

SUMMARY

[0005] Here we describe a universal, fluorescence label-free method for detecting viruses in a wide range of environmental conditions, with a single-particle sensitivity and enhanced specificity based on mechanical selection of specific interactions. The novel method enables fast, low-cost point-of-care diagnosis and home-based self-diagnosis of specific viruses and viral infection induced antibodies in suspected human samples. It also serves as a novel platform for discovering viral entry inhibitors.

[0006] The principle of the method described here also applies for fast, accurate and sensitive detection of any other multivalent targets such as biomarkers, exosomes, antibodies, pathogen-associated molecules, pollutants, etc. The method serves as a novel platform for disease diagnostics, biologics drug discovery, and environment monitoring.

[0007] The present disclosure relates to a method of detecting the presence or absence of an analyte in a test sample comprising the steps of:

a) incubating a plurality of motion-resistant particles and a plurality of force-driven particles with said test sample to form a test mixture, wherein said motion-resistant particles are coated with a first sensing element and said force-driven particles are coated with a second sensing element, both first and second sensing elements are capable of specific binding with the analyte, wherein when the analyte is present in the test sample, said analyte binds with said first and second sensing elements on said motion-resistant particles and said force-driven particles, respectively, to form bound motion-resistant /analyte/ force-driven particle conjugates;

b) adding the test mixture of step a) to a viscous medium and applying a force to the force-driven particles to separate said bound motion-resistant/analyte/force-driven particle conjugates, when present, from the unbound motion resistant particles and unbound force-driven particles.

[0007a] In a first aspect, the present disclosure relates to a method of detecting the presence or absence of an analyte in a test sample comprising the steps of:

a) incubating a plurality of motion-resistant particles and a plurality of force-driven particles with said test sample to form a test mixture, wherein said motion-resistant particles are coated with a first sensing element and said force-driven particles are coated with a second sensing element, both first and second sensing elements are capable of specific binding with the analyte, wherein when the analyte is present in the test sample, said analyte binds with said first and second sensing elements on said motion-resistant particles and said force-driven particles, respectively, to form bound motion-resistant/analyte/force-driven particle conjugates;

b) adding the test mixture of step a) to a viscous medium and applying a force to the force-driven particles to separate said bound motion-resistant/analyte/force-driven particle conjugates, when present, from the unbound motion-resistant particles and unbound force-driven particles, wherein said viscous medium has a viscosity in the range of 0.01 Pa.s to 10 Pa.s.

[0008] Advantageously, the method is universal and does not require the use of fluorescence labels for detecting a range of analytes including viruses in a wide range of environmental conditions. Mechanical selection achieved by the force application (such as mechanical force) further enhances the specificity of detection, as the specifically linked particle-particle conjugates dissociate under the application of the mechanical force slower than the non-specific ones. Counting the amount of specifically linked particle-particle conjugates results in the detection, with single-particle sensitivity and accuracy of detection. The method enables fast diagnosis of viral infections and may serve as a platform for discovering viral entry inhibitors. This is highly advantageous for clinical settings which require fast diagnosis to be carried out. Further, due to

the ease of using the method, the method can be used as a home-based self-diagnosis of viral infection.

[0009] Further advantageously, when applied to virus detection, the method does not require nucleic acids extraction from virus, reverse transcription (RT) for RNA viruses or PCR amplification, therefore avoiding significant loss of sample size during multiple-step procedures. The method can also be used for enriching the analyte in a low-concentration sample for downstream analysis such as sequencing. The method can be carried out on a small sample size, such as a finger-prick blood droplet. Due to its mechanically enhanced specificity and single-particle sensitivity, it can detect analyte in low concentrations, such as samples having a concentration in the picomolar to femtomolar.

[0010] The present disclosure relates to a sensing kit comprising
a plurality of motion-resistant particles; and
a plurality of force-driven particles,
wherein said motion-resistant particles and the force-driven particles are coated with a first sensing element and a second sensing element respectively, that can specifically bind to a target analyte in a test sample.

[0010a] In a second aspect, the present disclosure relates to a sensing kit comprising:
a plurality of motion-resistant particles;
a plurality of force-driven particles; and
a viscous medium having a viscosity in the range of 0.01 Pa.s to 10 Pa.s,
wherein said motion-resistant particles and the force-driven particles are coated with a first sensing element and a second sensing element respectively, that can specifically bind to a target analyte in a test sample.

[0011] The present disclosure relates to a system for detecting the presence or absence of an analyte in a test sample comprising:

a) a plurality of motion-resistant particles and a plurality of force-driven particles, wherein said motion-resistant particles are coated with a first sensing element and said force-driven particles are coated with a second sensing element, wherein both first and second sensing elements are capable of specific binding with the analyte, when present, to form bound motion-resistant/analyte/force-driven particle conjugates; and

b) a separation means comprising a viscous medium and a force that can separate said bound motion-resistant/analyte/force-driven particle conjugates, when present, from unbound motion-resistant particles and unbound force-driven particles.

[0011a] In a third aspect, the present disclosure relates to a system for detecting the presence or absence of an analyte in a test sample comprising:

a) a plurality of motion-resistant particles and a plurality of force-driven particles, wherein said motion-resistant particles are coated with a first sensing element and said force-driven particles are coated with a second sensing element, wherein both first and second sensing elements are capable of specific binding with the analyte, when present, to form bound motion-resistant/analyte/force-driven particle conjugates; and

b) a separation means comprising a viscous medium and a force that can separate said bound motion-resistant/analyte/force-driven particle conjugates, when present, from unbound motion-resistant particles and unbound force-driven particles, wherein said viscous medium has a viscosity in the range of 0.01 Pa.s to 10 Pa.s.

[0012] In a fourth aspect, the present disclosure relates to use of the sensing kit or the system as described herein for virus and biomarker detection.

[0013] Advantageously, the use of the sensing kit or the system may be designed, for example, for virus detection for actual clinical trials using human sample. The use of force, which can be a mechanical force (such as magnetic force generated by the magnetic field), through the viscous medium for selection and sorting may minimize the possibility of false positive and false negative

CLAIMS

1. A method of detecting the presence or absence of an analyte in a test sample comprising the steps of:

a) incubating a plurality of motion-resistant particles and a plurality of force-driven particles with said test sample to form a test mixture, wherein said motion-resistant particles are coated with a first sensing element and said force-driven particles are coated with a second sensing element, both first and second sensing elements are capable of specific binding with the analyte, wherein when the analyte is present in the test sample, said analyte binds with said first and second sensing elements on said motion-resistant particles and said force-driven particles, respectively, to form bound motion-resistant/analyte/force-driven particle conjugates;

b) adding the test mixture of step a) to a viscous medium and applying a force to the force-driven particles to separate said bound motion-resistant/analyte/force-driven particle conjugates, when present, from the unbound motion-resistant particles and unbound force-driven particles, wherein said viscous medium has a viscosity in the range of 0.01 Pa.s to 10 Pa.s.

2. The method of claim 1 further comprising step c) quantification of the bound motion-resistant/analyte/force-driven particle conjugates after separation of the bound motion-resistant/analyte/force-driven particle conjugates from the unbound motion-resistant particles and unbound force-driven particles.

3. The method of claim 1 or 2, wherein the size of said motion-resistant particles is in the range of 1 μm to 100 μm .

4. The method of any one of claims 1 to 3, wherein said motion-resistant particles are selected from the group consisting of polystyrene (PS), poly(lactic-co-glycolic acid) (PLGA), poly(methyl methacrylate) (PMMA), poly(ϵ -caprolactone) (PCL), polyacrylate (PA), polylactide (PLA), poly(Glycidyl Methacrylate) (PGMA), melamine resin, silica, hydrogel and lipid membrane vesicle.

5. The method of any one of claims 1 to 4, wherein the size of said force-driven particles is in the range of 10 nm to 50 μm .

6. The method of any one of claims 1 to 5, wherein when said force-driven particles are magnetic particles said magnetic particles are selected from the group consisting of iron, cobalt, nickel, alloy thereof, oxide thereof and combinations thereof.

7. The method of any one of claims 1 to 6, wherein said viscous medium is selected from the group consisting of glycerol, olive oil, linseed oil, motor oil, syrup, ferrofluid, a solution of crowding agents, a base liquid with one or more thickening agents, and mixture thereof.

8. The method of any one of claims 1 to 7, wherein said analyte has multiple equivalent binding sites for binding with said first sensing element and said second sensing element; and wherein said binding sites are different for the first sensing element and second sensing element.

9. The method of any one of claims 1 to 8, wherein step a) comprise the steps of
a1) incubating said motion-resistant or said force-driven particles with said test sample first to form a first incubation solution, and
a2) further incubating said first incubation solution with the other force-driven particles or other motion-resistant particles to form the bound motion-resistant/analyte/force-driven particle conjugates.

10. The method of claim 9, further comprising, after step a1) and before step a2), a step of vortexing said first incubation solution.

11. The method of claim 9 or 10, wherein the incubation time for step a1) is in the range of 10 minutes to 18 hours; and wherein the incubation time for step a2) is in the range of 10 minutes to 2 hours.

12. The method of claim 11, wherein the incubation time for step a1) is longer than the incubation time for step a2).

13. The method of any of claims 1 to 11, wherein said force is applied vertically or horizontally or both vertically and horizontally across said viscous medium.

14. The method of any of claims 1 to 11 and 13, wherein said force is applied for a duration of 10 seconds to 60 minutes.

15. The method of any of claims 1 to 11, 13 and 14, further comprising a step of collecting said bound motion-resistant/analyte/force-driven particle conjugates from said viscous medium after step b).

16. A sensing kit comprising:

a plurality of motion-resistant particles;

a plurality of force-driven particles; and

a viscous medium having a viscosity in the range of 0.01 Pa.s to 10 Pa.s,

wherein said motion-resistant particles and the force-driven particles are coated with a first sensing element and a second sensing element respectively, that can specifically bind to a target analyte in a test sample.

17. A system for detecting the presence or absence of an analyte in a test sample comprising:

a) a plurality of motion-resistant particles and a plurality of force-driven particles, wherein said motion-resistant particles are coated with a first sensing element and said force-driven particles are coated with a second sensing element, wherein both first and second sensing elements are capable of specific binding with the analyte, when present, to form bound motion-resistant/analyte/force-driven particle conjugates; and

b) a separation means comprising a viscous medium and a force that can separate said bound motion-resistant/analyte/force-driven particle conjugates, when present, from unbound motion-resistant particles and unbound force-driven particles,

wherein said viscous medium has a viscosity in the range of 0.01 Pa.s to 10 Pa.s.

18. Use of the sensing kit of claim 16 or the system of claim 17 for virus and biomarker detection.