

histidine-tag facilitates the attachment of the expressed protein to the particle. The Ga14 consensus sequences act to tether the encoding DNA to the particle via the interaction between the recognition motif and a particle-immobilized yeast DNA binding domain, which in this case is a GST-Ga14 fusion protein. The DNA binding domain of Ga14 (aa' 1-100) binds to the consensus sequence CGGatAgAagc-CgCCGAG and the GST binds to a glutathione moiety on the particle. Proteins are separately expressed in a cell-free translation system to reduce the abundance of irrelevant proteins and cell debris. Following protein expression, each expression mixture contains the encoding DNA and the expressed protein. Each protein expression mixture is divided into 2 aliquots. A single magnetic bead (4-10 microns in diameter) is added to a first aliquot and a quantity of NTA-glutathione-SAM-coated nanoparticles is added to a second aliquot. Particles are pelleted and washed to remove protein that is not particle-bound. Particles and beads are pooled together in subsets of 1000 species per pool and subjected to magnetic selection/dilution and electrochemical analysis. In this manner, unidentified proteins can be bound to the same bead or particle that also binds the corresponding encoding DNA.

#### Preparation of Nanoparticles

[0099] Gold nanoparticles (diameter=4-25 nm) are derivatized with heterologous self-assembled monolayers (SAMs) that present:

- [0100] a) NTA (nitrilo tri-acetic acid)-Ni<sup>++</sup> that captures histidine-tagged proteins;
- [0101] b) glutathione that binds to glutathione-S-transferase (GST) fusion proteins;
- [0102] c) octamethyl ferrocene, which is a redox active moiety that can deliver an electronic or electrochemical signal;
- [0103] d) carboxylates to prevent particle aggregation; and
- [0104] e) tri-ethylene glycol to resist non-specific adsorption. Virtually any biological species can be immobilized on a colloid.

#### Electrochemical Analysis

[0105] A model 630 electrochemical analyzer from CH Instruments (Austin, Tex.) is used to detect interactions between species immobilized on magnetic beads and species immobilized on colloids that also bear redox active metals. The instrument is modified to facilitate multiplexed detection. In this case, the redox active metals are ferrocene derivatives. Pads of the electrode array are individually addressable and act as the working electrode. In this case, the pads are gold-coated and derivatized with conductive self-assembled monolayers. A Ag vs. Ag/Cl reference electrode is used with a Pt auxiliary. Electrodes are scanned using Alternating Current Voltammetry (ACV) with a 25 mV overpotential at a frequency of 10 Hz..

#### Design of Electrode Array Sandwiched between Individually Addressable Electromagnets

[0106] Electrode arrays 100 (FIG. 6) having 300-500 electrode pads 110 are constructed by plating gold over

Ni<sup>++</sup>. Electrode pads are 50-500 microns on edge and are sandwiched between sets of individually addressable Helmholtz electromagnets 120 such that a magnetic field gradient can be generated to recruit, then hold magnetic beads at the pad surface (see FIG. 6). The direction of the current is reversed to drive the magnetic field to zero when it is desirable to release magnetic beads from the surface to wash away or redistribute. To ensure that the interaction reservoir is thermally isolated from the electromagnet arrays so that heat does not denature proteins in solution, a layer of insulative material 130 is placed between the electromagnets and the interaction reservoir 140.

#### Calculation of Protein Sets and Electrode Pad Number

[0107] To generate the protein interaction map of the entire proteome, one needs to divide the proteome into subsets, which are then tested for interaction with every other subset. Assuming that there are about 50,000 proteins of interest, the proteome is divided into 50 sets of 1000 proteins each. Each group of 1000 proteins is then tested for interaction with every other group of 1000, resulting in 50x50 matrix or 2500 separate experiments. The number of proteins in each subset determines the number of electrode pads in each array. If we assume that each protein has a single binding partner, then each protein has a 1/50 chance of finding that partner when tested for interaction with one of the 50 subsets of proteins. However, each protein probably has on average 5 relevant binding partners, increasing the probability of finding a binding partner within a subset to 1/10. That means that for 2000 proteins in one pooled interaction mix, 200 will deliver a positive signal, which implies that the electrode array should have 300-500 pads. Low-level signals from non-specific binding events are minimal because of competitive inhibition by relevant binders. However, the occurrence of false positives is minimized when a signal threshold is set, wherein signals below the threshold are counted as negatives. Relative affinities are determined by comparison of the degree of interaction of a first binding species and a second binding species with a third target protein to which the first and second bind.

#### EXAMPLE 2

##### Determining the Binding Partners of a Single Target Protein with a Large Pool of Candidate Binding Partners

[0108] This prophetic example describes how to identify proteins, from a large pool of putative binding partners, which interact with a single target protein. Proteins from the large pool are prepared as described above in Example 1, and immobilized only on signaling nanoparticles. The target protein is immobilized on a set of magnetic beads. Because the identity of the target protein is known, it is not necessary to co-immobilize its encoding DNA. Interacting partners are selected by electrochemical analysis as described above.

#### EXAMPLE 3

##### Selection of Interacting Protein Partners by FACS Analysis

[0109] This example describes how to identify the binding partners of a single target protein. The target protein is