2023](#). The method of any one of claims [17-1919-22](#), wherein the biologically relevant molecular crowding agent comprises human serum albumin (HSA) , IgG, transferrin, fibrinogen, IgA, α 2-macroglobulin, IgM, α 1-antitrypsin, haptoglobin, α 1-acid glycoprotein, apolipoprotein A-1, apolipoprotein A-11, or any other protein components found in blood or serum samples.

[2124](#). The method of claim [203](#), wherein the molecular crowding agent is present at a physiologically relevant concentration.

[2225](#). The method of claim [203](#), wherein the molecular crowding agent is present at a concentration of between about 1 g/L and about 100g/L.

[2326](#). The method of any one of claims [17-2219-25](#), further comprising, determining an amount of binding at two or more concentrations of the biologically relevant molecular crowding agent.

[2427](#). The method of any one of claims [17-2319-26](#), wherein the biologically relevant molecular crowding agent comprises serum and/or plasma.

[2528](#). The method of any one of claims [17-2419-27](#), further comprising, determining an amount of binding at two or more pHs to determine a pH dependence of binding.

[2629](#). The method of any one of claims [17-2519-28](#), further comprising, determining an amount of binding at two or more salt concentrations to determine a salt dependence of binding.

[2730](#). The method of any one of claims [17-2619-29](#), wherein the target molecule comprises an antibody and the capture molecule comprises an antigen that specifically binds to the antibody.

[2831](#). The method of any one of claims [17-2619-29](#), wherein the target molecule comprises an antigen and the capture molecule comprises an antibody that specifically binds to the antigen.

[2932](#). The method of any one of claims [17-2619-29](#), wherein the target molecule

comprises a receptor or ligand binding fragment thereof and the capture molecule comprises a ligand that specifically binds to the receptor or ligand binding fragment thereof.

3033. The method of any one of claims 17-26~~19-29~~, wherein the target molecule comprises a ligand and the capture molecule comprises a receptor or ligand binding fragment thereof that specifically binds to the ligand.

3134. The method of any one of claims 17-30~~19-33~~, wherein the capture molecule is coupled to the surface of the sensor with a linker.

3235. The method of claim 3134, wherein the linker comprises biotin and streptavidin or avidin.

3336. The method of ~~any one of claims 19-29~~31, wherein the ~~linker~~target molecule ~~comprises an antibody and the capture molecule comprises~~ comprises anti-IgG Fc.

3437. The method of claim 3336, wherein the anti-IgG Fc is anti-human IgG Fc.