

mutations in DNA sequences are used to study the function of the proteins that are encoded by these genes. Chemical genetics instead involves the use of small molecules that alter the function of proteins to which they bind, thus either inactivating or activating protein function. This, or course, is the basis of action of most currently approved small molecule drugs. The present invention involved the development of "chip-like" technology to enable the rapid detection of interactions between small molecules and specific proteins of interest. The methods and composition of the present invention can be used to identify small molecule ligands for use in chemical genetic research. One of ordinary skill in the art will realize that the inventive compositions and methods can be utilized for other purposes that require a high density protein format.

[0119] B. Signal Transduction

[0120] Another preferred embodiment of the binding assays performed in this invention is to study modulation of protein-protein interaction by small molecules. These assays measure either the facilitation or competition for cognate binding by different molecules in order to help understand aspects of binding dynamics under varying conditions. In an exemplary embodiment, a capture protein is attached on a support surface in microarray, cognate ligands are added to bind to the capture protein. The binding between the capture protein and its cognate ligand is monitored and compared in the presence or absence of a small molecule that may be a drug candidate. In a preferred embodiment, various capture proteins's interaction with various ligands affected by various small molecules are investigated in a multi-plex fashion on a microarray chip.

[0121] Protein interactions often occur through domains that are sometimes called binding motifs. It is in these regions that small molecules that are effective at regulating protein interactions are most likely to work. However, proteins within a family tend to share homologous sequences that contribute to forming binding motifs and proteins that contain these motifs often have similar functions. A problem in screening for drugs that regulate such protein functions is obtaining specificity in these screens as the targets among the binding motif family of proteins are similar in structure, and have similar binding features. The protein microarray technology disclosed here permits efficient and easily repeatable steps for determine specificity of small molecules for regulating large numbers of motif-containing protein family members, and will greatly facilitate the process of drug screening.

[0122] In an exemplary embodiment, regulation of the Bcl-2 family, known to affect cell apoptosis, is studied. These proteins share homology to combinations of four Bcl-2 homology regions (BH1-4). The Bcl-2 family proteins function to either protect cells against apoptosis or to promote apoptosis by regulating membrane behavior and ion channel function at the mitochondria and the endoplasmic reticulum. The anti-apoptotic family members, Bcl-2, Bcl-XL, and Mcl-1 contain all four domains. The largest group of pro-apoptotic members, Bad, Bik, Bid, Bag-1, HRK, and Noxa contain only BH-3 domains, while pro-apoptotic proteins Bax and (Multidomain pro-apoptotic proteins) contain BH-1, BH-2, and BH-3 domains.

[0123] Methods of the invention can be used to screen for small molecules that regulate the function of an entire family

of apoptosis-regulating proteins. Such a small molecule may mimic the function of a BH-3 protein and serve as a drug candidate. Referring to FIG. 3A and 3B, recombinant fusion proteins from the Bcl-2 family of apoptosis regulating proteins may be prepared by standard methods and printed in microarrays as binding element 30 on either BSA-NHS glass slides or an aldehyde derivatised glass slide 10 as described earlier through a linker 20. Ligands 80 for these proteins such as a full length Bcl-XL protein may be added in the absence or presence of a small molecule 90 such as a BH-3 containing peptide from the Bcl-2 family protein BAK or a small molecule that mimics a BH-3 containing peptide. The ligand 80 may be labeled with a fluorescent dye (e.g. CY5). Concentration of the printed proteins, the ligands, or the small molecule may be varied, by itself or in combinations with others. The slides may then be read using an optical reader such as the Arrayworx scanner and/or confirmed through mass spectrometry using commercially available mass spectrometry chips. The increase or decrease in the signal obtained from bound ligand can be used to chart the regulatory roles of the small molecule, whether it is up-regulatory or down-regulatory. Using the method of the invention, multiple capture molecules, multiple ligands and multiple small molecules can be screened side by side on a single array support (e.g. a 96 well plate), greatly increasing efficiency in drug screening. A more detailed example can be found in the Example Section E (iii).

[0124] Another example of the invention's application in studying signal transduction is to screen for small molecules that inhibit protein-protein binding in the apoptotic pathway through the BH-4 region of multidomain-containing BCL-2 family members.

[0125] C. Protein Expression

[0126] To date, there are no published reports on microarray-based detection of proteins in labeled cell extracts. Labeling and detection of cell surface proteins would allow parallel profiling of multiple cell surface antigens. State of the art in cell surface molecule profiling is by flow cytometry or fluorescence microscopy, currently allowing 2-5 different antigens to be profiled in a single sample. Antibody arrays in theory allow the detection of an unlimited number of antigens. Furthermore, antibody arrays have the potential for detecting intracellular proteins and protein modifications such as phosphorylation in parallel with expression.

[0127] In an exemplary embodiment, monoclonal antibodies to cell surface proteins such as c-ErbB2, EGFR, and transferrin receptor are arrayed on a BSA-NHS slide by a GMS 417 arrayer. Live cells from a cancerous cell line such as the epidermoid carcinoma cell line A-431 or breast cancer cell line SK-BR-3 may be used as sample cells. Cell surface proteins are preferably labeled with a dye that contains a hydrophilic polymer moiety such as a polyethyleneglycol, which has shown good specificity, low background, and does not label proteins inside cells. An example of such a dye is fluorescein-PEG2000-NHS dye available from Shearwater. Following labeling and wash, cells are lysed (e.g., in SDS). Total labeled proteins are then incubated on the antibody microarray for binding to occur before the slides are scanned by an optical reader. As a result, it was confirmed that the A-431 cell line over-expresses EGFR but not ErbB2. Likewise, it was confirmed that the SK-BR-3 cell line over-expresses ErbB2, but not EGFR.