

containing samples between two flat substrate surfaces with microarrays formed on both surfaces according to the invention.

[0036] B. Material

[0037] Various materials, organic or inorganic or a combination of both, can be used as support for this invention. Suitable substrate materials include, but are not limited to, glasses, ceramics, plastics, metals, alloys, carbon, papers, agarose, silica, quartz, cellulose, polyacrylamide, polyamide, and gelatin, as well as other polymer supports, other solid-material supports, or flexible membrane supports. Polymers that may be used as substrate include, but are not limited to: polystyrene; poly(tetra)fluoroethylene (PTFE); polyvinylidenedifluoride; polycarbonate; polymethylmethacrylate; polyvinylethylene; polyethyleneimine; polyoxymethylene (POM); polyvinylphenol; polylactides; polymethacrylimide (PMI); polyalkenesulfone (PAS); polypropylene; polyethylene; polyhydroxyethylmethacrylate (HEMA); polydimethylsiloxane; polyacrylamide; polyimide; and various block co-polymers. The substrate can also comprise a combination of materials, whether water-permeable or not, in multi-layer configurations. A preferred embodiment of the substrate is a plain 2.5 cm×7.5 cm glass slide with surface Si—OH functionalities.

[0038] C. Surface Preparation/Reactive Groups

[0039] In order to allow attachment by a linker or directly by a binding element, the surface of the substrate may need to undergo initial preparation in order to create suitable reactive groups. Such reactive groups could include simple chemical moieties such as amino, hydroxyl, carboxyl, carboxylate, aldehyde, ester, ether (e.g. thio-ether), amide, amine, nitrile, vinyl, sulfide, sulfonyl, phosphoryl, or similarly chemically reactive groups. Alternatively, reactive groups may comprise more complex moieties that include, but are not limited to, maleimide, N-hydroxysuccinimide, sulfo-N-hydroxysuccinimide, nitrilotriacetic acid, activated hydroxyl, haloacetyl (e.g., bromoacetyl, iodoacetyl), activated carboxyl, hydrazide, epoxy, aziridine, sulfonylchloride, trifluoromethyl diaziridine, pyridyldisulfide, N-acylimidazole, imidazolecarbamate, vinylsulfone, succinimidylcarbonate, arylazide, anhydride, diazoacetate, benzophenone, isothiocyanate, isocyanate, imidoester, fluorobenzene, biotin and avidin. Techniques of placing such reactive groups on a substrate by mechanical, physical, electrical or chemical means are well known in the art, such as described by U.S. Pat. No. 4,681,870, incorporated herein by reference.

[0040] To achieve high-density arrays, it may be necessary to “pack” the support surface with reactive groups to a higher density. One preferred method in the case of a glass surface is to first “strip” the surface with reagents such as a strong acid, and then to apply or reapply reactive groups to the surface.

[0041] In the case of a glass surface, the reactive groups can be silanes, Si—OH, silicon oxide, silicon nitride, primary amines or aldehyde groups. Slides treated with an aldehyde-containing silane reagent are preferred in immobilizing many binding elements and are commercially available from TeleChem International (Cupertino, Calif.) under the trade name “SuperAldehyde Substrates.” The aldehyde groups on the surface of these slides react readily with

primary amines on proteins to form a Schiff base linkage. Since typical proteins display many lysine residues on their surfaces, as well as the generally more reactive α -amines at their N-termini, they can attach to the slide in a variety of orientations, permitting different sides of the protein to interact with other proteins or small molecules in solution. After arraying binding elements such as proteins onto these aldehyde slides, a buffer containing bovine serum albumin (BSA) may be applied to the slide to block later non-specific binding between analytes and unreacted aldehyde groups on the slide.

[0042] II. Linkers

[0043] Once the initial preparation of reactive groups on the substrate is completed (if necessary), linker molecules optionally may be added to the surface of the substrate to make it suitable for further attachment chemistry.

[0044] As used herein, the term “linker” means a chemical moiety which covalently joins the reactive groups already on the substrate and the binding element to be eventually immobilized, having a backbone of chemical bonds forming a continuous connection between the reactive groups on the substrate and the binding elements, and having a plurality of freely rotating bonds along that backbone. Linkers may be selected from any suitable class of compounds and may comprise polymers or copolymers of organic acids, aldehydes, alcohols, thiols, amines and the like. For example, polymers or copolymers of hydroxy-, amino-, or di-carboxylic acids, such as glycolic acid, lactic acid, sebacic acid, or sarcosine may be employed. Alternatively, polymers or copolymers of saturated or unsaturated hydrocarbons such as ethylene glycol, propylene glycol, saccharides, and the like may be employed. Preferably, the linker should be of an appropriate length that allows the binding element, which is to be attached, to interact freely with molecules in a sample solution and to form effective binding.

[0045] The linker in the present invention comprises at least two reactive groups with the first to bind the substrate and the second to bind the binding element. The two reactive groups may be of the same chemical moiety. The at least two reactive groups of linkers may include any of the chemical moieties described above of reactive groups on the substrate. And one preferred second group comprises a maleimide group. Another preferred embodiment for a linker's second group is a vinyl sulfone group. It is believed that the hydrophilicity of these groups helps limit nonspecific binding by analytes such as proteins when further assay is conducted in an aqueous buffer.

[0046] Methods for binding the linker to the surface of the substrate will vary depending on the reactive groups already on the substrate and the linker selected, and will vary as considered appropriate by one skilled in the art. For example, siloxane bonds may be formed via reactions between the trichlorosilyl or trisalkoxy groups of a linker and the hydroxyl groups on the support surface.

[0047] The linkers may be either branched or unbranched, but this and other structural attributes of the linker should not interfere stereochemically with relevant functions of the binding elements, such as a ligand-antiligand interaction.

[0048] Protection groups, known to those skilled in the art, may be used to prevent linker's end groups from undesired or premature reactions. For instance, U.S. Pat. No. 5,412,