

[0081] C. Labeling

[0082] Binding elements may be tagged with fluorescent, radioactive, chromatic and other physical or chemical labels or epitopes. For certain preferred embodiments where quantified labeling is possible, this yields great advantage for later assays.

[0083] In a preferred embodiment, a fluorescent dye containing a hydrophilic polymer moiety such as polyethyleneglycol is used.

[0084] V. Samples for Assays

[0085] Upon formation of microarrays of binding elements on the solid support, large quantities of samples may be applied to the support surface for binding assays. Examples of such samples are as follows:

[0086] A. Body Fluids/Tissue and Biopsy Samples

[0087] Samples to be assayed using the microarrays of the present invention may be drawn from various physiological, environmental or artificial sources. In particular, physiological samples such as body fluids of a patient or an organism may be used as assay samples. Such fluids include, but are not limited to, saliva, mucous, sweat, whole blood, serum, urine, genital fluids, fecal material, marrow, plasma, spinal fluid, pericardial fluids, gastric fluids, abdominal fluids, peritoneal fluids, pleural fluids and extraction from other body parts, and secretion from other glands. Alternatively, biological samples drawn from cells grown in culture may be employed. Such samples include supernatants, whole cell lysates, or cell fractions obtained by lysis and fractionation of cellular material.

[0088] B. Cell Extracts

[0089] Extracts of cells and fractions thereof, including those directly from a biological entity and those grown in an artificial environment, can also be used to screen for molecules in the lysates that bind to a particular binding element.

[0090] C. Normal v. Diseased Samples

[0091] Any of the above-described samples may be derived from cell populations from a normal or diseased biological entity.

[0092] D. Treated v. Untreated Samples

[0093] Any of the above-described samples may be derived from cell populations which have or have not been treated with compounds or other treatments which are believed or suspected of being either deleterious or beneficial, and differences between the treated and untreated populations may be used to assess the effects of the treatment.

[0094] E. Labeling

[0095] Specific molecules in a given sample may be modified to enable later detection by using techniques known to one of ordinary skill in the art, such as using fluorescent, radioactive, chromatic and other physical or chemical labels. In a preferred embodiment, a fluorescent dye containing a hydrophilic polymer moiety such as polyethyleneglycol (e.g. fluorescein-PEG2000-NHS) is used. Labeling can be accomplished through direct labeling of analytes in the sample, or through labeling of an affinity tag

that recognizes an analyte (indirect labeling). Direct labeling of sample analytes with different fluorescent dyes makes it possible to conduct multiple assays from the same spot (e.g., measuring target protein's expression level and phosphorylation level). When the analyte is a phage-displayed ligand, the phage may be pre-labeled for detecting binding between the ligand and the microarray of binding elements.

[0096] Under the direct-labeling approach, sample over-labeling has long been recognized as a serious problem. Over-labeling of proteins can cause aggregation of protein conjugate, which tends to result in non-specific staining; it can also reduce antibody's specificity for its antigen by disrupting antibody's epitope-recognition function, causing loss of signal. It is well known in the art that, to mitigate over-labeling, one need to either shorten reaction time for the labeling process or increase substrate:label ratio. A solution to over-labeling is to first digest a whole protein into peptides and then label the termini of the peptides, which avoids labeling any internal epitopes. Accordingly, the labeling process may proceed to completion without one having to worry about over-labeling and thus giving a researcher more complete control over the labeling process. Moreover, if the potential labeling sites on a peptide is known, it is possible to quantify labeled peptide once the peptide is captured through affinity reagents that recognize an internal epitope. An application of this method would be to quantify labeled peptides digested from whole proteins in cell extracts for quantitative analysis of protein expression levels.

[0097] In a preferred embodiment, whole proteins are digested with trypsin before subjected to labeling by a succinimidyl ester dye such as Cy3, Cy5 or an Alexa dye. A succinimidyl ester dye labels primary amines, such as the one in lysine. Trypsin cleaves after lysines and generates peptides with lysines at their C-terminus. Therefore, peptides resulting from trypsin digestion fall into two categories: those without lysine and having a primary amine at the N-terminus, and those with a lysine at the C-terminus and hence primary amines at both termini. None of the peptide would have any internal lysine. As a result, a succinimidyl ester dye will only label tryptic peptides at their termini without labeling any internal epitope.

[0098] In an alternative embodiment, one may use a protease other than trypsin to digest a whole protein and still use a succinimidyl ester dye for labeling as long as the peptide to be captured does not contain an internal lysine. That way, labeling will still only occur at a terminus of the selected peptide. Such a peptide may be used as a preferential panning peptide. To take advantage of a preferential panning peptide, an immunoglobulin is first raised against the peptide. Second, a sample, e.g., from a whole cell lysate, is digested with a protease or a combination of proteases that will generate that specific panning peptide, resulting in a library of peptides. These peptides are then labeled to completion with a succinimidyl ester dye. A large excess of reactive labeling reagent may be used to ensure complete labeling of the non-lysine containing peptide. Then, the labeled peptides are applied to the immunoglobulin for capture.

[0099] Because the amount of labeling on a preferential panning peptide is known, one can quantify the amount of such peptide in a given sample through the amount of label