

087, incorporated herein by reference, describes the use of photo-removable protection groups on a linker's thiol group.

[0049] In a preferred embodiment, the linker comprises a BSA molecule. An example of such an embodiment is a BSA-NHS slide suitable for making microarrays. Although appropriate for some applications, slides functionalized with aldehyde groups, further blocked with BSA, are not suitable when peptides or small proteins are arrayed, presumably because the BSA obscures the molecules of interest. For such applications, BSA-NHS slides are preferred. **FIGS. 1A and 1B** illustrate a method of making such a slide. First, a molecular monolayer of BSA is attached to the surface of a glass slide. Specifically shown in **FIG. 1A**, a glass slide **10** with hydroxyl groups is silanated with aminopropyl triethoxy silane (step **1**) before being activated with N,N'-disuccinimidyl carbonate (step **2**). The activated amino group on the slide in turn forms covalent bonds with linker **20**, which is BSA (step **3**). Then, the surface of the BSA is activated with N,N'-disuccinimidyl carbonate (step **4**), resulting in activated carbamate and ester, such as a N-hydroxy succinimide (NHS) group. Referring to **FIG. 1B**, the activated lysine, aspartate, and glutamate residues on the BSA react readily with the surface amines on the binding element **30**, which is a capture protein here (step **5**) to form covalent urea or amide linkages. Any remaining reactive groups on BSA are subsequently quenched with glycine (step **6**). The result is a binding element **30** (a capture protein here) immobilized to a support **10** through a linker **20** (a BSA molecule here). In contrast to the BSA-blocked slides with aldehyde functionality, proteins or peptides arrayed on BSA-NHS substrates are displayed on top of the BSA monolayer, rendering them accessible to macromolecules in solution.

[0050] III. Binding Elements

[0051] The binding elements of the present invention may be chosen from any of a variety of different types of naturally occurring or synthetic molecules, including those having biological significance ("biomolecules").

[0052] For example, the binding elements may include naturally occurring molecules or molecule fragments such as nucleic acids, nucleic acid analogs (e.g., peptide nucleic acid), polysaccharides, phospholipids, capture proteins including glycoproteins, peptides, enzymes, cellular receptors, and immunoglobulins (e.g., antibodies, antibody fragments,) antigens, naturally occurring ligands, other polymers, and combinations of any of the above. And it is also contemplated that natural product-like compounds, generated by standard chemical synthesis or from split-and-pool library or parallel syntheses, may be utilized as binding elements.

[0053] A. Antibodies and Antibody Fragments

[0054] Antibodies and antibody fragments are preferred candidates for binding elements. These include antigen-binding fragments (Fabs), Fab' fragments, pepsin fragments (F(ab')₂ fragments), scFv, Fv fragments, single-domain antibodies, dsFvs, Fd fragments, and diabodies, as well as full-length polyclonal or monoclonal antibodies. Antibody-like fragments, such as modified fibronectin, CTL-A4, and T cell receptors are contemplated here as well. Once the microarray has been formed, the antigen binding domains of the antibodies or antibody fragments may be utilized to

screen for molecules with the specific antigenic determinants recognized by the antibodies or antibody fragments.

[0055] In a preferred embodiment, to study cellular translocation events and cell surface expression, phage-displayed scFv that trigger cell internalization of a surface receptor can be directly selected from large non-immune phage libraries by recovering and amplifying phage particles from within the cells. See Becerril et al. (1999), *Biochem Biophys Res Commun.* 255(2): 386-93, the entire disclosure of which is incorporated by reference herein.

[0056] B. Receptors

[0057] Naturally occurring biological receptors, or synthetically or recombinantly modified variants of such receptors, also may be used as the binding elements of the invention. Classes of receptors that can be used as binding elements include extracellular matrix receptors, cell-surface receptors and intracellular receptors. Specific examples of receptors include fibronectin receptors, fibrinogen receptors, mannose 6-phosphate receptors, erb-B2 receptors, and EGF (epidermal growth factor) receptors.

[0058] C. Receptor Ligands

[0059] Similarly, naturally occurring biological receptor ligands, or synthetically or recombinantly modified variants of such ligands, also may be used as binding elements to screen for their specific binding partners, or for other, non-natural binding partners. Classes of such ligands include hormones, growth factors, neurotransmitters, antigens and can be phagedisplayed.

[0060] D. Modifications for Coupling to Substrate/Linkers

[0061] As will be apparent to those of skill in the art, the binding elements may be modified in order to facilitate attachment, through covalent or non-covalent bonds, to the reactive groups on the surface of the substrate, or to the second reactive groups of a linker attached to the substrate. As examples of such modifications, nucleophilic S-, N- and O- containing groups may be added to facilitate attachment of the binding element to the solid support via a Michael addition reaction to the linker.

[0062] To preserve the binding affinity of an binding element, it is preferred that the binding element is modified so that it binds to the support substrate at a region separate from the region responsible for interacting with the binding element's cognate ligand. If the binding element binds its ligand at a first terminus, attaching the binding element to the support at a second or opposite terminus, or somewhere in between the termini may be such a solution. In a preferred embodiment, where the binding element is an scFv, the present invention provides a modification method such that the scFv can be attached to the surface of a glass slide through binding with an electrophilic linker, such as a maleimide group, without interfering with the scFv's antigen-binding activity. According to this method which is detailed in Example C (i), an scFv is first engineered so that its carboxy-terminus includes a cysteine residue which can then form a covalent bond with an electrophilic linker such as the maleimide group. Similarly, a binding element's N-terminus can be engineered to include a reactive group for attachment to the support surface.