CLAIMS

1. A microfluidic device comprising:

an enclosure comprising a substrate and microfluidic circuit material, the enclosure defining a flow region and at least one sequestration pen, each located within the enclosure, wherein the at least one sequestration pen comprises an isolation region with a single opening to a connection region, wherein the connection region fluidically connects the isolation region and the flow region, the connection region having a proximal opening to the flow region and a distal opening to the isolation region; and

at least one in situ-generated capture structure disposed within the at least one sequestration pen, wherein the at least one in situ-generated capture structure comprises a solidified polymer network.

2. The microfluidic device of claim 1, wherein the solidified polymer network comprises one or more functionalized sites.

3. The microfluidic device of claim 1 or 2, wherein the solidified polymer network comprises an assay reagent or assay analyte.

4. The microfluidic device of any one of claims 1-3, wherein the at least one in situ-generated capture structure is disposed within the isolation region of the sequestration pen.

5. The microfluidic device of claim 3 or 4, wherein the assay reagent is non-covalently attached to the solidified polymer network.

6. The microfluidic device of any one of claims 3-5, wherein the assay reagent comprises a protein, a nucleic acid, an organic molecule, and/or a saccharide.

7. The microfluidic device of claim 6, wherein the assay reagent comprises an antibody or an antigen.

8. The microfluidic device of claim 3 or 4, wherein the assay analyte comprises an antibody or a cytokine.

9. The microfluidic device of any one of claims 1-8, wherein two or more in situ-generated capture structures are disposed in the at least one sequestration pen.

10. The microfluidic device of any one of claims 1-9, wherein the solidified polymer network comprises a photoinitiated polymer.

11. The microfluidic device of any one of claims 1-10, wherein the solidified polymer network comprises a synthetic polymer, a modified synthetic polymer, a biological polymer, or any combination thereof.

12. The microfluidic device of any one of claims 1-11, wherein the substrate is configured to generate dielectrophoresis (DEP) forces within the enclosure.

13. A method of assaying a micro-object in a microfluidic device comprising at least a first in situ-generated capture structure comprising:

disposing a micro-object within the microfluidic device in a region proximal to the at least first in situ-generated capture structure comprising a solidified polymer network, wherein the solidified polymer network comprises an assay reagent or assay analyte;

contacting the assay reagent or assay analyte with the micro-object or a biological product of the micro-object; and

detecting an interaction of the assay reagent or assay analyte with the micro-object or the biological product.

14. The method of claim 13, wherein the microfluidic device comprises: an enclosure comprising a substrate and microfluidic circuit material, the enclosure defining a flow region and at least one sequestration pen; and

wherein the at least first in situ-generated capture structure is disposed within the at least one sequestration pen.

15. The method of claim 13 or 14, wherein the at least one sequestration pen comprises an isolation region with a single opening to a connection region, wherein the connection region fluidically connects the isolation region and the flow region, the connection region having a proximal opening to the flow region and a distal opening to the isolation region, and further wherein the at least first in situ-generated capture structure is disposed within the isolation region of the sequestration pen.

16. The method of any one of claims 13-15, wherein the assay reagent or assay analyte is non-covalently attached to the solidified polymer network.

17. The method of any one of claims 13-16, wherein the biological product comprises a protein, an oligonucleotide, an organic molecule, or a saccharide.

18. The method of any one of claims 13-17, wherein the step of contacting the assay reagent or assay analyte with the biological product or the micro-object further comprises forming a non-covalent complex.

19. The method of any one of claims 13-21, wherein the step of detecting the interaction further comprises introducing a detection reagent having a detectable label to the region proximal to the at least first in situ-generated capture structure.

20. The method of claim 19, wherein the detectable label is configured to be concentrated to the at least first in situ-generated capture structure when the assay reagent or assay analyte interacts with the biological prodsauct or the micro-object.

21. The method of any one of claims 13-23, wherein the step of detecting comprises detecting a fluorescent signal from the at least first in situ-generated capture structure.

22. The method of any one of claims 13-24, wherein the microfluidic device further comprises a second in situ-generated capture structure disposed within the at least one sequestration pen, wherein the second in situ-generated capture structure comprises a second solidified polymer network and further wherein the second solidified polymer network comprises a second assay reagent or assay analyte, wherein each of the first and second in situ-generated capture structures comprises a different assay reagent or assay analyte, and wherein the step of detecting comprises detecting a first biological product of the micro-object and a second biological product of the micro-object, wherein the first biological product is different from the second biological product.

23. The method of any one of claims 13-25, wherein the step of disposing the micro-object within the microfluidic device in the region proximal to the at least first in situ-generated capture structure comprises moving the micro-object using dielectrophoresis force.

24. The method of claim 13, wherein the solidified polymer network comprises an assay reagent, and wherein the assay reagent comprises a protein.

25. The method of claim 24, wherein the assay reagent comprises an antigen or an antibody.

26. The method of claim 13, wherein the biological product of the micro-object is an antibody or an antigen.

27. A kit comprising a microfluidic device comprising:

an enclosure comprising a substrate and microfluidic circuit material, the enclosure defining a flow region and at least one sequestration pen, wherein the at least one sequestration pen comprises an isolation region with a single opening to a connection region, wherein the connection region fluidically connects the isolation region and the flow region, the connection region having a proximal opening to the flow region and a distal opening to the isolation region; and

a functionalized pre-polymer that can be controllably activated to form a solidified polymer network.

28. The kit of claim 27, further comprising an assay reagent or assay analyte.

29. A method of preparing a microfluidic device comprising at least a first in situ-generated capture structure, the method comprising:

providing the microfluidic device, wherein the microfluidic device comprises an enclosure comprising a substrate and microfluidic circuit material, the enclosure defining a flow region and at least one sequestration pen fluidically connected to the flow region;

introducing a first flowable functionalized pre-polymer into the flow region; and

activating solidification of the first flowable functionalized pre-polymer at at least one selected area of the at least one sequestration pen, thereby forming the at least a first in situ-generated capture structure therein.

30. The method of claim 29, wherein the at least a first in-situ generated capture structure comprises a solidified polymer network comprising one or more functionalized sites, wherein the one or more functionalized sites comprise a biotin, avidin or streptavidin moiety.

31. The method of claim 29 or 30 further comprising: introducing a first functionalized assay reagent or assay analyte to the at least first in situ-generated capture structure within the at least one sequestration pen; and

associating the first functionalized assay reagent or assay analyte to the functionalized sites of the solidified polymer network of the at least first in situ-generated capture structure.

32. The method of any one of claims 29-31, further comprising: introducing a second functionalized assay reagent or assay analyte.

33. The method of claim 32, wherein the second functionalized assay reagent or assay analyte is detectably distinguishable from the first functionalized assay reagent or assay analyte.

34. The method of claim 32, wherein the second functionalized assay reagent or assay analyte associates with a second functionalized site on a second in situ-generated capture structure in the at least one sequestration pen.

35. The method of claim 34 further comprising introducing the second in situ-generated capture structure in the at least one sequestration pen, wherein introducing the second in situ-generated capture structure comprises the steps of:

introducing a second flowable functionalized pre-polymer into the flow region; and

activating solidification of the second flowable functionalized pre-polymer at at least a second selected area of the at least one sequestration pen, thereby forming the second in situ-generated capture structure therein.