

SAM formation on colloids. In this case, detergent can be and preferably is removed after SAM formation and is no longer present on the colloid, in the SAM, or elsewhere during binding interactions or other use of the colloids.

[0044] A target molecule can be attached to an electronic signaling colloid and then incubated with magnetic beads that each present a separate drug candidate species. Following incubation, the magnetic beads can be magnetically attracted to a sensing electrode and analyzed by, for example, ACV. An interaction between a drug on a magnetic bead and a target molecule on an electronic signaling colloid renders the resultant complex both recruitable and detectable. Complexes are magnetically attracted to an electrode and electronically analyzed. Various techniques for producing SAMs on colloids and assays involving beads and colloidal particles are described in International Patent Application PCT/US00/01997, filed Jan. 25, 2000, entitled "Rapid and Sensitive Detection of Aberrant Protein Aggregation in Neurodegenerative Diseases," by Bamdad, et al., published Jul. 27, 2000 as WO 00/43791 and in International Patent Application PCT/US00/01504, filed Jan. 21, 2000, entitled "Assays Involving Colloids and Non-Colloidal Structures," by Bamdad, et al., published Jul. 27, 2000 as WO 00/43783.

[0045] Small molecule drug libraries contain millions of discrete compounds, which poses a logistical challenge. Current practice is to pool drug candidates, test, analyze the individual components of pools that registered a positive, and repeat until a single interacting species has been identified. This practice is very time consuming. Another approach is to modify each drug candidate with a unique tag that codes for its identity and a fluorescent label. Target molecules, immobilized on polystyrene beads, are incubated with pooled drug candidates, then rinsed. The polystyrene beads that captured a drug candidate fluoresce and can be microscopically separated from the others. The captured drugs are then decoded to identify the small molecule that interacted. The problem with this approach is that the many labels that have been added to the drug candidates participate in the interactions, leading to many false positives.

[0046] An alternate approach is to synthesize drugs on, or attach to, magnetic beads. The encoding label is then attached to the bead not the drug candidate. Pools of drug-presenting magnetic beads are then mixed with colloids that present both the target molecule and an electronic label (such as a ferrocene-thiol incorporated into a SAM on the colloid). The solution is retained over an array of microelectrodes. A magnetic field can be separately applied to each electrode pad of the array. First, magnetic fields are applied to each electrode in the array to attract the magnetic beads. The array is then electronically analyzed (ACV preferred). Pads that register a positive, indicate that, at that address, a drug candidate on a magnetic bead has captured a target molecule on a signaling colloid. The magnetic field at spatial addresses that registered a positive remain "turned on", while the other magnetic fields are released, and an exit valve is opened to wash away magnetic beads bearing drug candidates that did not interact. The exit valve is closed and more solution is added to dilute the drug candidates in situ. The process is repeated several times to ensure that each positive results from a single magnetic bead, which bears a single drug candidate. These positives are collected and analyzed to identify the interacting drug candidates. Beads

that bear drug candidates can be encoded or drugs can be released from the beads and submitted to analysis techniques such as mass spec, NMR, sequencing and the like.

[0047] Referring now to the figures, one technique for determination of binding interactions will be described. FIG. 1 illustrates, schematically, first and second articles 20 and 22, respectively. Articles 20 and 22 can be any articles that can immobilize a chemical or biological species for binding study, and can be drawn magnetically from one place to another via a magnetic field. Polymeric magnetic beads that are typically used in biochemical analyses are convenient to use for articles 20 and 22, and the articles will be referred to as magnetic beads hereinafter with the understanding that other articles can be used.

[0048] Magnetic bead 20 carries a first chemical or biological agent 24 (D) immobilized relative to the bead, and magnetic bead 22 similarly carries an immobilized second chemical or biological agent 26 (D').

[0049] In FIG. 1 binding partners 28 (P) also are provided, each immobilized relative to a signaling entity 30. As illustrated, signaling entity 30 is a colloid particle. In the embodiment illustrated, binding partner 28 has binding affinity for first chemical or biological agent 24, but not for second biological or chemical agent 26. Accordingly, exposure of all components illustrated in FIG. 1 to each other, for example via mixing in a typically biochemical assay fluid medium, will result in binding of binding partner 28 to first agent 24, and corresponding immobilization of bead 20 relative to signaling entity 30. Signaling entity 30 does not become immobilized relative to bead 22.

[0050] A variety of signaling entities can be immobilized relative to surfaces of a variety of articles, such as colloid particles, if desired. Signaling entities such as fluorescent-conjugated antibodies and other fluorescent fusion proteins, including green fluorescent proteins, can easily be attached to surfaces of gold colloids and other surfaces that also present putative binding partners either through affinity tags, EDC/NHS chemistry or by binding to a His-tagged protein A or G presented on NTA-SAM-coated colloids according to the invention. Signaling entities such as fluorescent moieties also can be co-immobilized on a colloid via a biotin terminated ligand, or may be fastened via a chelate/metal/metal binding tag linkage. A fluorescent moiety may also be fastened by attaching it to an antibody and using a chelate/metal/metal binding tag with His-protein G to bind the antibody. The moieties can then be directly detected. In a preferred embodiment, the signaling entity is electroactive, specifically a redox-active complex, which can be electrochemically detected by, for example, alternating current voltammetry (ACV).

[0051] Although any of a variety of binding interactions can be studied in accordance with the invention, a preferred technique involves high-throughput detection of drug candidate/target molecule interactions. In such an arrangement, first and second agents 24 and 26 (D and D' respectively) are drug candidates and binding partner 28 is a target protein that may bind to one drug candidate but may not bind to other drug candidates. This specific assay will be discussed with respect to the remainder of the figures, but it is to be understood that the invention is not limited to drug candidate/target protein interactions.

[0052] In a typical drug screening assay, a very large number (from thousands to millions) of drug candidates can