

[0093] Colloids that do not present an electroactive signaling element or that display additional signaling elements can also be used with aspects of this invention. For example, colloids can be derivatized to present electroactive as well as fluorescent moieties (including, by definition herein, phosphorescent moieties). Each set of colloids can present a different color of fluorescence. FACS (fluorescence activated cell sorting) analysis can be used to sort hetero-particle complexes after magnetic selection, but prior to identifying molecular species attached to each bead. Colloid decorated magnetic beads can also be separated by electromagnetic methods. Additionally, colloids that do not bear an auxiliary signaling element can be used with aspects of the invention. These colloid-decorated beads can be identified visually, because the agglomeration of gold colloids onto particles colors them red. Additionally, nanoparticles that inherently fluoresce, for example as a function of their size, can also be used both to signal and to identify the surface-attached biomolecule.

[0094] One attribute of an aspect of the invention over the state of the art is that it enables the MULTIPLEXED identification and isolation of interacting species. This capability is especially useful in the field of proteomics because of the vast number of possible interaction between the gene products, which are the proteins. With the number of genes in the human genome now estimated to be about 40,000, determining interaction networks by sequential pair-wise testing will involve a minimum of 8×10^8 experiments. The isolation of interacting species attached to particle-like supports simplifies the analysis of interacting species, including the identification of interacting motifs. Additionally, the attachment of putative binding partners to solid supports enables the recovery and reuse of particles that presented species that did not interact.

EXAMPLE 1

Massively Parallel Analysis of Protein-Protein Interactions: Elucidating the Interaction Map of the Human Proteome

[0095] The following prophetic example describes how to perform massively parallel analysis of protein-protein interactions, which is particularly useful when proteins are as yet uncharacterized. Here, this method is used to elucidate the protein interaction map of the human proteome. A subset of, or the entire set of, proteins of the proteome is expressed with affinity tags to facilitate attachment of the expressed protein to sets of particles. Particle-immobilized proteins are pooled together and allowed to interact. Interacting pairs are selected from the pooled mixture by a reiterative magnetic selection/dilution process. Following the selection/dilution process, the identity of interacting partners is determined. The selection step reduces the complexity of the problem by eliminating the need to analyze non-interacting proteins.

[0096] Proteins and their encoding DNA molecules are indirectly connected to each other by co-immobilizing both on a common particle or bead, wherein each particle (or bead) presents a single protein species and its encoding DNA. Connecting the expressed protein to its encoding DNA expedites the identification of each set of interacting proteins after the selection/dilution process. Each protein and its encoding DNA are immobilized on two different kinds of particles: a recruitable particle and a signaling

particle. The sizes of the particles are also different such that smaller signaling particles can form satellites around each larger recruitable particle. In this example, each protein and its encoding DNA are immobilized on a single 4-10 micron magnetic bead as well as on a multitude of fluid suspendable nanoparticles, that are 4-40 nm in diameter and bear electroactive signaling entities. When a first species on a magnetic bead biologically interacts with a second species on a signaling nanoparticle, the recruitable particle becomes "connected" to the signaling particle. These hetero-particle-complexes are then magnetically recruited to a sensing electrode (see FIG. 6), where they can deliver a signal, such as an electronic or electrochemical signal. To facilitate multiplexed analysis, the particles are magnetically attracted to an electrode array that has a number of individually addressable electrode pads. Beneath each electrode pad is an individually controllable electromagnet, such that the magnetic field above each pad can be selectively turned off and on. However, magnetic particles that are not connected to signaling particles, which cannot deliver a signal, may also be recruited to the same electrode pad that delivers a positive signal. These non-signaling magnetic beads are electromagnetically released from the pad and washed out of the interaction reservoir. Signaling nanoparticles that do not interact with species on magnetic particles remain in a homogeneous suspension and do not deliver a signal but are also washed out of the interaction reservoir. Both of these purging functions are accomplished by maintaining the magnetic fields beneath electrode pads that deliver a positive signal, to retain interacting complexes, while the magnetic fields beneath pads that do not deliver a positive signal are driven to zero to release non-interacting magnetic beads. A port 150 (FIG. 6) is opened and fluid is washed out of the interaction reservoir carrying away non-interacting magnetic beads and non-interacting nanoparticles. The port is closed, all magnetic fields are driven to zero and more buffer is added through a second port 160 along with mechanical agitation to resuspend and redistribute the particles. All the magnetic fields beneath all the electrode pads are turned on again and the selection/dilution process is repeated until, statistically, each pad that delivers a positive signal contains a single magnetic bead bound to a multitude of signaling nanoparticles.

[0097] To determine the identity of interacting proteins, an array of magnetic pins, whose dimensions correspond to that of the electrode array is juxtaposed over the electrode array and the magnetic fields beneath the entire electrode array are driven to zero such that each magnetic pin captures a single hetero-particle complex. The loaded pin array is dipped into a multi-well plate, of compatible dimensions, each well of which is filled with solution containing DNA amplification reagents. The respective encoding DNA sequences (immobilized on the nanoparticles and the bead) are amplified by PCR or similar technique and sequenced to reveal the identity of each set of interacting proteins. Individual aspects of the experiment are detailed below.

Preparation of Proteins

[0098] A DNA sequence that encodes each protein member of the proteome is inserted into a bacterial protein expression vector. The expression vector carries an affinity tag, (His)₆ in this case, tandem repeats of Ga14 consensus sequences, and 2 sequences that flank the protein identification sequence, to which PCR primers can bind. The