

The slide is then briefly dipped in ethanol or like solutions and centrifuged to remove excess silanol. The adsorbed silane layer is then cured (e.g., one hour at 115° C.). After cooling, the slide is washed in ethanol or like solutions to remove uncoupled reagent.

**[0141]** A simple, semi-quantitative method can be used to verify the presence of amino groups on the slide surface. An amino-derivatized slide is washed briefly with 5 mL of 50 mM sodium bicarbonate, pH 8.5. The slide can then be dipped in 5 mL of 50 mM sodium bicarbonate, pH 8.5 containing 0.1 mM sulfo-succinimidyl-4-0-(4,4'-dimethoxytrityl)-butyrate (s-SDTB; Pierce, Rockford, Ill.) and shaken vigorously for 30 minutes. (The s-SDTB solution may be prepared by dissolving 3.03 mg of a s-SDTB in 1 mL of DMF and diluting to 50 ML with 50 mM sodium bicarbonate, pH 8.5). After a 30-minute incubation, the slide can then be washed with 20 mL of distilled water and subsequently treated with 5 mL of 30% perchloric acid. The development of an orange-colored solution will indicate that the slide has been successfully derivatized with amines; no color change has been seen for untreated glass slides. Quantitation of the 4,4'-dimethoxytrityl cation ( $E_{498nm}=70,000 \text{ M}^{-1}\text{cm}^{-1}$ ) released by the acid treatment has indicated an approximate density of 2 amino groups per  $\text{nm}^2$ .

**[0142]** B. Addition of Linkers to Substrates

**[0143]** (i) BSA as Linker

**[0144]** BSA-NHS slides, displaying activated amino and carboxyl groups on the surface of an immobilized layer of bovine serum albumin (BSA), were fabricated as follows: 10.24 g N,N'-disuccinimidyl carbonate (100 mM) and 6.96 ml N,N'-diisopropylethylamine (100 mM) were dissolved in 400 ml anhydrous N,N'-dimethylformamide (DMF). Thirty polylysine slides, such as CMT-GAP slides (Coming Incorporated, Coming, N.Y.), displaying amino groups on their surface, were immersed in this solution for 3 hr at room temperature. These slides were rinsed twice with 95% ethanol and then immersed in 400 ml of phosphate buffered saline (PBS), pH 7.5 containing 1% BSA (w/v) for 12 hr at room temperature. Slides were further rinsed twice with  $\text{ddH}_2\text{O}$ , twice with 95% ethanol, and centrifuged at 200 g for 1 min to remove excess solvent. Slides were then immersed in 400 ml DMF containing 100 mM N,N'-disuccinimidyl carbonate and 100 mM N,N'-diisopropylethylamine for 3 hr at room temperature. Slides were rinsed four times with 95% ethanol and centrifuged as above to yield BSA-NHS slides. Slides were stored in a desiccator under vacuum at room temperature for up to two months without noticeable loss of activity.

**[0145]** (ii) A Maleimide Group as Linker

**[0146]** Maleimide-derivatized slides were manufactured as follows: after the surface of a plain glass slide was "packed" (re-silanated, for instance) as described in the Example A(i), the resulting slides were transferred to slide-sized polydimethylsiloxane (PDMS) reaction vessels. One face of each slide was treated with 20 mM N-succinimidyl 3-maleimido propionate in 50 mM sodium bicarbonate buffer, pH 8.5, for three hours. (This solution was prepared by dissolving the N-succinimidyl 3-maleimido propionate in DMF and then diluting 10-fold with buffer). After incubation, the plates were washed several times with distilled water, dried by centrifugation, and stored at room tempera-

ture under vacuum until further use. The resulting slide surface was equipped with a maleimide end.

**[0147]** C. Preparation of Binding Elements

**[0148]** (i) Production and Purification of Cysteine-Tagged scFv

**[0149]** The scFv C6.5 binds to the extracellular region of the human tumor antigen c-erbB-2 with a  $K_d$  of  $1.6 \times 10^{-10}$  M. This antibody was isolated using affinity driven selection as described in Schier et al. (1996), *J. Mol. Biol.* 255(1):28-43.

**[0150]** The gene for the scFv C6.5 was then subcloned into a pUC-119-(Hexa-His)-Cys expression vector, which results in the addition of a hexa-His tag followed by a single cysteine to the COOH-terminus of the scFv. The protein was expressed and purified using immobilized metal affinity chromatography (IMAC). Binding affinity mutants of C6.5 were made by mutagenizing the complementary binding region (CDR), and the affinity constants of the derivative mutants [C6.5ML 3-4 ( $K_d=3.4 \times 10^{-9}$ ) and C6.5G98 ( $K_d=1.6 \times 10^{-9}$ )], were determined using BiaCore (described in Schier et al 1996b). The cysteine tagged scFv C6.5, C6.5ML3-4, and C6.5 G98. were used to demonstrate ligand capture by scFv which have been chemically coupled to glass surfaces. The reduced sulfhydryl of the COOH terminal cysteine of these scFv yields a thiol that can be used to couple the scFv to glass surfaces that have been functionalized with maleimide groups.

**[0151]** (ii) Reducing an scFv for Conjugation to a Maleimide Linker

**[0152]** Purified scFv were reduced with 5 mM cysteamine (SIGMA) for 1 hour at 25° C. and exchanged into phosphate buffered saline (PBS), pH 7.0 using a P10 spin column.

**[0153]** D. Assays Employing Microarrays

**[0154]** (i) Scanning Slides for Fluorescence

**[0155]** Slides were scanned using an Array WoRoX™ slide scanner (AppliedPrecision, Issaquah, Wash.). Slides were scanned at a resolution of 5  $\mu\text{m}$  per pixel. Double filters were employed for both the incident and emitted light. Fluorescein fluorescence was observed using a FITC/FITC excitation/emission filter set, Cy3 fluorescence was observed using a Cy3/Cy3 excitation/emission filter set, and Cy5 fluorescence was observed using a Cy5/Cy5 excitation/emission filter set.

**[0156]** E. Applications of Microarrays

**[0157]** (i) Affinity Capture of Labeled Peptides on scFv Modified Glass Surfaces.

**[0158]** Steady state trypsin cleavage of cell surface proteins was performed on SKBR3 (human breast carcinoma) or SKOV3 cells at 4° C. using TPKC-treated trypsin. Tryptic digests were examined using MALDI mass spectrometry, which is shown in FIG. 4A for SKOV3 cells. About 0.5  $\mu\text{l}$  of the digest was loaded onto a MALDI surface and embedded with matrix consisting of cinnamic acid saturated 50% acetonitril, 0.5% Trifluor, and acetic acid. Digests were treated with protease inhibitors and incubated with 1  $\mu\text{g}$  of purified 6 $\times$  His-scFv against the transferrin receptor ectodomain. The scFv-peptide complex was purified from the digests using Ni-NTA sepharose beads. The beads were