

MICROARRAYS OF FUNCTIONAL BIOMOLECULES AND USES THEREFOR

RELATED APPLICATION

[0001] This application is based on and claims priority of U.S. Provisional Patent Application No. 60/222,763, filed on Aug. 3, 2000, the disclosure of which is hereby incorporated by reference.

FIELD OF THE INVENTION

[0002] The present invention relates to the field of diagnostic and analytical chemistry, and particularly to devices for screening complex chemical or biological samples to identify, isolate or quantify components within a sample based upon their ability to bind to specific binding elements. The invention is particularly related to the production and use of arrays, preferably microarrays, of binding elements which are of biological significance or which bind to ligands of biological significance.

BACKGROUND OF THE INVENTION

[0003] To construct high-density arrays of functional biomolecules for efficient screening of complex chemical or biological samples or large numbers of compounds, the binding elements need to be immobilized onto a solid support. A variety of methods are known in the art for attaching biological molecules to solid supports. See generally, *Affinity Techniques, Enzyme Purification: Part B, Meth. Enz.* 34 (ed. W. B. Jakoby and M. Wilchek, Acad. Press, N.Y. 1974) and *Immobilized Biochemicals and Affinity Chromatography, Adv. Exp. Med. Biol.* 42 (ed. R. Dunlap, Plenum Press, N.Y. 1974). Arenkov et al., for example, have described a way to immobilize proteins while preserving their function by using microfabricated polyacrylamide gel pads to capture proteins, and then accelerating diffusion through the matrix by microelectrophoresis (Arenkov et al. (2000), *Anal Biochem* 278(2):123-31). The patent literature also describes a number of different methods for attaching biological molecules to solid supports. For example, U.S. Pat. No. 4,282,287 describes a method for modifying a polymer surface through the successive application of multiple layers of biotin, avidin, and extenders. U.S. Pat. No. 4,562,157 describes a technique for attaching biochemical ligands to surfaces by attachment to a photochemically reactive arylazide. Irradiation of the azide creates a reactive nitrene that reacts irreversibly with macromolecules in solution, resulting in the formation of a covalent bond. The high reactivity of the nitrene intermediate, however, results in both low coupling efficiencies and many potentially unwanted products due to nonspecific reactions. U.S. Pat. No. 4,681,870 describes a method for introducing free amino or carboxyl groups onto a silica matrix, in which the groups may subsequently be covalently linked to a protein in the presence of a carbodiimide. In addition, U.S. Pat. No. 4,762,881 describes a method for attaching a polypeptide chain to a solid substrate by incorporating a light-sensitive unnatural amino acid group into the polypeptide chain and exposing the product to low-energy ultraviolet light.

[0004] There remains, however, a need for more efficient and easy-to-make array systems that identifies, isolates and/or quantifies components within complex samples, as well as to screen large numbers of compounds based upon their ability to bind to a variety of different binding partners.

SUMMARY OF THE INVENTION

[0005] The present invention provides microarray assay systems where binding elements of interest are immobilized on a substrate and are able to interact with and bind to sample analytes. The microarrays are useful for screening large libraries of natural or synthetic compounds to identify natural binding partners for the binding elements, as well as to identify non-natural binding partners which may be of diagnostic or therapeutic interest. The invention is particularly useful in providing microarrays of antibodies or antibody fragments such as scFv, which have previously not been successfully incorporated into high-density arrays while maintaining their specific binding activity. The invention also provides methods for using such microarrays, methods for selecting epitopes for the antibodies or antibody fragments useful in such arrays, and methods for analyzing the data obtained from assays conducted on the microarrays.

[0006] Preferably, the immobilized binding elements are arranged in an array on a solid support, such as a silicon-based chip or glass slide. The surface of the support is chosen to possess, or are chemically derivatized to possess, at least one reactive chemical group that can be used for further attachment chemistry. There may be optional flexible molecular linkers interposed between the support and the binding elements. Examples of such linkers include bovine serum albumin (BSA) molecules, maleimide and vinyl sulfone groups.

[0007] In certain embodiments of the invention, a binding element is immobilized on a support in ways that separate the binding element's region responsible for binding to its cognate ligand and the region where it is linked to the support. In a preferred embodiment, the two regions are two separate termini, and the binding element is engineered to form covalent bond, through one of the termini, to a linker molecule on the support. Such covalent bond may be formed through a Schiff-base linkage, a linkage generated by a Michael addition, or a thioether linkage. In a particularly preferred embodiment, an antibody fragment is engineered to comprise a reduced cysteine at its carboxyl terminus.

[0008] In preferred embodiments, the microarrays comprise an array of immobilized yet functional binding elements at a density of at least 1000 spots per cm². In some embodiments, to prevent dehydration, the invention provides for adding a humectant such as glycerol to the layer of immobilized binding elements. In other embodiments, the invention provides for the addition of a blocking agent solution such as BSA to the substrate surface.

[0009] In another aspect, the present invention provides methods of labeling an antigen such that the labeling will not interfere with the antigen's binding with an antibody or antibody fragment. In a preferred embodiment, the antigen is labeled at its terminal amines after protease digestion. In a particularly preferred embodiment, the antigen is digested with trypsin before being labeled with a succinimidyl ester dye.

[0010] In a further aspect, the present invention provides a method for detecting a phosphorylated protein by fragmenting a candidate protein into a plurality of peptides wherein one of the peptides comprises a known or suspected phosphorylation site, and using an antibody or antibody fragment to select the peptide through an epitope close to the phosphorylation site.