

substrates and/or inhibitors, carbohydrates (including glycoproteins and glycolipids)/lectins, carbohydrates and other binding partners, proteins/proteins; and protein/small molecules. These may be wild-type or derivative sequences.

**[0099]** In a preferred embodiment, the analyte is nucleic acid. The binding ligand for nucleic acids include sequence-specific binding ligands, as well as generic binding ligands. Sequence-specific binding ligands include, but is not limited to, a substantially complementary nucleic acid, or a sequence-specific nucleic-acid binding protein. As outlined below, this complementarity need not be perfect; there may be any number of base pair mismatches which will interfere with hybridization between the target sequence and the single stranded nucleic acids of the present invention. However, if the number of mutations is so great that no hybridization can occur under even the least stringent of hybridization conditions, the sequence is not a complementary target sequence. Thus, by "substantially complementary" herein is meant that the probes are sufficiently complementary to the target sequences to hybridize under normal reaction conditions. Generic binding ligands include, for example, single-stranded DNA binding proteins (SSB proteins), which can be expected to bind to all single-stranded DNA in a sample; poly-dT oligonucleotides, which can bind to substantially all the mRNA in the sample.

**[0100]** In a preferred embodiment, the analyte is protein. In this embodiment, the binding ligands include proteins, peptides, or small molecules. These binding ligands can be specific to a particular protein.

**[0101]** Alternatively, they may be recognizable by a particular class of proteins or even all proteins. For example, a specific binding ligand for a protein analyte can be specific antibodies or fragments thereof. When analyte is an enzyme, binding ligands can also be substrates, inhibitors, and other proteins that bind the enzyme, i.e. components of a multi-enzyme (or protein) complex. When target analyte is nucleic acid binding protein, the binding ligand can be a single-stranded or double-stranded nucleic acid.

**[0102]** In a preferred embodiment, the analyte is a cell. Binding ligands for a particular cell type generally comprise an antibody that recognize an epitope that serves to identify a particular cell type and distinguish it from other cell types. Suitable epitopes in this embodiment include, but are not limited to, components of the cell membrane, such as membrane-bound proteins or glycoproteins, including cell surface antigens of either host or viral origin, histocompatibility antigens or membrane receptors.

**[0103]** In a preferred embodiment, the binding ligands may be directly conjugated to the magnetic particles. Alternatively, the binding ligands and magnetic particles may be joined by means of a coupling agent. As used herein, coupling agents include various bifunctional cross-linking or coupling agents, i.e., molecules containing two reactive groups or ends, which may be separated by a spacer. The coupling agent contains both a binding ligand for the target analyte and a binding group for the molecule conjugated on the magnetic particle, thus brings the two together.

**[0104]** The method of attachment of the capture binding ligands to the attachment linker (either an insulator or conductive oligomer) will generally be done as is known in the art, and will depend on both the composition of the

attachment linker and the capture binding ligand. In general, the capture binding ligands are attached to the attachment linker through the use of functional groups on each that can then be used for attachment. Preferred functional groups for attachment are amino groups, carboxy groups, oxo groups and thiol groups. These functional groups can then be attached, either directly or indirectly through the use of a linker, sometimes depicted herein as "Z". Linkers are well known in the art; for example, homo- or hetero-bifunctional linkers as are well known (see 1994 Pierce Chemical Company catalog, technical section on cross-linkers, pages 155-200, incorporated herein by reference). Preferred Z linkers include, but are not limited to, alkyl groups (including substituted alkyl groups and alkyl groups containing heteroatom moieties), with short alkyl groups, esters, amide, amine, epoxy groups and ethylene glycol and derivatives being preferred, with propyl, acetylene, and C<sub>2</sub> alkene being especially preferred. Z may also be a sulfone group, forming sulfonamide linkages.

**[0105]** In a preferred embodiment, the coupling agent is a linker molecule. The linker can be an organic moiety such as a hydrocarbon chain (CH<sub>2</sub>)<sub>n</sub>, or can comprise an ether, ester, carboxamide, or thioether moiety, or a combination thereof. The linker can also be an inorganic moiety such as siloxane (O—Si—O). The length of the linker is selected so that the magnetic particle does not interfere with the molecular interaction between the target analyte and its binding ligand.

**[0106]** In a preferred embodiment, the coupling agent comprises at least two parts, one part comprising a binding ligand for the analyte to be labeled, another part comprising an epitope that can be recognized by a binding ligand conjugated on the magnetic particle. This embodiment is particularly advantageous because a single kind of conjugated magnetic particle can be used for the labeling of a variety of target analytes. For example, Miltenyi Biotech streptavidin magnetic colloid labels can be used. These labels, together with a coupling agent comprising a biotinylated antibody can be used to label a cell or a protein that can be recognized by the biotinylated antibody. Similarly, the Miltenyi labels and a coupling agent comprising a biotinylated nucleic acid can be used to label a nucleic acid that is complementary to the biotinylated nucleic acid.

**[0107]** Labeling reactions comprising more than one reaction step can be done in a variety of sequences. For example, the conjugated magnetic particles can first bind to the coupling agent, and the coupling agent/magnetic particle complex then reacts with the analyte in the sample. Alternatively, the coupling agent can first react with the analyte in the sample, and conjugated magnetic particles are subsequently introduced to the reaction. It is also possible that the analyte, the conjugated magnetic particle, and the coupling agent are allowed to bind to each other in a single reaction.

**[0108]** It should be noted that the labeled analytes may have various ratios of volume or numbers with regard to the labels. Thus, for large analytes such as cells, a multiplicity of labels may be attached to the cellular surface. On the other hand, if the analyte to be labeled is a single molecule, a multiplicity of such molecules may reside on a single label. Attaching a large nonmagnetic material, such as a cell to a magnetic particle alters the magnetic characteristics of the label to some extent due to the increased volume of the