

colloids for analysis by a variety of methods, including but not limited to, competitive inhibition of the affinity tag-bead interaction, enzymatic digestion, acid elution and laser desorption. For example, proteins expressed with histidine-tags to facilitate attachment to surfaces presenting nitrilo triacetic acid/nickel (NTA-Ni) groups, can be released via the introduction of imidazole. Alternatively, proteins can be removed from supports using trypsin cleavage. Molecules can also be removed from particles by laser ionization desorption then directly input into an automated analysis system such as MS.

[0073] One method of the invention provides for massively parallel analyses of large numbers of putative interacting species. Such a system is especially useful for deciphering the protein interaction network of the gene products from the entire human genome.

[0074] In this case, cDNA libraries encoding the entire genome, a subset or a library of disease-associated genes are provided in distinct aliquots in separate locations. Each cDNA fragment is separately inserted into a protein expression vector. To facilitate attachment of the expressed protein to beads or colloidal particles, affinity tag vectors can be used. Cells are separately transfected with the plasmid DNA and protein expression is induced. At this point, proteins bearing an affinity tag are separately mixed with beads or colloids that bear a binding partner for the affinity tag. In this way, each particle will present a single protein species. For example, a first set of proteins, bearing histidine tags, is attached to a first set of magnetic beads bearing nitrilo triacetic acid (NTA) nickel moieties; NTA-Ni(II) binds stretches of histidines. A second set of proteins, bearing glutathione-S-transferase (GST) fusion moieties, is attached to gold colloids which present glutathione which is the binding partner of GST. Different particle types, i.e. beads versus colloids, need not bear different affinity tags. Each particle, however, bears a label that uniquely identifies the protein displayed on its surface. In a preferred embodiment, this label is a DNA sequence and gold colloids also display an electroactive signaling moiety.

[0075] According to methods of the invention described above, magnetic beads bearing a first protein set are mixed with gold colloids bearing a second protein set and an electroactive signaling moiety. The solution is placed over an electrode array. Beneath each pad of the electrode array is a separately controllable electromagnet. Proteins displayed on colloids are allowed to interact with proteins displayed on magnetic beads. Recall that if a protein attached to an electronic signaling colloid interacts with a protein on a magnetic bead, then the complex will transduce an electronic signal when recruited to the electrode. Magnetic beads alone are incapable of providing this signal. After some incubation period, all the electromagnetics are turned on in unison. Electromagnets, under pads that register a positive signal, are held "ON", while those that failed to generate a signal are released to release beads that did not interact with a colloid-immobilized partner. A port is opened and released beads are washed away. Fluid is introduced to the interaction chamber to dilute the remaining beads and electromagnets under pads that registered a positive are released. The process is repeated until one is assured that statistically, only beads decorated with signaling colloids remain.

[0076] Each magnetic bead and each colloid can bear a DNA strand that encodes the identity of the attached protein. At this point, pieces of single stranded, composite DNA are added to the interaction chamber. Each DNA strand is comprised of two parts: one end is complementary to a DNA sequence on a magnetic bead and the other end is complementary to a sequence on a colloid. All possible combinations of sequences on beads and colloids are represented. The DNA is allowed to freely interact with the bead-colloid complexes. Unbound DNA is washed out of the interaction chamber. DNA strands that simultaneously bind to a complementary sequence on a magnetic bead and a complementary sequence on a colloid represent "solutions" to the problem of which proteins interact with each other. Rather than perform peptide micro sequencing to reveal the identity of the interacting pairs, one merely elutes the bound DNA solutions and submits them to DNA sequencing. The sequence of each DNA strand will be the complement of the DNA code that identifies each protein.

[0077] Enzymes that digest single stranded DNA can be added to the solution after hybridization and rinsing to remove unbound portions of DNA. In this way, strands of DNA that bound to only one particle and not the other are not read as solutions. Enzymes having 3' and/or 5' exonuclease activity, such as *E. coli* DNA Pol I, which digest single stranded DNA from the ends, are preferred since they will not digest DNA nicks.

[0078] The length of DNA required to encrypt the protein set is a function of the number of distinct species in the set. Also, the length of the DNA "tag" is directly proportional to the number of mismatched bases that will be allowed without abolishing hybridization. For this reason, short DNA strands are preferred. Conditions such as temperature and salt concentration, along with the addition of chemicals like formamide, can also be optimized to ensure that only perfectly matched DNA will hybridize.

[0079] Alternatively, the magnetic bead-colloid complexes can be separately released from the interaction chamber, prior to the introduction of DNA "solution" strands, by sequential release of the electromagnets beneath each electrode pad. Each interacting complex can then be diverted to a separate location, where all possible DNA solutions are introduced. To relieve steric interference with DNA hybridization, the interacting protein partners can be dissociated from each particle and removed from the isolated location, prior to the introduction of DNA solution strands. DNA strands are allowed to simultaneously hybridize to beads and colloids in the absence of protein-protein interactions. Non-interacting DNA species are rinsed away, by for example pelleting the particles and removing the supernatant. Buffer suitable for the dissociation of DNA hybrids are added, particles pelleted and the DNA contained in the supernatant, which encodes the identity of the interacting proteins, are then sequenced by standard sequencing methods or by hybridization to DNA array chips.

[0080] To prevent proteins on magnetic beads from binding to each other before the introduction of proteins on colloids, magnetic beads can be added (en masse or separately) and magnetically drawn to the electrode array immediately. After an incubation period, electromagnets are released and incubated free in solution before the first round of selection and dilution.