

be provided, immobilized with respect to magnetic beads. Preferably, each magnetic bead carries only one type of immobilized drug candidate, and there may be only one bead per drug candidate provided, or many beads per drug candidate. One advantage of the invention, as will become more fully understood from the description below, is that only one bead need be provided for each drug candidate. That is, a plurality of beads can be provided in an assay, for each bead carries a different immobilized drug candidate.

[0053] In the figures, signaling entity 30 is shown as a colloid particle, and use of a colloid particle as a signaling entity, or as a structure for assistance in the immobilization of a different signaling entity (described below) with respect to target protein 28, is preferred. In particular, colloid particles exposing gold surfaces, such as gold colloid particles, are particularly convenient. Although colloid particles will be discussed in connection with protein 28 hereinafter, it is understood that the protein, or other binding partner, can be immobilized with respect to a signaling entity without use of a colloid particle.

[0054] Immobilization of drug candidates 24 and 26 to magnetic beads 20 and 22 can be carried out by any technique known in the art. Such techniques are routine. Drug candidates should be presented at the surfaces of the magnetic beads in a concentration sufficient to facilitate adequate binding and signaling, which can be easily tailored by those of ordinary skill in the art with the knowledge of requirements of assays of the invention.

[0055] Binding partners 28 can be immobilized relative to colloid particles 30 via thiol linkage. That is, binding partners 28 can incorporate a thiol, or can be chemically attached or otherwise immobilized to a thiol, which will bind to a gold surface of colloid particles 30. In one preferred embodiment proteins 28 are attached to self-assembled monolayers (SAMs) formed on surfaces of gold colloid particles 30 via a metal binding tag/metal/chelate linkage. In such a case a metal binding tag can be attached to binding partner 28, and the colloid can carry a SAM presenting a chelate coordinating a metal to which the binding tag binds. Other affinity tags can be used to attach binding partners 28 to SAM species on the colloid particles. In another embodiment SAMs on colloid particles present carboxylate groups, and binding partners 28 incorporate or are immobilized relative to primary amines which can be linked to the SAMs via EDC/NHS chemistry. Affinity tag/binding partner linkage, or EDC/NHS chemistry can be used to link essentially any species to essentially surface of the invention. Preferably such SAMs include sufficient exposure of protein 28 to facilitate binding to drug candidate 24, the remainder of the self-assembled monolayer comprising non-specific binding-inhibiting species such as polyethylene-glycol-terminated SAM-forming species. The selective attachment of various species to self-assembled monolayer-forming species, to form self-assembled monolayers at surfaces exposing desired chemical or biochemical functionality, are known from references noted above and additional documents including U.S. Pat. No. 5,512,131 and International Patent Publication WO 96/29629, published Jun. 26, 1996, each of which is incorporated herein by reference. Chemistry for attachment of proteins 28 to colloid particles 38 also is described in co-pending, commonly owned International Patent Application Serial No. PCT/US00/01504 of Bamdad

and Bamdad, filed Jan. 21, 2000, published as WO 00/34783 on Jul. 27, 2000 and incorporated herein by reference.

[0056] In another technique, SAMs can be formed on colloid particles 30 that incorporate carboxy-terminated thiols. EDC/NHS coupling chemistry then can be used to attach any molecule that presents a primary amine to the SAM. Most proteins 28 will include primary amines, and attachment of primary amines to molecules for subsequent attachment to SAMs on colloid particle 30 is thereby facilitated. Typical EDC/NHS linkage can be carried out as follows.

[0057] A dimethyl formamide (DMF) solution containing 20-40 micromolar NTA (nitrilotriacetic acid) C11 thiol, 540-580 micromolar COOH—C11 thiol and +/-40 micromolar ferrocenyl-C11 thiol is prepared. Colloid particles are incubated in the solution. Following incubation, the DMF solution is removed, and the colloid particles are introduced into a second DMF solution containing 400 micromolar polyethylene glycol C11 thiol and heat cycled as described above. 30 microliters of the colloids are pelleted and resuspended in 100 microliters of phosphate buffer. To the buffer solution is added 0.5 micromoles N-ethyl-N' (3-dimethyl aminopropyl) carbodimide (EDC), and 0.1 micromoles of N-hydroxy succinimide plus a primary-amine-containing binding partner 28 to be linked to the colloids. The colloid solution plus binding partner is incubated for 10 minutes, during which time the binding partner attaches to the SAMs on the colloids. Removal of the solution, followed by washing and re-suspension followed. To avoid linkage of binding partner to more than one colloid particle, the volume of the solution in which the reaction occurs can be doubled or tripled appropriately. The binding partner can be demonstrated to be immobilized with respect to the colloids by exposure to agarose beads carrying a target immobilized thereto, and observing agglomeration.

[0058] In FIG. 2, the components illustrated in FIG. 1 are shown suspended in a fluid medium following binding of protein 28 to drug candidate 24. The components are suspended in the vicinity of a first location 40 and a second location 42, to which magnetic particles 20 and 22 can be magnetically drawn. Locations 40 and 42 can be predetermined areas of a surface of a single article, or surfaces of different articles. Whether locations 40 and 42 are of the same or of a different article is not important in connection with the invention, so long as the locations are separate and distinguishable to the extent that immobilization of a signaling entity at one location is distinguishable from immobilization at another location (as will become apparent from the description below). In a preferred embodiment, first and second locations 40 and 42 are surfaces of first and second electrodes 44 and 46, respectively, wherein an electromagnet is associated with each of the electrodes. Electromagnets can be positioned to draw beads 20 and 22 to the vicinity of the first and second locations 40 and 42. As illustrated, electromagnets 48 and 50 are shown in association with each of electrodes 44 and 46, respectively, and are positioned behind the electrodes with respect to components of the assay. Electrical circuitry addressing the electrodes and electromagnets is conventional, and is not shown.

[0059] Magnetic beads 20 and 22 are magnetically drawn to first and second locations 40 and 42 by activation of electromagnets 48 and 50. Typically, beads 20 and 22 are