

[0063] E. Coupling to Substrates/Linkers

[0064] Methods of coupling the binding element to the reactive end groups on the surface of the substrate or on the linker include reactions that form linkage such as thioether bonds, disulfide bonds, amide bonds, carbamate bonds, urea linkages, ester bonds, carbonate bonds, ether bonds, hydrazine linkages, Schiff-base linkages, and noncovalent linkages mediated by, for example, ionic or hydrophobic interactions. The form of reaction will depend, of course, upon the available reactive groups on both the substrate/linker and binding element.

[0065] As discussed in the Examples section below, a Michael addition may be employed to attach compounds to glass slides, and plain glass slides may be derivatized to give surfaces that are densely functionalized with maleimide groups. Compounds containing thiol groups, such as an scFv modified to include a cysteine at the carboxy-terminus, may then be reacted with the maleimides to form a thioether linkage.

[0066] IV. Formation of Microarrays

[0067] In one aspect, the present invention provides methods for the generation of arrays, including high-density microarrays, of binding elements immobilized on a substrate directly or via a linker. According to the methods of the present invention, extremely high density microarrays, with a density over 100, preferably over 1000, and further preferably over 2000 spots per cm², can be formed by attaching a biomolecule onto a support surface which has been functionalized to create a high density of reactive groups or which has been functionalized by the addition of a high density of linkers bearing reactive groups.

[0068] A. Spotting

[0069] The microarrays of the invention may be produced by a number of means, including "spotting" wherein small amounts of the reactants are dispensed to particular positions on the surface of the substrate. Methods for spotting include, but are not limited to, microfluidics printing, microstamping (see, e.g., U.S. Pat. No. 5,515,131 and U.S. Pat. No. 5,731,152), microcontact printing (see, e.g., PCT Publication WO 96/29629) and inkjet head printing. Generally, the dispensing device includes calibrating means for controlling the amount of sample deposition, and may also include a structure for moving and positioning the sample in relation to the support surface.

[0070] (i) Volume/Spot Size

[0071] The volume of fluid to be dispensed per binding element in an array varies with the intended use of the array, and available equipment. Preferably, a volume formed by one dispensation is less than 100 nL, more preferably less than 10 nL, and most preferably about 1 nL. The size of the resultant spots will vary as well, and in preferred embodiments these spots are less than 20,000 μm in diameter, more preferably less than 2,000 μm in diameter, and most preferably about 150-200 μm in diameter (to yield about 1600 spots per square centimeter).

[0072] (ii) Viscosity Additives

[0073] The size of a spot in an array corresponding to a single binding element spot may be reduced through the addition of media such as glycerol or trehalose that increase

the viscosity of the solution, and thereby inhibit the spreading of the solution. Hydrophobic boundaries on a hydrophilic substrate surface can also serve to limit the size of the spots comprising an array.

[0074] Adding a humectant to the solution of the binding element may also effectively prevent the dehydration of the microarrays, once they are created on the surface of the substrate. Because dehydration can result in chemical or stereochemical changes to binding elements, such as oxidation or, in the case of proteins, denaturation, the addition of a humectant can act to preserve and stabilize the microarray and maintain the functionality of binding elements such as scFv. For example, in some preferred embodiments, scFv are coupled to maleimide-derivatized glass in phosphate-buffered saline (PBS) solutions with 40% glycerol. The glycerol helps maintain continued hydration which, in turn, helps to prevent denaturation.

[0075] (iii) Blocking Agents

[0076] Solutions of blocking agents may be applied to the microarrays to prevent non-specific binding by reactive groups that have not bound to a binding element. Solutions of bovine serum albumin (BSA), casein, or nonfat milk, for example, may be used as blocking agents to reduce background binding in subsequent assays.

[0077] (iv) Robotics

[0078] In preferred embodiments, high-precision, contact-printing robots are used to pick up small volumes of dissolved binding elements from the wells of a microtiter plate and to repetitively deliver approximately 1 nL of the solutions to defined locations on the surfaces of substrates, such as chemically-derivatized glass microscope slides. Examples of such robots include the GMS 417 Arrayer, commercially available from Affymetrix of Santa Clara, Calif., and a split pin arrayer constructed according to instructions downloadable from <http://cmgm.stanford.edu/pbrown>. The chemically-derivatized glass microscope slides are preferably prepared using custom slide-sized reaction vessels that enable the uniform application of solution to one face of the slide as shown and discussed in the Examples section. This results in the formation of microscopic spots of compounds on the slides. It will be appreciated by one of ordinary skill in the art, however, that the current invention is not limited to the delivery of 1 nL volumes of solution, to the use of particular robotic devices, or to the use of chemically derivatized glass slides, and that alternative means of delivery can be used that are capable of delivering picoliter or smaller volumes. Hence, in addition to a high precision array robot, other means for delivering the compounds can be used, including, but not limited to, ink jet printers, piezoelectric printers, and small volume pipetting robots.

[0079] B. In Situ Photochemistry

[0080] In forming arrays or microarrays of molecules on the surface of a substrate, in situ photochemistry maybe used in combination with photoactivatable reactive groups, which may be present on the surface of the substrate, on linkers, or on binding elements. Such photoactivatable groups are well known in the art.