

TABLE 1

Examples	Catalytic element	Substrate	Signal	Detection
1	Alkaline phosphatase (AP)	Lumigen APS-5 and others	Light (450 nm).	Photo sensor
2	Horse-radish peroxidase (HRP)	Lumigen PS-atto, Lumigen TMA-6, Lumigen PS-3, etc H ₂ O ₂ , oxidizable compound Amplex Red (10-acetyl-3,7-dihydroxyphenoxazine) + H ₂ O ₂ 3,5,3',5'-tetramethylbenzidine (or its analogues) + H ₂ O ₂	Light Electron Fluorescence from resorufin Absorption (450 nm)	Photo sensor Electrical sensor excitation at 530-571 nm, emission at 590-600 nm UV-Vis
3	Glucose oxidase Glucose oxidase (GO) + Horseradish peroxidase (HRP)	Glucose, O ₂ a) Glucose, O ₂ for GO b) Amplex Red for HRP	Electron Fluorescence from resorufin	Electrical sensor excitation at 530-571 nm, emission at 590-600 nm
4	Luciferase (firefly)	ATP + MG2+ + O ₂ + luciferin (or its analogues: aminoluciferin, quinolinyl luciferin)	light (560 nm)	Photo sensor
	Luciferase (Renilla)	Coelenterazine + O ₂	light (475 nm)	Photo sensor
	Luciferase (Bacterial)	Aldehyde + FMNH ₂ + O ₂	light (490 nm)	Photo sensor

[0070] In other embodiments, the signal particle is itself detectable by the detection element in the absence of a catalytic element and reaction substrate. Typically, in such situation, the signal particle will comprise a SERS-active nanoparticle or a fluorescent nanoparticle, which can, for example, comprise a nanoparticle coupled to a surface-enhanced fluorescent tag. The SERS-active nanoparticle is detectable by Raman in the detection zone. The fluorescent nanoparticle can be, for example, a Qdot or other fluorescent nanoparticles, such as SEF nanoparticles or FluoDots, which are detectable by examining fluorescence in the detection zone.

[0071] Alternatively, detection of the analyte can occur by fluorescence quenching. In one embodiment, the signal particle comprises a nanoparticle coupled to an affinity agent and an ODN sequence. The detection zone contains a FRET pair of double stranded ODNs that contain donor or acceptor on one strand each, and where one of the single strands is complementary to the ODN sequence on the nanoparticle. Interaction between the ODN and the FRET pair results in a decrease in fluorescence, thus indicating the presence of the analyte.

[0072] In a further embodiment, the analyte is detected by Fluorescence Resonance Energy Transfer (FRET). FRET is an energy transfer mechanism between two fluorescent molecules. A fluorescent donor is excited at its specific fluorescence excitation wavelength. By a long-range dipole-dipole coupling mechanism, this excited state is then nonradiatively transferred to a second molecule, the acceptor, where it is then released as a photon. In one embodiment, a sandwich binding complex is formed between a magnetic particle, an analyte, and a nanoparticle coated with one partner of a FRET pair in one zone, the sandwich binding complex is moved to a second zone where another partner of the FRET pair is present, and FRET is detected.

[0073] In a further embodiment, fluorescence is detected through the use of complimentary segments of the fluorophore. For example, a sandwich binding complex is formed between a magnetic particle, an analyte, and a nanoparticle coated with half of a fluorescent protein (such as GFP or an analog) in one zone, the sandwich binding complex is moved

to a second zone where the other half of the fluorescent protein is present, the fluorescent protein self-assembles, and fluorescence is detected.

[0074] Fluorogenic detection can also be employed. In one embodiment, a binding complex is formed between a magnetic particle, an analyte, and an antibody-enzyme fusion protein in one zone, and it is moved to a second zone containing a fluorogenic reaction substrate, where the enzyme reacts with the reaction substrate to produce a detectable reaction product.

[0075] Time-resolved fluorescence can similarly be used in the invention. In one embodiment, a sandwich binding complex is formed between a magnetic particle, an analyte, and a nanoparticle encoded with Eu³⁺ or Tb³⁺ or another lanthanide in one zone; the binding complex is moved to another zone and detected by time-resolved fluorescence.

[0076] Other types of fluorescence can also be used, such as fluorescence polarization, and fluorescence life time studies.

[0077] Additionally, binding complex formation can be detected by chemiluminescence. As described above, a sandwich binding complex can be formed between a magnetic particle, an analyte, and a nanoparticle coated with a catalytic element in one zone, with chemiluminescent detection in another fluidic zone. Alternatively, a binding complex can be formed between a magnetic particle, an analyte, and an antibody-catalytic element fusion protein in one zone, with chemiluminescent detection in another zone. In a further embodiment, a binding complex can be formed between a magnetic particle, an analyte, and a silver nanoparticle or nanorod coated with affinity agents (such as antibodies) for recognition of the analyte and chelating agents for its metal ions (such as Eu³⁺) in one zone, moving the binding complex to another zone where metal ions such as Eu³⁺ are present, and detecting the binding complex by chemiluminescence.

[0078] Binding complex formation is also detectable via UV-visible spectroscopy. For example, a binding complex can be formed between a magnetic particle, an analyte and an antibody-catalytic element (such as horseradish peroxidase)