

beads carrying signaling entities can be isolated from all other beads and the identity of their immobilized drug candidates determined.

[0064] The techniques of the invention allow for a reduction in labor in screening large numbers of possible binding interactions. For example, the screening of ten million drug candidates against a single potential binding protein can be carried out using ten thousand surface locations, or much fewer, for example only one thousand locations, or even one hundred surface locations or less. Of course, a drug library can alternatively be simultaneously screened for interaction with a panel of proteins. In this case, hetero-particle complexes may be subsequently analyzed to determine the identity of not only the drug, but also the protein to which it binds.

[0065] This advantage (efficient screening) of the invention defines one aspect of the invention which involves providing a plurality of binding partner candidates, such as drug candidates, each immobilized relative to separate articles, and studying binding of the binding partner candidates with a target immobilized with respect to a signaling entity and, in a process involving one tenth the number of surface locations relative to the number of binding partner candidates, determining, specifically, binding between at least one binding partner candidate and the target.

[0066] In another aspect of the invention, magnetic beads can be modified to present proteinaceous molecules, then tested according to methods of the invention for their ability to interact with (a) target species, which may also be proteinaceous, attached to a population of gold colloids. A complex sample mixture such as a cell lysate or a natural products sample can be fractionated, then fractions containing a single or a few distinct molecular species can be attached to magnetic beads. Attachment to magnetic beads can be facilitated by non-specific adsorption, or chemical coupling to functionalized beads. These beads may or may not be gold coated to facilitate the formation of SAMs on their surfaces. Using methods of the invention, a small number of beads are identified that present species that interact with colloid-immobilized target molecules. Interacting species can then be desorbed or cleaved from the particles and identified using standard biochemical analysis techniques, such as mass spectroscopy (MS) or matrix laser desorption ionization (MALDI) MS, or peptide sequencing techniques. Proteins that have been attached to magnetic beads via interaction with an affinity tag can be released for analysis by competitive inhibition of the interaction with the affinity tag. For example, magnetic beads bearing NTA-Ni moieties selectively capture histidine tagged species. The His-tagged species can be released from the bead by competitively inhibiting the interaction with added imidazole.

[0067] Alternatively, small numbers of distinct molecular species, from complex mixtures, can be attached to magnetic beads without fractionation; a low number of molecules can be presented on each bead by using beads that present a small number of functional groups or by using a large number of beads with a low concentration of the complex mixture.

[0068] Additionally, molecular biology techniques can be used to present a single or a small number of distinct species on beads. In this way, the in situ magnetic selection-dilution techniques, described herein, are particularly applicable to

the study of proteomics. A set of magnetic beads can be generated to present the gene products of a cDNA library, then tested for their ability to interact with target species immobilized on a population(s) of colloids that may also bear auxiliary signaling elements. cDNA libraries are comprised of the fragments of genome that specifically code for proteins. cDNA libraries can be readily generated using standard techniques and are widely available from cell lines of interest. To facilitate the attachment of the proteins to beads or colloids, a cDNA library can be inserted, en mass or separately, into an affinity tag expression vector. For example, affinity tag vectors are used to produce histidine-tagged or glutathione-S-transferase (GST)-tagged proteins. Cells can then be transfected with the resultant plasmid DNAs. As those skilled in the art are aware, bacterial cells generally take up a single plasmid; in this way a pool of cDNA plasmids can be simultaneously transfected into cells, grown on agarose, and each colony will statistically contain a single species of transfected plasmid DNA. Colonies are picked and mini cultured, according to standard methods.

[0069] Cell cultures are then grown and induced to express the encoded proteins, according to standard techniques. To expedite the process, cell cultures (each representing a single clone) can be pooled together, grown and induced, en mass, to express the encoded proteins. The pooled proteins are then attached to a single bead. This means that when assayed with colloids presenting a target species, a bead that generated a positive signal simultaneously presents the interacting species as well as irrelevant species. Therefore, if pooled transfectants are used, the procedure can be repeated to determine which component of the bead-attached mixture actually interacted with the colloid-immobilized species, as follows. The bead-colloid mixtures can be subjected to standard analysis techniques, such as peptide micro sequencing, to determine which culture pool had been attached to that particular bead. The identification of a single protein from the mixture attached to the bead identifies the pool from which the transfectant was derived. Aliquots of the individual transfectants are reserved such that each transfectant can be separately grown and induced to express protein, which then is separately attached to a set of beads. The assay then is repeated to identify single interacting components.

[0070] Protein purification can be simplified by using magnetic beads that display binding partners of the affinity tag that was incorporated into the proteins. Alternatively, expressed proteins can be attached to magnetic beads via affinity tag interaction from a crude mixture or after standard purification.

[0071] A description of the analysis of interacting species, such as colloid-bead-immobilized species follows.

[0072] Magnetic beads that present species that interact with colloid-immobilized species, become coated with a colloid layer, due to the completion of the interaction between the species. These beads generate an electronic signal. After the in situ magnetic selection-dilution process, magnetic beads together with their attached colloids can be subjected to analysis techniques, such as MS, MALDI MS, peptide sequencing (s.a. Edman micro sequencing) and the like, which may also include enzymatic cleavage steps, to determine the identity of the putative interacting species. Immobilized proteins can be removed from the beads and