

[0011] In yet another aspect, the present invention provides a method for identifying a small molecule that regulates protein-protein interaction. According to this aspect, a capture protein is attached to a support surface and exposed to its ligand and at least one small molecule. The presence or the absence of binding between the capture protein and the ligand is then detected to determine the regulatory effect of the small molecule. In a preferred embodiment, a microarray of capture proteins that act in the same cellular pathway are attached to the support surface to profile the regulatory effect of a small molecule on all these proteins in a parallel fashion.

[0012] In yet a further aspect, the present invention provides a method for studying a cellular event by attaching a capture molecule on a support surface to capture a cellular organelle contained in a solution such as a whole-cell lysate.

[0013] These and other aspects of the invention will be apparent to one of ordinary skill in the art from the following detailed disclosure, and description of the preferred embodiments.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIG. 1A illustrates exemplary steps of treating a support surface to attach a BSA molecule to it and activating the BSA molecule.

[0015] FIG. 1B illustrates exemplary steps of attaching a capture protein to the activated BSA molecule.

[0016] FIG. 2 illustrates proximal phospho-affinity mapping.

[0017] FIG. 3A and 3B illustrate an embodiment where small molecule regulating protein-protein interaction is studied.

[0018] FIG. 4A is a mass spectrometry profile of the steady state surface proteins from a trypsin digest of SKOV3 cells.

[0019] FIG. 4B is a mass spectrometry diagram showing peptide being affinity captured by scFv H7 on Ni-NTA SELDI surface.

[0020] FIG. 4C is a mass spectrometry diagram showing the result of a control experiment.

[0021] FIG. 4D illustrates the capture of transferrin receptor ectodomain tryptic peptide that is labeled with CY-5.

[0022] FIG. 5 are mass spectrometry diagrams showing binding by a fusion protein as a capture molecule versus the negative control.

[0023] FIG. 6 are mass spectrometry diagrams showing a small molecule competes a ligand off an binding elements on a SELDI surface.

[0024] FIG. 7A and 7B show fluorescence units detected from ligand bound to immobilized binding elements in the presence or absence of a small molecule.

[0025] FIG. 8 shows fluorescence scans of microarrays that have captured labeled EGFR, TIR or ErbB2 at various dilutions.

[0026] FIG. 9 is a fluorescence scan showing labeled cell surface proteins from cell lysate being captured by antibody microarrays.

[0027] FIG. 10 are fluorescence scans of microarrays where the capture of unlabeled antigen is detected through a second labeled antibody.

[0028] FIG. 11 are fluorescence scans detecting the binding of antigens from cell lysates. The detection is through a second labeled antibody.

DETAILED DESCRIPTION OF THE INVENTION

[0029] The present invention depends, in part, upon the discovery of new methods of producing arrays, particularly microarrays, of naturally occurring or artificially produced biological macromolecules which may be used to screen samples, including both biological and artificial samples, to identify, isolate or quantify molecules in such samples that associate with the immobilized binding elements. Towards this end, the present invention provides methods and products to enable the high-throughput screening of very large numbers of compounds to identify those compounds capable of interacting with biological macromolecules.

[0030] The present invention has particularly significant applications in immunoassays, which pave the way for extensive and efficient screening using antibodies and similar molecules. Antibodies have long played an essential role in determining protein function, in identifying the spatiotemporal pattern of gene expression, in identifying protein-protein interactions, and for in vitro and in vivo target validation by phenotypic knockout. However, whereas individual antibodies are useful for monitoring individual proteins from biological samples, the present invention provides for the generation of large arrays of antibodies, antibody fragments, or antibody-like binding elements formatted for high throughput analysis. This technology, which enables comprehensive profiling of large numbers of proteins from normal and diseased-state serum, cells, and tissues, provides a powerful diagnostic and drug discovery tool.

[0031] One aspect of the present invention concerns improvements in methods of attaching a biomolecule to a solid support through a chemical linker, while retaining the biological functions of that molecule, particularly in the case of a capture protein or an antibody fragment.

[0032] I. Substrate/Support

[0033] The microarrays of the present invention are formed upon a substrate or support. Although the characteristics of these substrates may vary widely depending upon the intended use, the basic considerations regarding the shape, material and surface modification of the substrates are described below.

[0034] A. Shape

[0035] The substrates of the invention may be formed in essentially any shape. Although it is preferred that the substrate has at least one surface which is substantially planar or flat, it may also include indentations, protuberances, steps, ridges, terraces and the like. The substrate can be in the form of a sheet, a disc, a tubing, a cone, a sphere, a concave surface, a convex surface, a strand, a string, or a combination of any of these and other geometric forms. One can also combine several substrate surfaces to make use of the invention. One example would be to sandwich analyte-