CLAIMS

1. A method for detecting an analyte using surface enhanced Raman spectroscopy (SERS), the method comprising
   a) contacting one or more analyte-binding molecules with the analyte under conditions that allow binding of the analyte to the one or more analyte-binding molecules to form a first mixture, wherein the analyte comprises haptoglobin and the one or more analyte-binding molecules comprise haemoglobin,
   b) contacting a liquid reagent comprising a peroxidase substrate and a peroxide source with the first mixture to form a second mixture, while maintaining pH of the second mixture at 10 or less,
   c) quenching the second mixture to form a third mixture,
   d) contacting the third mixture with a SERS-active substrate, and
   e) detecting a surface enhanced Raman signal from the third mixture and/or a surface of the SERS-active substrate, wherein the one or more analyte-binding molecules are attached to a support, wherein the support comprises a non-SERS active material or a SERS-active material, wherein the non-SERS active material comprises an inorganic oxide particle having a magnetic core or a polymeric particle having a magnetic core, and wherein the SERS-active material comprises gold nanoparticles.

2. The method according to claim 1, wherein the one or more analyte-binding molecules further comprise an antibody.

3. The method according to claim 1, wherein contacting the one or more analyte-binding molecules with the analyte is carried out for a time period in the range of about 2 minutes to about 10 minutes.

4. The method according to claim 1, wherein pH of the first mixture is adjusted to be in the range of about 2.6 to about 2.8.

5. The method according to claim 1, wherein the peroxidase substrate is selected from the group consisting of 3,3’,5,5’-tetramethylbenzidine, o-phenylenediamine, biphenyl-

4,4'-dithiol, 5-bromo-4-chloro-3-indolyl phosphate, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), and combinations thereof.

6. The method according to claim 1, wherein the peroxide source is selected from the group consisting of hydrogen peroxide, carbamide peroxide, sodium perborate, sodium percarbonate, urea peroxide, and combinations thereof.

7. The method according to claim 1, wherein pH of the second mixture is maintained in the range of about 2.6 to about 2.8.

8. The method according to claim 1, wherein contacting the liquid reagent with the first mixture is carried out for a time period in the range of about 1 minute to about 5 minutes.

9. The method according to claim 1, wherein quenching the second mixture is carried out by at least one of heating the second mixture or adding a quenching agent to the second mixture.

10. The method according to claim 9, wherein heating the second mixture is carried out at a temperature in the range of about 80°C to about 95°C.

11. The method according to claim 9, wherein the quenching agent is selected from the group consisting of hydrochloric acid, sulfuric acid, saponins, sodium dodecyl sulfate, cetyl trimethyl ammonium bromide, N-laurylsarcosine, dodecyltrimethylammonium bromide, 8-anilino-1-naphthalenesulfonic acid, protoporphyrin, bilirubin, taurodeoxycholic acids, dicoumarol, 2-mercaptobenzothiazole, catalase enzyme, and combinations thereof.

12. The method according to claim 1, wherein the SERS-active substrate comprises gold nanoparticles.

13. The method according to claim 12, wherein the gold nanoparticles are attached on a chromatographic paper.

14. The method according to claim 1, wherein the SERS-active substrate comprises

a) a plurality of nanostructures attached on a support, and

b) a first metallic layer deposited on the plurality of nanostructures.

15. The method according to claim 14, wherein the SERS-active substrate further comprises a second metallic layer deposited on the first metallic layer to form a metallic bilayer.

16. The method according to claim 15, wherein the first metallic layer and the second metallic layer are independently gold or silver.

17. The method according to claim 16, wherein the first metallic layer is silver, and the second metallic layer is gold.

18. The method according to claim 1, wherein the analyte is contained in a sample, wherein the sample is a bodily fluid and the detection is *in vitro*.

19. The method according to claim 1, wherein the surface enhanced Raman signal from the third mixture and/or the surface of the SERS-active substrate is correlated with amount of the analyte.