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(54) Title: COMPOSITION AND METHODS FOR ENTRAPPING A PROTEIN ON A SURFACE

(57) Abstract: The present invention provides a formulation to link protein to a solid support that comprises one or more proteins, Oligo-dT and one or more non-volatile, water-soluble protein solvents, solutes or combination thereof in an aqueous solution. Further provided is a method of attaching a protein to a surface of a substrate. The formulations provided herein are contacted onto the substrate surface, printed thereon and air dried. The substrate surface is irradiated with UV light to induce thymidine photochemical crosslinking via the thymidine moieties of the Oligo-dT.

COMPOSITIONS AND METHODS FOR ENTRAPPING PROTEIN ON A SURFACE

Cross-Reference to Related Application

This international application claims benefit of priority under 35 U.S.C. §119(e) of provisional application U.S. Serial No. 61/823,065, filed May 14, 2013, now abandoned, the entirety of which is hereby incorporated by reference.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates generally to the fields of microarrays and protein chemistry. More specifically, the present invention relates to a formulation and methods for entrapping protein on a surface.

Description of the Related Art

It is well known that proteins may be attached to surfaces, typically by covalent attachment of the protein directly to the solid substrate, or by covalent attachment to polymers that had previously been attached to the surface, or by physical entrapment of the protein into pores within the solid surface itself, or by simple adsorption of the protein to the surface of the microarray. Although such means of attachment allow for a higher concentration of protein, there is a loss in functionality due to chemical modification of the surface. Sol-gels have been used to entrap proteins on solid supports. However, acceptable sol-gels are limited to those without undesirable properties of gelling in the pin during printing, irreproducible spot sizes, cracking, poor adhesion, incompatibility with entrapped components, or reducing activity of the entrapped protein. None of these methods of attachment or entrappment enable site-addressable, self-assembly of a 3 dimensional protein structure on a microarray.

Thus, there is a recognized need in the art for improved formulations and methods for physically entrapping protein on a microarray surface without direct attachment, binding or adsorption to the surface. Specifically, the prior art is deficient in aqueous crosslinkable formulations comprising Oligo-dT and protein(s) that can be entrapped and preserved in a native protein state in a high concentration on the microarray. The present invention fulfills this longstanding need and desire in the art.

FIG. 4 depicts SAPE (Cy-3) signals normalized to OligoT (Cy-5) signals from a BSA microarray printed on an epoxy silane microarray surface, as in FIG. 2B.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term, "a" or "an" may mean one or more. As used herein in the claim(s), when used in conjunction with the word "comprising", the words "a" or "an" may mean one or more than one. Some embodiments of the invention may consist of or consist essentially of one or more elements, method steps, and/or methods of the invention. It is contemplated that any method, compound, composition, or device described herein can be implemented with respect to any other device, compound, composition, or method described herein.

As used herein, the term "or" in the claims refers to "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or".

As used herein, the term "about" refers to a numeric value, including, for example, whole numbers, fractions, and percentages, whether or not explicitly indicated. The term "about" generally refers to a range of numerical values, e.g., +/- 5-10% of the recited value, that one of ordinary skill in the art would consider equivalent to the recited value, e.g., having the same function or result. In some instances, the term "about" may include numerical values that are rounded to the nearest significant figure.

In one embodiment of the present invention, there is provided a formulation to link protein to a solid support, comprising: one or more proteins; Oligo-dT; and one or more non-volatile, water-soluble protein solvents, solutes or combination thereof in an aqueous solution. In one aspect, the water soluble protein solvent may comprise glycerol or the water soluble protein solvent and solutes may comprise glycerol and at least one of sucrose, trehalose or sorbitol. In this aspect the sucrose, trehalose or sorbitol may be present at a mass ratio of about 0.5:1 up to about 4:1 relative to glycerol. In another aspect, the water soluble protein solvent may comprise propanediol or the water soluble protein solvent and solids may comprise propanediol and at least one of sucrose, trehalose or sorbitol. In this aspect the mass ratio of the sucrose, trehalose or sorbitol is as described *supra* relative to propanediol. In yet another aspect, the water soluble protein solvents and solids may comprise glycerol and propanediol and at least one of sucrose, trehalose or sorbitol. In yet another aspect, the formulation may be applied to or disposed on a solid support such as an amino-silane layer upon an underlying surface. In this aspect representative underlying surfaces may be, but are not limited to a metal surface, a glass surface or a ceramic surface. In yet another aspect, the formulation may be applied

complement of the solution is allowed to evaporate away, yielding a concentrated, water-depleted phase comprising Oligo-dT, protein, solvents, solutes which is then crosslinked, photochemically, to entrap the protein within the resulting crosslinked, polymeric Oligo-dT network. The non-volatile, water soluble solvents and solutes are chosen so that, subsequent to evaporative water loss, the resulting water-depleted phase remains principally non-crystalline, thereby mitigating protein damage by microcrystal formation. Proteins are linked to an underlying surface, indirectly, rather than by direct chemical linkage to the surface and in a way such that, subsequent to evaporative water depletion and UV crosslinking, the protein becomes entrapped in the crosslinked Oligo-dT network which was created around it. The above combination of indirect photochemical Oligo-dT network entrapment, plus retention of a non-crystalline phase upon water depletion, gives rise to preservation of a native protein state on the solid support, which is then available, subsequent to rehydration, to bind to analytes applied in water solution, as the basis for a binding, or diagnostic or public health screening assay.

More particularly, the Oligo-dT polymer chains are utilized as a linker mediating protein attachment to an underlying microarray surface. Oligo-dT is used a protein surface linker based on its capacity to engage in photocrosslinking. Briefly, Oligo-dT can readily be co-printed in @ 0.5x to 10x mole excess with any number of proteins of interest, e.g., albumin, antibodies, enzymes, HLA or any other water soluble protein. At time of printing, the anionic Oligo-dT adsorbs, non-covalently, to the underlying cationic amino-silane surface via formation of electrostatic bonds. If applied to an epoxysilane coated surface, it can associate with the surface via a combination of covalent linkage to the epoxide and h-bonding to ring opened epoxide diols. The microarray spot is then allowed to air-dry over several minutes. In the present invention, protein printing occurs with one of several water-soluble (but non-volatile) solutes, in a buffered water solution, which upon air drying, becomes a water-depleted fluid, which retains solubilization of the protein and eliminates buffer salt crystal formation, which would have occurred if the water-soluble non-aqueous solutes were not added.

Several solutes and solute mixtures can be used, such as for example, including but not limited to glycerol; Glycerol with propanediol; propanediol; glycerol with sorbitol; glycerol with propane diol and sorbitol; propanediol and sorbitol; glycerol with trehalose; glycerol with propane diol and trehalose; and propanediol and trehalose. Protein can be printed at one of several concentrations (250 ug/ml – 5 ug/ml); 500 ug/ml; 250 ug/ml; 100 ug/ml; 50 ug/ml; 20 ug/ml; and 10 ug/ml.

In all cases, after printing and air-drying, the resulting microarrays (FIG. 1A) can be subjected to standard UV-Crosslinking at @300 mjoule, to photo-crosslink link the Oligo-dT (via T-T bonding) into a loosely-crosslinked matrix and in some cases to covalently link

OligoT	50-mer 25uM Glycerol	50-mer 25uM Glycerol	50-mer 25uM Glycerol	50-mer 25uM Glycerol	50-mer 25uM Glycerol	50-mer 25uM Glycerol
OligoT	50-mer 100uM G-PD	50-mer 100uM G-PD	50-mer 100uM G-PD	50-mer 100uM G-PD	50-mer 100uM G-PD	50-mer 100uM G-PD
OligoT	50-mer 50uM G-PD	50-mer 50uM G-PD	50-mer 50uM G-PD	50-mer 50uM G-PD	50-mer 50uM G-PD	50-mer 50uM G-PD
OligoT	50-mer 25uM G-PD	50-mer 25uM G-PD	50-mer 25uM G-PD	50-mer 25uM G-PD	50-mer 25uM G-PD	50-mer 25uM G-PD
OligoT	50-mer 100uM PD	50-mer 100uM PD	50-mer 100uM PD	50-mer 100uM PD	50-mer 100uM PD	50-mer 100uM PD
OligoT	50-mer 50uM PD	50-mer 50uM PD	50-mer 50uM PD	50-mer 50uM PD	50-mer 50uM PD	50-mer 50uM PD
OligoT	50-mer 25uM PD	50-mer 25uM PD	50-mer 25uM PD	50-mer 25uM PD	50-mer 25uM PD	50-mer 25uM PD
OligoT	50-mer 100uM Trehalose	50-mer 100uM Trehalose	50-mer 100uM Trehalose	50-mer 100uM Trehalose	50-mer 100uM Trehalose	50-mer 100uM Trehalose
OligoT	50-mer 50uM Trehalose	50-mer 50uM Trehalose	50-mer 50uM Trehalose	50-mer 50uM Trehalose	50-mer 50uM Trehalose	50-mer 50uM Trehalose
OligoT	50-mer 25uM Trehalose	50-mer 25uM Trehalose	50-mer 25uM Trehalose	50-mer 25uM Trehalose	50-mer 25uM Trehalose	50-mer 25uM Trehalose
Right 6 x13 segment of 12 x 12 Microarray						
Protein conc	20 ug/ml	20 ug/ml	10 ug/ml	10 ug/ml	5 ug/ml	5 ug/ml
OligoT	50-mer 100uM Glycerol	50-mer 100uM Glycerol	50-mer 100uM Glycerol	50-mer 100uM Glycerol	50-mer 100uM Glycerol	50-mer 100uM Glycerol
OligoT	50-mer 50uM Glycerol	50-mer 50uM Glycerol	50-mer 50uM Glycerol	50-mer 50uM Glycerol	50-mer 50uM Glycerol	50-mer 50uM Glycerol
OligoT	50-mer 25uM Glycerol	50-mer 25uM Glycerol	50-mer 25uM Glycerol	50-mer 25uM Glycerol	50-mer 25uM Glycerol	50-mer 25uM Glycerol
OligoT	50-mer 100uM G-PD	50-mer 100uM G-PD	50-mer 100uM G-PD	50-mer 100uM G-PD	50-mer 100uM G-PD	50-mer 100uM G-PD
OligoT	50-mer 50uM G-PD	50-mer 50uM G-PD	50-mer 50uM G-PD	50-mer 50uM G-PD	50-mer 50uM G-PD	50-mer 50uM G-PD
OligoT	50-mer 25uM G-PD	50-mer 25uM G-PD	50-mer 25uM G-PD	50-mer 25uM G-PD	50-mer 25uM G-PD	50-mer 25uM G-PD
OligoT	50-mer 100uM PD	50-mer 100uM PD	50-mer 100uM PD	50-mer 100uM PD	50-mer 100uM PD	50-mer 100uM PD

depleted phase will concentrate the protein and Oligo-dT molecules in it about 100-fold. Below, several calculations are displayed for the result of such concentration, for a representative 50kd protein with a diameter of 14nm which is similar to a globular protein such as bovine albumin.

Microarray fabrication, of the kind to be exploited in the present invention is a type of nanotechnology, where individual microarray elements or "spots" are applied to a surface as nanoliter droplets, which typically form spots on a surface that are about 100 microns in diameter. The present invention describes by calculation, the effect of depositing a typical 1 nanoliter droplet on a microarray surface, under conditions such that, as described above, air drying of the water in it will cause non-volatile solutes, such as glycerol, or propanediol alone or with solids like trehalose or sorbitol, to be concentrated 100-fold within the 100um spot, thereby reducing the thickness of the spot.

Microarray Spot Fluid Thickness Calculation: Subsequent to Air-Drying

Assume $1x10^{-9}$L print volume = $1x10^{-6}cm^3$.

Assume 100-fold air-drying to $1x10^{-11}$ L = $1x10^{-8}cm^3$.

Assume Spot Diameter = $1x10^{-2}$cm (100um).

Spot Area = $1x10^{-4}cm^2$

Film Thickness = Print Volume after Air-Drying/Spot Area = [$1x10^{-8}cm^3$] / $1x10^{-4}cm^2$ = $1x10^{-4}$cm = 1um

Assuming 1nL print of 1% non-volatile solutes:

Final Spot Shape upon 100-fold evaporative concentration.

Spot Width = 100um and Spot Thickness = 1um

Surface Coverage Calculations

1). Assuming that a Protein is @14nm in diameter, its 2D projection = $150nm^2$.

2). If the Microarray spot is 100um wide, its area = $0.75x10^{-8}$ m^2 = $75x10^{+8}$ nm^2.

3). So one 100um spot will be covered by @$0.5x10^{+8}$ Protein molecules, as a one-molecule thick layer

4). If contact print volume = 1nL, at:

5uM = $3x10^{+9}$ molecules per spot yields 30 molecule deep Protein layer, post evaporation;

2uM = $1.5x10^{+9}$ molecules per spot yields 12 molecule deep Protein layer, post evaporation;

1uM = $0.75x10^{+9}$ molecules per spot yields 1.5 molecule deep Protein layer, post evaporation;

0.4uM = $0.3x10^{+9}$ molecules per spot yields 0.6 molecule deep Protein layer, post evaporation;

@75nm, or about 5x the protein diameter (FIG. 1A). If printed at @10uM, Oligo-dT will also concentrate 100 fold, to yield an average separation of only about 60nm, or about 4x the diameter of the protein, thus yielding a dense network of Oligo-dT molecules surrounding the protein molecules between them (FIG. 1A). If desired, the effective pore size of the Oligo-dT network could be increased to an average separation of 100 nm if the Oligo-dT were applied at 2 uM prior to 100-fold air dying, or conversely, if the Oligo-dT were applied at 50 uM, in the original water-containing phase the pore size upon drying could be reduced to about 35 nm.

While still water-free, due to the dense proximity of Oligo-dT strands, photochemical crosslinking will be efficient: between Oligo-dT strands and also between Oligo-dT and the protein (FIG. 1B). Interestingly, at 250 ug/ml, the same calculation suggest that protein molecules will on average "pile" to form a layer on the microarray surface that is about 12 protein molecules deep within the water depleted, 1 um thick microarray spot. Thus, FIG. 1B represents about 200um (about $1/5^{th}$) of such a 1 um-thick desiccated microarray spot. Upon rehydration of that 1 um thick Oligo-dT + protein layer, wetting will cause the layer to swell, the separation between protein molecules to increase, thus preparing the protein, while still entrapped in the crosslinked Oligo-dT matrix, for subsequent microarray-based binding interaction with water-soluble analytes of interest.

EXAMPLE 3

Protein Attachment to Solid Surfaces

The present invention is novel in that protein is not linked to the surface directly, nor to a preformed polymer field, or to pores in the solid support or by adsorption to the microarray surface. Instead, the protein is applied to the microarray surface with Oligo-dT, which although not a high polymer, forms an extended polymeric matrix within a microarray spot, subsequent to controlled evaporative concentration, followed by photochemical crosslinking.

The physical and chemical entrapment of protein within that Oligo-dT matrix is created locally, only within the spots comprising sites of microarray fluid deposition, thereby allowing site-addressable, self-assembly of a 3 dimensional protein structure such as that in FIGS. 1A-1B. The key components of the invention are the length of the Oligo-dT (typically @50bases), the ratio of Oligo-dT to protein (typically 1/1 to 10/1 on a mole basis), and the ability to control the final concentration of protein and Oligo-dT after ordinary evaporation, by including water-miscible, non-volatile solvents and solutes, typically at 1% by mass, so that the protein & Oligo-dT will concentrate @ 100-fold prior to UV crosslinking.

OligoT	50-mer 25 µM G-S	50-mer 25 µM G-S	50-mer 25 µM G-S	50-mer 25 µM G-S	control	control
OligoT	50-mer 100 µM glycerol	50-mer 100 µM glycerol	50-mer 100 µM glycerol	50-mer 100 µM glycerol	50-mer 25 µM glycerol	50-mer 25 µM glycerol
OligoT	50-mer 25 µM G-PD	50-mer 25 µM G-PD	50-mer 25 µM G-PD	50-mer 25 µM G-PD	50-mer 100 µM G-PD	50-mer 100 µM G-PD
OligoT	50-mer 100 µM trehalose	50-mer 100 µM trehalose	50-mer 100 µM trehalose	50-mer 100 µM trehalose	50-mer 25 µM trehalose	50-mer 25 µM trehalose
OligoT	50-mer 25 µM G-S	50-mer 25 µM G-S	50-mer 25 µM G-S	50-mer 25 µM G-S	control	control
Right 6 x13 segment of 12 x 12 Microarray						
[BSA] conc	50 µg/ml	20 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	20 µg/ml
OligoT	50-mer 25 µM glycerol	50-mer 25 µM glycerol	50-mer 100 µM G-PD	50-mer 100 µM G-PD	50-mer 100 µM G-PD	50-mer 100 µM G-PD
OligoT	50-mer 100 µM PD	50-mer 100 µM PD	50-mer 25 µM PD	50-mer 25 µM PD	50-mer 25 µM PD	50-mer 25 µM PD
OligoT	50-mer 25 µM trehalose	50-mer 25 µM trehalose	50-mer 100 µM G-S	50-mer 100 µM G-S	50-mer 100 µM G-S	50-mer 100 µM G-S
OligoT						
OligoT	50-mer 25 µM glycerol	50-mer 25 µM glycerol	50-mer 100 µM G-PD	50-mer 100 µM G-PD	50-mer 100 µM G-PD	50-mer 100 µM G-PD
OligoT	50-mer 100 µM PD	50-mer 100 µM PD	50-mer 25 µM PD	50-mer 25 µM PD	50-mer 25 µM PD	50-mer 25 µM PD
OligoT	50-mer 25 µM trehalose	50-mer 25 µM trehalose	50-mer 100 µM G-S	50-mer 100 µM G-S	50-mer 100 µM G-S	50-mer 100 µM G-S
OligoT						
OligoT	50-mer 25 µM glycerol	50-mer 25 µM glycerol	50-mer 100 µM G-PD	50-mer 100 µM G-PD	50-mer 100 µM G-PD	50-mer 100 µM G-PD
OligoT	50-mer 100 µM PD	50-mer 100 µM PD	50-mer 25 µM PD	50-mer 25 µM PD	50-mer 25 µM PD	50-mer 25 µM PD
OligoT	50-mer 25 µM trehalose	50-mer 25 µM trehalose	50-mer 100 µM G-S	50-mer 100 µM G-S	50-mer 100 µM G-S	50-mer 100 µM G-S
OligoT						

TABLE IV

Adjuvant	Abbreviation	Sodium Phosphate pH 8.4 (mM)	Biotinylated BSA (μg/ml)	50 mer Oligo-T (μM)	Cy5 Oligo-T (μM)
Glycerol 1%	G1	2	250	100	1
	G2	2	100	100	1
	G3	2	50	100	1
	G4	2	20	100	1
	G5	2	250	25	1
	G6	2	100	25	1
	G7	2	50	25	1
	G8	2	20	25	1
Glycerol-Propanedio 0.5%-0.5%	GP1	2	250	100	1
	GP2	2	100	100	1
	GP3	2	50	100	1
	GP4	2	20	100	1
	GP5	2	250	25	1
	GP6	2	100	25	1
	GP7	2	50	25	1
	GP8	2	20	25	1
Propanediol 1%	PD1	2	250	100	1
	PD2	2	100	100	1
	PD3	2	50	100	1
	PD4	2	20	100	1
	PD5	2	250	25	1
	PD6	2	100	25	1
	PD7	2	50	25	1
	PD8	2	20	25	1
Trehalose 1%	T1	2	250	100	1
	T2	2	100	100	1
	T3	2	50	100	1
	T4	2	20	100	1
	T5	2	250	25	1
	T6	2	100	25	1
	T7	2	50	25	1
	T8	2	20	25	1
Glycerol-Sorbitol 0.5%-0.5%	GS1	2	250	100	1
	GS2	2	100	100	1
	GS3	2	50	100	1
	GS4	2	20	100	1
	GS5	2	250	25	1
	GS6	2	100	25	1
	GS7	2	50	25	1
	GS8	2	20	25	1
Control Trehalose 0.5%	C1	150	250	0	1
	C2	150	250	100	1

In Tables Va, Vb and Vc the numerical values used to generate FIGS. 3 and 4 are provided.

TABLE Vb

Test Condition	SAPE @ 0.1 μg/ml + 1% casein				
	635nm Signal Intensity Post Printing	635nm Signal Intensity Post SAPE binding	Signal Decrease (%)	532nm SAPE Signal Intensity	SAPE/Cy5 Signal Intensity Ratio
G1	12131	7829	35	924	0.12
G2	5625	3932	30	961	0.24
G3	1081	891	18	749	0.84
G4	1150	1140	1	647	0.57
G5	1326	822	38	937	1.14
G6	501	372	26	1071	2.88
G7	611	480	21	1077	2.24
G8	150	139	7	135	0.97
GP1	15734	10458	34	1177	0.11
GP2	7716	5523	28	1398	0.25
GP3	6435	4714	27	1415	0.30
GP4	137	124	9	123	0.99
GP5	12493	6449	48	867	0.13
GP6	3741	2450	35	909	0.37
GP7	3796	2701	29	859	0.32
GP8	3034	2173	28	791	0.36
PD1	65535	65535	0	835	0.01
PD2	49436	40764	18	847	0.02
PD3	15169	8673	43	665	0.08
PD4	3159	2420	23	401	0.17
PD5	50343	30750	39	1201	0.04
PD6	35607	14057	61	1227	0.09
PD7	18201	9594	47	1072	0.11
PD8	21455	10282	52	940	0.09
T1	65535	9538	85	799	0.08
T2	65535	15743	76	559	0.04
T3	65535	17025	74	193	0.01
T4	65535	19488	70	381	0.02
T5	65535	9878	85	657	0.07
T6	65535	17010	74	573	0.03
T7	65535	24874	62	662	0.03
T8	65535	31337	52	376	0.01
GS1	64880	25578	61	1071	0.04
GS2	10017	5088	49	1120	0.22
GS3	1718	824	52	1112	1.35
GS4	2662	1400	47	1143	0.82
GS5	23631	2382	90	814	0.34
GS6	6148	2138	65	412	0.19
GS7	2023	686	66	193	0.28
GS8	2577	1150	55	135	0.12
C1	65535	65535	0	609	0.01
C2	65535	65535	0	466	0.01

independent of the supporting non-aqueous solvent and solutes added. However, when the resulting SAPE/Cy-5 ratio was obtained, it was seen that glycerol (G) glycerol-propanediol (GP) and glycerol-sorbitol (GS) each provide for substantial biotin-BSA interaction with its cognate streptavidin-phycoerythrin (SAPE) conjugate. In contrast, it is seen that Trehalose (T) and to a lesser extent Propanediol (PD) provide for very poor BSA interaction due to poor protein association to the surface or protein disruption on the surface or both.

The above Examples demonstrate that Oligo-dT mediated UV crosslinking allows a protein such as BSA to be linked to a microarray surface to form, upon air-drying, a principally water free phase containing a number of nonvolatile water miscible solvents and solutes. The data show that, for BSA, certain solvent-solute pairs, e.g., glycerol and glycerol propane diol, appear to be superior to trehalose and propandiol in the present case of BSA attachment to the microarray surface.

11. The formulation of claim 1, wherein the solid support is an epoxy-silane layer disposed upon an underlying surface.

12. The formulation of claim 11, wherein said underlying surface is a metal.

13. The formulation of claim 1, wherein the non-volatile solvent and solutes are formulated in water at about 1% by mass.

14. The formulation of claim 1, wherein the protein is an immunoglobulin, a glycoprotein, a viral protein, an intact virus, albumin, an HLA, or an enzyme.

15. A formulation to link protein to a solid support, comprising:

one or more proteins;

Oligo-dT; and

glycerol or glycerol and at least one of sucrose, trehalose or sorbitol in an aqueous solution.

16. The formulation of claim 15, where the Oligo-dT is about 50 bases long and is contained in the formulation in a concentration of about 1 μg/ml to about 100 μg/ml.

17. The formulation of claim 15, where the protein is about 50 kD to 250 kD in mass and is contained in the formulation in a concentration of at least 10 μg/ml.

18. The formulation of claim 15, wherein the solid support is an amino-silane layer or epoxy-silane layer disposed upon an underlying surface comprising a metal surface, a glass surface or a ceramic surface.

19. The formulation of claim 15, wherein the protein is an immunoglobulin, a glycoprotein, a viral protein, an intact virus, albumin, an HLA, or an enzyme.

20. A method of attaching a protein to a surface of a substrate, comprising the steps of:

contacting the formulation of claim 1 onto the substrate surface;

printing the formulation onto the substrate surface;

air-drying the substrate surface;

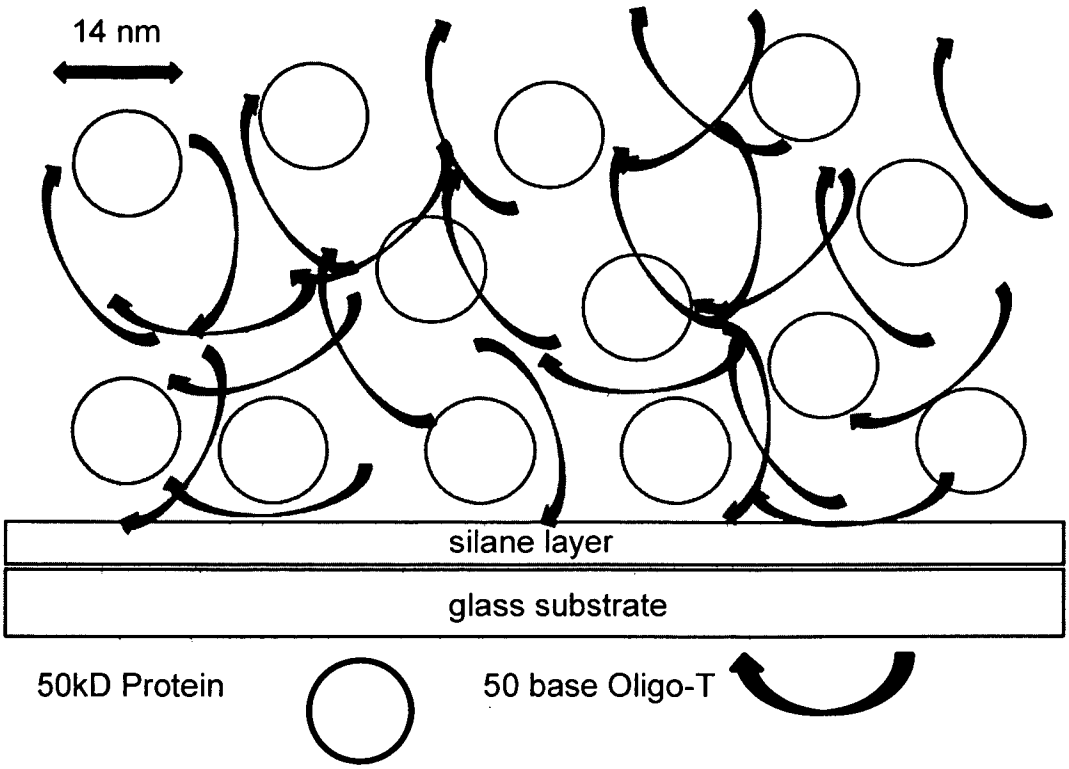

14 nm
silane layer
glass substrate
50kD Protein
50 base Oligo-T

FIG. 1A

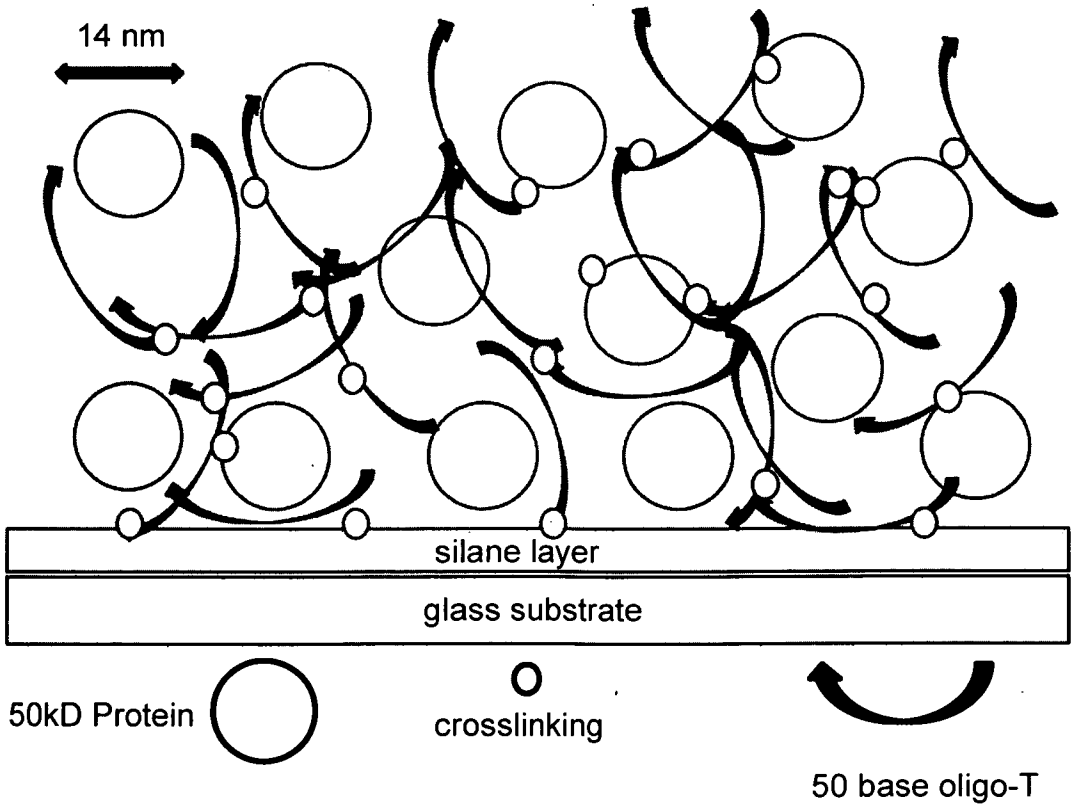

14 nm
silane layer
glass substrate
50kD Protein
crosslinking
50 base oligo-T

FIG. 1B

635nm = Cy5 532nm = SAPE

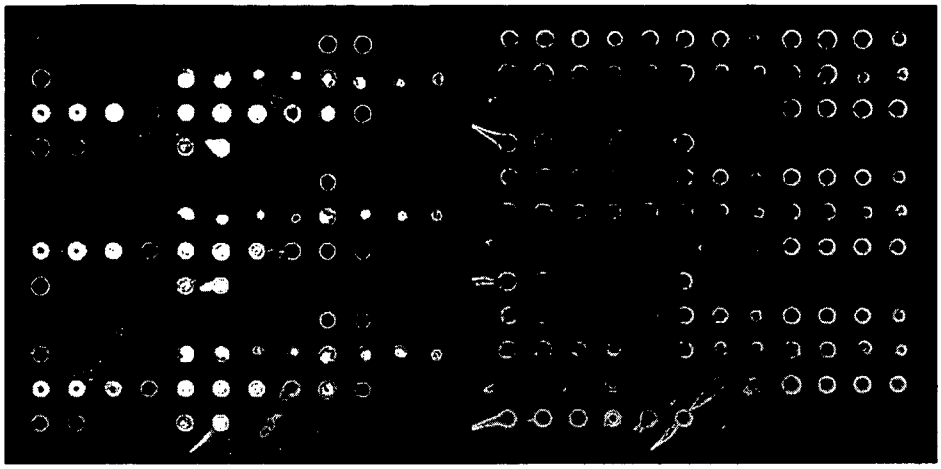
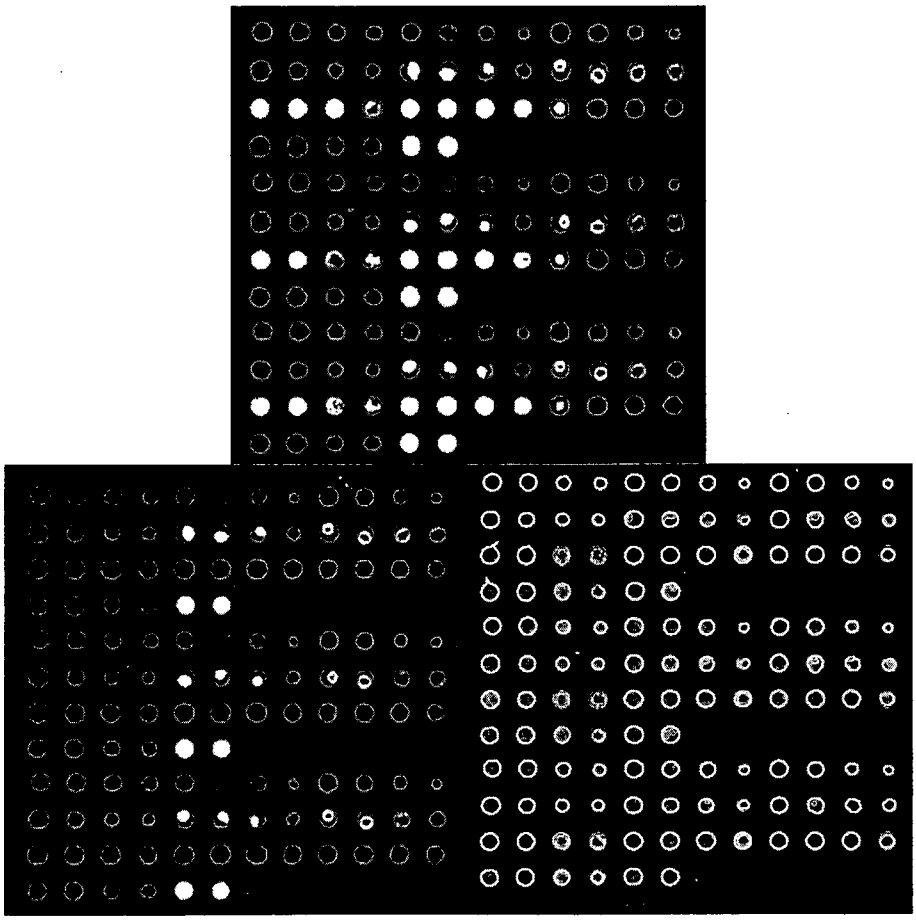


FIG. 2A

635nm = Cy5



635nm = Cy5 532nm = SAPE

FIG. 2B

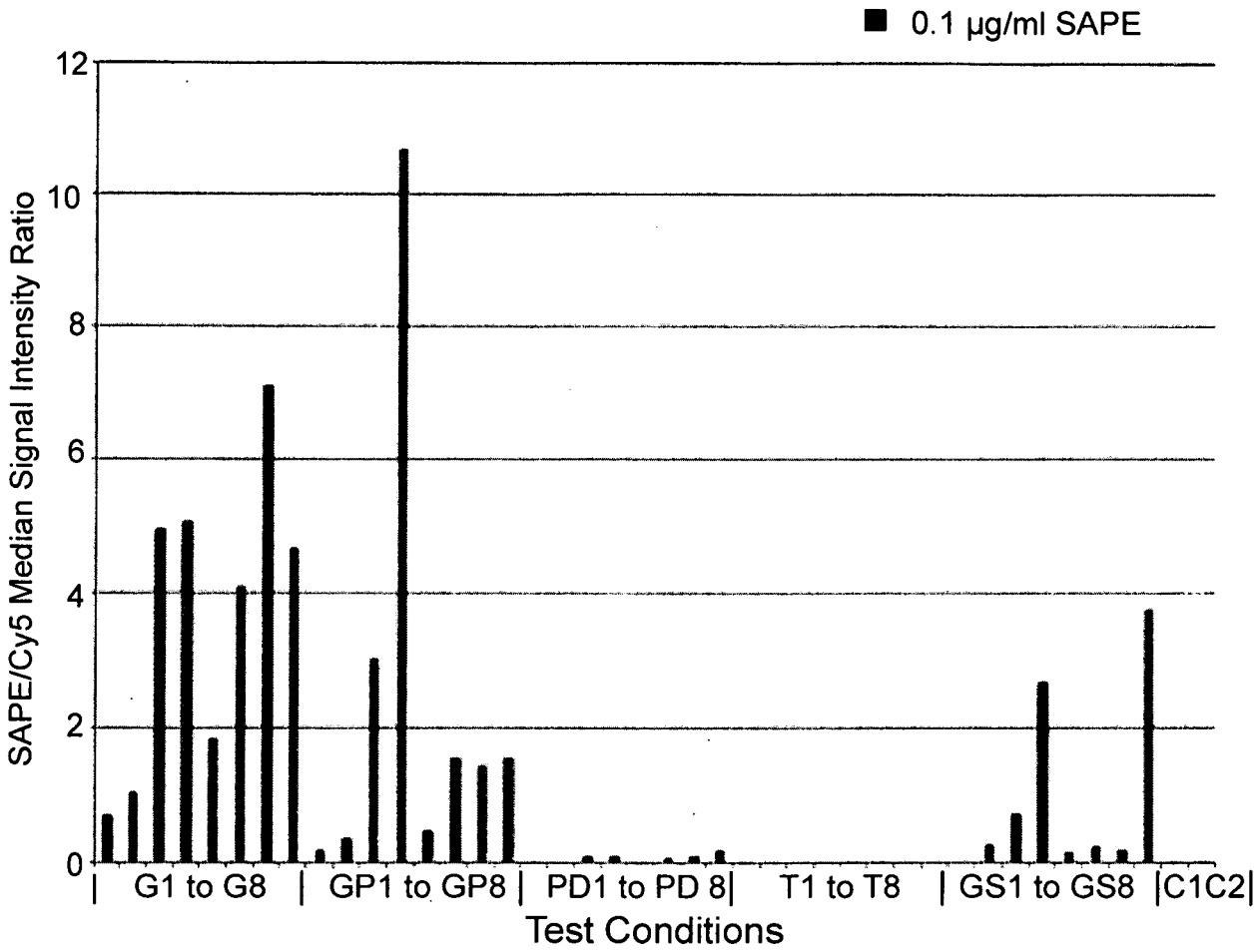

0.1 µg/ml SAPE
SAPE/Cy5 Median Signal Intensity Ratio
12
10
8
6
4
2
0
G1 to G8
GP1 to GP8
PD1 to PD 8
T1 to T8
GS1 to GS8
C1C2
Test Conditions

FIG. 3

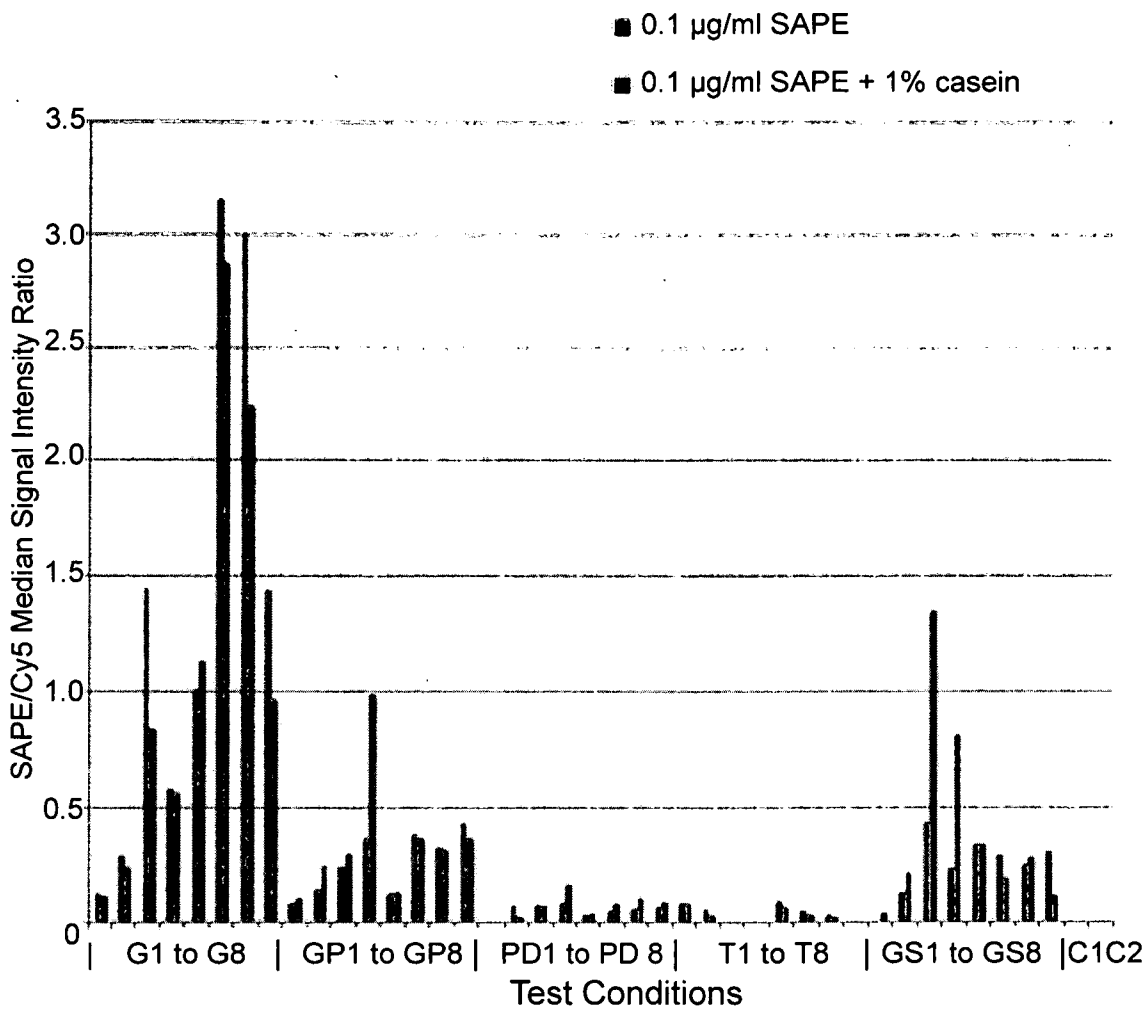

0.1 µg/ml SAPE
0.1 µg/ml SAPE + 1% casein
SAPE/Cy5 Median Signal Intensity Ratio
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
G1 to G8
GP1 to GP8
PD1 to PD 8
T1 to T8
GS1 to GS8
C1C2
Test Conditions

FIG. 4