

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 said biologically relevant molecular crowding agent and 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, 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.

3. The method of claim 2, wherein the molecular crowding agent is present at a physiologically relevant concentration.

4. The method of claim 2, wherein the molecular crowding agent is present at a concentration of between about 1 g/L and about 100g/L.

5. The method of any one of claims 1-4, further comprising, determining an amount of binding at two or more concentrations of the biologically relevant molecular crowding agent.

6. The method of any one of claims 1-5, wherein the biologically relevant molecular crowding agent comprises serum and/or plasma.

7. The method of any one of claims 1-6, further comprising, determining an amount

of binding at two or more pHs to determine a pH dependence of binding.

8. The method of any one of claims 1-7, further comprising, determining an amount of binding at two or more salt concentrations to determine a salt dependence of binding.

9. The method of any one of claims 1-8, wherein the target molecule comprises an antibody and the capture molecule comprises an antigen that specifically binds to the antibody.

10. The method of any one of claims 1-8, wherein the target molecule comprises an antigen and the capture molecule comprises an antibody that specifically binds to the antigen.

11. The method of any one of claims 1-8, 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.

12. The method of any one of claims 1-8, 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.

13. The method of any one of claims 1-12, wherein the capture molecule is coupled to the surface of the sensor with a linker.

14. The method of claim 13, wherein the linker comprises biotin and streptavidin or avidin.

15. The method of claim 13, wherein the linker comprises anti-IgG Fc.

16. The method of claim 15, wherein the anti-IgG Fc is anti-human IgG Fc.

17. 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;

(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.

18. The method of claim 17, 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.

19. The method of claim 18, wherein the threshold is a normalized response to an ideal, dilute, or semi-dilute solution.

20. The method of any one of claims 17-19, 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.

21. The method of claim 20, wherein the molecular crowding agent is present at a physiologically relevant concentration.

22. The method of claim 20, wherein the molecular crowding agent is present at a concentration of between about 1 g/L and about 100g/L.

23. The method of any one of claims 17-22, further comprising, determining an amount of binding at two or more concentrations of the biologically relevant molecular crowding agent.

24. The method of any one of claims 17-23, wherein the biologically relevant molecular crowding agent comprises serum and/or plasma.

25. The method of any one of claims 17-24, further comprising, determining an amount of binding at two or more pHs to determine a pH dependence of binding.

26. The method of any one of claims 17-25, further comprising, determining an amount of binding at two or more salt concentrations to determine a salt dependence of binding.

27. The method of any one of claims 17-26, wherein the target molecule comprises an antibody and the capture molecule comprises an antigen that specifically binds to the antibody.

28. The method of any one of claims 17-26, wherein the target molecule comprises an antigen and the capture molecule comprises an antibody that specifically binds to the antigen.

29. The method of any one of claims 17-26, 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.

30. The method of any one of claims 17-26, 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.

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

32. The method of claim 31, wherein the linker comprises biotin and streptavidin or avidin.

33. The method of 31, wherein the linker comprises anti-IgG Fc.

34. The method of claim 33, wherein the anti-IgG Fc is anti-human IgG Fc.