Related methods comprise: modifying an exposed surface of a substrate to incorporate a first chemical group; reacting the first chemical group with a mixture of a water-soluble protective coating and a functionalized polymer molecule comprising a first reactive group to form a functionalized polymer coating layer covalently bound to the exposed surface of the substrate and mixed with the water-soluble protective coating; and grafting a primer to the functionalized polymer coating layer by reacting the primer with a second reactive group of the functionalized polymer coating layer. In such examples, then the functionalized polymer coating layer and water-soluble protecting layer are applied at the same time. In such examples, the functionalized polymer coating layer is cured following application of the mixture of the functionalized polymer molecule and the water-soluble protecting coating. In some examples, the mixture is from 5wt% to about 95wt% of the water-soluble coating relative to the functionalized polymer molecule, or about 10wt% to about 80wt%, or about 25wt% to about 75wt%, or about 25wt%, 50wt%, or 75wt%. The water-soluble protecting coating can protect the surface during the curing step. In some examples, a mixture comprising a water-soluble coating precursor and a functional polymer molecule is applied. In some examples, the mixture further comprises a solvent, such as ethanol. In some examples, the water-soluble coating precursor is KOLLICOAT® IR and the functionalized polymer molecule is PAZAM. In one example, applying a mixture of 5% w/v KOLLICOAT® IR, 0.25% w/v PAZAM, and 5% ethanol in water during the functionalized polymer coating step improved CFR intensity and read 1 cycle 1 intensity, and maintained % passing filter of resulting grafted primers that were loaded at concentrations of 0.8, 1.1, 1.5, and 5 µM, relative to a control (0.25% w/v PAZAM and 5% ethanol in water). In some examples, the mixture is 5% w/v water-soluble coating precursor (e.g., KOLLICOAT® IR), 0.25% w/v functional polymer molecule (e.g., PAZAM), and 5% ethanol. In such examples, the water-soluble coating may remain in place during the primer grafting step. For patterned substrates, the water-soluble coating may be removed during a subsequent polishing step (which employs aqueous conditions), or during rehydration of the flow cell before grafting (the functionalized polymer coating dehydrates during curing).

[0066] In some examples of the methods or flow cells described herein, the chemical group incorporated on the exposed surface of the substrate is optionally substituted alkenyl, ~~optionally substituted alkenyl,~~ optionally substituted cycloalkenyl, optionally substituted norbornenyl, optionally substituted cyclooctynyl, optionally substituted bicyclononynyl,

[0086] As used herein, “cycloalkyl” means a fully saturated carbocyclyl ring or ring system. Examples include cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl.

[0087] As used herein, “cycloalkylene” means a fully saturated carbocyclyl ring or ring system that is attached to the rest of the molecule via two points of attachment.

[0088] As used herein, “cycloalkenyl” or “cycloalkaene” means a carbocyclyl ring or ring system having at least one double bond, wherein no ring in the ring system is aromatic. Examples include cyclohexenyl or cyclohexene and norbornene or norbornenyl. Also as used herein, “heterocycloalkenyl” or “heterocycloalkene” means a carbocyclyl ring or ring system with at least one heteroatom in ring backbone, having at least one double bond, wherein no ring in the ring system is aromatic.

[0089] As used herein, “cycloalkynyl” or “cycloalkyne” means a carbocyclyl ring or ring system having at least one triple bond, wherein no ring in the ring system is aromatic. An example is cyclooctyne. Another example is bicyclononyne. Also as used herein, “heterocycloalkynyl” or “heterocycloalkyne” means a carbocyclyl ring or ring system with at least one heteroatom in ring backbone, having at least one triple bond, wherein no ring in the ring system is aromatic.

[0090] The term “depositing,” as used herein, refers to any suitable application technique, which may be manual or automated, and results in modification of the surface properties. Generally, depositing may be performed using vapor deposition techniques, coating techniques, grafting techniques, or the like. Some specific examples include chemical vapor deposition (CVD), spray coating (e.g., ultrasonic spray coating), spin coating, dunk or dip coating, doctor blade coating, puddle dispensing, flow through coating, aerosol printing, inkjet printing, or the like. In some aspects, the water-soluble protective coating is applied by spray coating. In other aspects, the water-soluble protecting coating is applied by flow through coating.

[0091] As used herein, the term “depression” refers to a discrete concave feature in a patterned substrate having a surface opening that is completely surrounded by interstitial region(s) of the patterned substrate surface. Depressions can have any of a variety of shapes at their opening in a surface including, as examples, round, elliptical, square, polygonal, hexagonal, star shaped (with any number of vertices), etc. The cross-section of a depression taken orthogonally with the surface can be curved, square, polygonal, hyperbolic, conical, angular, etc.

[0221] The example shown in Figs. 5E though 5H will now be described. In Fig. 5E, the primer 22 is grafted to the functionalized polymer coating layer 20 in the depression(s) 14, 14'. Any of the primers described herein may be used. In this example, grafting may be accomplished by dunk coating, spray coating, puddle dispensing, or by another suitable method that will attach the primer(s) to the functionalized polymer coating layer 20 in at least some of the depressions 14, 14'. Each of these example techniques may utilize the primer solution or mixture described herein, which may include the primer(s), water, a buffer, and a catalyst.

[0222] As shown in Fig. 5F, after the primer 22 is grafted to the functionalized polymer coating layer 20 in the depressions 14, 14', the water-soluble protective coating 24 is formed on the surface chemistry 20, 22 and on at least a portion of the patterned substrate. The water-soluble protective coating 24 may be formed on the exposed surface of the patterned substrate 12 that is not part of a bonding region 25. In this example, the water-soluble protective coating 24 is selectively deposited or patterned on the interstitial regions 16 between adjacent depressions 14, 14', but not at the edge/periphery of the patterned substrate 12 where the bonding region 25 is located. The selective deposition/patterning of the water-soluble protective coating 24 may be accomplished using the aqueous solution, as described herein. In this example, the material in the aqueous solution may be any of the examples disclosed herein (i.e., a water-soluble non-cationic synthetic polymer; a water-soluble natural polysaccharide or a derivative thereof; a water-soluble natural protein or a derivative thereof; a water-soluble salt; a water-soluble small molecule compound selected from the group consisting of a water-soluble surfactant, a sugar, an antioxidant, a chelator, a buffer, a glycol; or a cyclodextrin). In an example, the water-soluble material may be the polyvinyl alcohol/polyethylene glycol graft copolymer, sucrose, dextran, polyacrylamide, glycols, ethylenediaminetetraacetic acid sodium salt, tris(hydroxymethyl)aminomethane with ethylenediaminetetraacetic acid, (tris(2-carboxyethyl)phosphine), tris(3-hydroxypropyltriazolylmethyl)amine, bathophenanthrolinedisulfonic acid disodium salt, hydroxyl functional polymers, glycerol, or saline sodium citrate. After the aqueous solution is deposited, it may be dried to form the water-soluble protective coating 24.

[0223] As depicted in Fig. 5G, the lid 26 may then be bonded to the bonding region 25. The lid 26 may be any of the materials and in any of the configurations described herein. The lid 26 may also be bonded to the bonding region 25 via any of the techniques described herein.

[0224] In the example shown in Fig. 5G, the lid 26 includes a top portion 27 integrally formed with sidewall(s) 29. The sidewall(s) 29 are bonded to the bonding region 25 of the patterned substrate 12 through the spacer layer 28.

[0225] Together, the lid 26 and the patterned substrate 12 (with the surface chemistry 20, 22) define the flow channel 30, which is in selective fluid communication with the depressions

14, 14'. The flow channel 30 may serve to, for example, selectively introduce fluid to the protective coating 24 in order to remove the coating 24, and to selectively introduce reaction components or reactants to the surface chemistry 20, 22 (after the protective coating 24 is removed) in order initiate designated reactions in/at the depressions 14, 14'.

[0226] When the lid 26 is bonded to the silanized, coated, polished, and grafted patterned substrate, an example of the flow cell 10' is formed, as shown in Fig. 5G. In this example, the protective coating 24 remains in place on the surface chemistry 20, 22 and some of the patterned substrate surface. The flow cell 10' can be shipped, stored, etc. with the protective coating 24 in place.

[0227] When it is desirable to utilize the flow cell 10' in an application (e.g., a sequencing operation), the protective coating 24 may be removed via the dissolution process described in reference to reference numeral 108. The water solubility of the protective coating 24 enables it to be removed via dissolution in the aqueous solution, which is not deleterious to the underlying surface chemistry 20, 22 or patterned substrate 12. Fig. 5H depicts the flow cell 10' after the protective coating 24 is removed.

[0228] The example shown in Figs. 5I though 5L will now be described. In Fig. 5I, the initial water-soluble protective coating 24' may be selectively deposited, or patterned, such that the functionalized polymer coating layer 20 is covered and such that the bonding region 25 of the patterned substrate 12 remains exposed. In this example, the initial water-soluble protective coating 24' is selectively deposited or patterned on the interstitial regions 16 between adjacent depressions 14, 14', but not at the edge/periphery of the patterned substrate 12 where the bonding region 25 is located. The selective deposition/patterning of the initial water-soluble protective coating 24' may be accomplished as described herein using the aqueous solution described in reference to reference numeral 104'' (which includes water and, in some instances, up to about 15% (mass to volume) of the polyvinyl alcohol/polyethylene glycol graft copolymer, sucrose, or polyethylene glycol).

[0229] The initial protective coating 24 protects the functionalized polymer coating layer 20 during any assembly processes that are subsequently performed. As depicted in Fig. 5J, the assembly process may include bonding the lid 26 to the bonding region 25. While not shown, when the substrate wafer is used, the assembly process may include bonding and dicing. The lid 26 may be any of the materials and may have any of the configurations described herein. The lid 26 may be bonded to the bonding region 25 via any of the techniques described herein.

~~What is claimed is:~~Claims

1. A flow cell, comprising:

a substrate ~~comprising an exposed surface~~;

a lid bonded to a bonding region of the substrate, wherein the lid and the substrate at least partially define a flow channel therebetween and wherein the lid bonded to the substrate at the bonding region forms a boundary of the flow channel;

an input port and an output port formed in the lid or the substrate;

surface chemistry positioned on at least a portion of a surface of the substrate in the flow channel, the surface chemistry including:

a functionalized polymer coating layer covalently bound to the exposed surface of the substrate through a chemical group on the exposed surface;

a primer grafted to the functionalized polymer coating layer; and

a water-soluble protective coating on the primer and the functionalized polymer coating layer.

2. The flow cell of claim 1, wherein the functionalized polymer coating layer is covalently bound to the exposed surface due to reaction of the chemical group on the exposed surface with a first reactive group of the functionalized polymer coating layer.

3. The flow cell of claim 1 or claim 2, wherein the primer is grafted to the functionalized polymer coating layer due to reaction of the primer with a second reactive group of the functionalized polymer coating layer.

~~4. The flow cell of any one of claims 1 to 3, further comprising a lid bonded to a bonded region of the substrate, wherein the lid and the substrate at least partially define a flow channel.~~

~~5~~4. The flow cell of ~~claim 4~~ any one of claims 1 to 3, wherein the substrate is a patterned substrate comprising depressions separated by interstitial regions, and wherein the flow channel is in selective communication with the depressions.

~~6~~5. The flow cell of any one of claims 1 to ~~4~~3, wherein the substrate is a patterned substrate comprising depressions separated by interstitial regions.

~~7~~6. The flow cell of ~~any one of claims 5 to 6~~ claim 4 or 5, wherein the water-soluble protective coating is in the depressions and on at least a portion of the interstitial regions.

~~8~~7. The flow cell of any one of claims 1 to ~~4~~3, wherein the substrate is a non-patterned substrate.

~~9~~8. A method of preparing a flow cell, comprising:

providing a substrate with an exposed surface comprising a first chemical group, wherein the providing optionally comprises modifying the exposed surface of the substrate to incorporate the first chemical group;

reacting the first chemical group with a first reactive group of a functionalized polymer molecule to form a functionalized polymer coating layer covalently bound to the exposed surface of the substrate;

bonding a lid to a bonding region of the substrate, wherein the lid and the substrate at least partially define a flow channel including the exposed surface;

grafting a primer to the functionalized polymer coating layer by reacting the primer with a second reactive group of the functionalized polymer coating layer; and

forming a water-soluble protective coating on the primer and the functionalized polymer coating layer.

~~10~~9. The method of claim ~~9~~8, wherein the substrate is a non-patterned substrate~~, and wherein the method further comprises bonding a lid to a bonding region of the non-patterned substrate, wherein the lid and the non-patterned substrate at least partially define a flow channel including the exposed surface~~.

~~11~~10. The method of claim ~~10~~9, wherein the modifying of the exposed surface, the reacting of the first chemical group with the first reactive group, the grafting of the primer, and the forming of the water-soluble protective coating involve respective flow through processes.

~~12~~11. The method of claim ~~9~~8, wherein:

the substrate is a non-patterned substrate; and

the modifying of the exposed surface comprises plasma ashing~~; and~~

~~after the modifying of the exposed surface, the method further comprises bonding a lid to a bonding region of the non-patterned substrate, wherein the lid and the non-patterned substrate at least partially define a flow channel including some of the exposed surface~~.

~~13~~12. The method of claim ~~9~~8, wherein:

the substrate is a patterned substrate comprising depressions separated by interstitial regions;

the modifying of the exposed surface involves attaching a silane or a silane derivative comprising the first chemical group to the exposed surface to form silanized depressions and silanized interstitial regions;

the reacting forms the functionalized polymer coating layer in the silanized depressions and on the silanized interstitial regions; and

prior to the grafting of the primer, the method further comprises removing the functionalized polymer coating layer from the silanized interstitial regions.

~~14~~13. The method of claim ~~13~~12, wherein:

the grafting attaches the primer to the functionalized polymer coating layer in the silanized depressions, thereby forming functionalized depressions; and

the forming yields the water-soluble protective coating on the functionalized depressions and at least some of the interstitial regions.

~~15~~14. The method of claim ~~13~~12, wherein after the removing of the functionalized polymer coating layer, the method ~~further~~ comprises bonding ~~a~~ the lid to ~~a~~ the bonding region of the patterned substrate, wherein the lid and the patterned substrate at least partially define a flow channel including the silanized depressions having the functionalized polymer coating thereon.

~~16~~15. The method of claim ~~15~~14, wherein the grafting of the primer and the forming of the water-soluble protective coating involve respective flow through processes.

~~17~~16. The method of claim ~~9~~8, wherein after the functionalized polymer coating layer is formed and before the grafting of the primer and the forming of the water-soluble protective coating, the method further comprises:

patterning an initial water-soluble protective coating on the functionalized polymer coating layer such that a bonding region of the substrate remains exposed;

bonding ~~a~~ the lid to the bonding region of the substrate to form a flow channel that is in selective fluid communication with the initial water-soluble protective coating; and

removing the initial water-soluble protective coating.

~~18~~17. The method of claim ~~9~~8, wherein the forming of the water-soluble protective coating involves applying an aqueous solution including up to about 15% mass to volume, or from about 1 to about 15% mass to volume, or from about 1 to about 10% mass to volume, or from about 1 to about 5% mass to volume, or from about 2 to about 5% mass to volume, or from about 4 to about 8% mass to volume, or from about 5 to about 7.5% ~~(~~mass to volume~~)~~, of a water-soluble material.

~~19~~18. The method of claim ~~18~~17, wherein the percentage of the water-soluble material in the aqueous solution is adjusted based on a primer density of the primer.

~~20~~19. The flow cell of any one of claims 1-~~8~~7 ~~or the method of any one of claims 9-19~~, wherein the water-soluble protective coating comprises a non-cationic synthetic polymer; a natural polysaccharide or a derivative thereof~~;~~ a natural protein or a derivative thereof~~;~~ a water-soluble salt; or a small molecule compound selected from the group consisting of a water-soluble surfactant, a sugar, an antioxidant, a chelator, a buffer, a glycol, glycerol, and a cyclodextrin; or a combination thereof.

~~21~~20. The flow cell ~~or method~~ of claim ~~20~~19, wherein the water-soluble protective coating comprises:

(a) the non-cationic synthetic polymer ~~comprising~~ which further comprises polyacrylamide, poly(acrylic acid), polyacrylate, poly(methacrylic acid), poly(vinyl pyrrolidone), poly(vinyl alcohol), poly (methacrylamide), a poly(N-alkyl acrylamide), a poly(N-dialkyl acrylamide),

poly(N-(2-hydroxypropyl)methacrylamide), poly(divinyl ether-maleic anhydride), a poly(phosphate), a poly(2-alkyl-2-oxazoline), poly(hydroxyethyl methacrylate), poly(2-hydroxyethyl acrylate), polyethylene glycol, a polyether, poly(sulfobetaine methacrylate), poly(vinyl ether-maleic acid), a hydroxyl functional polymer, a non-natural polypeptide, ~~or~~ a silicone, or a combination thereof;

(b) the natural polysaccharide or derivative thereof ~~comprising~~ which further comprises starch, carboxymethylcellulose, xanthan gum, pectin, dextran, carrageenan, guar gum, cellulose, hydroxypropylmethyl cellulose (HPMC), hydroxypropyl cellulose (HPC), hydroxyethyl cellulose (~~H~~REC), methyl cellulose, carboxymethylhydroxyethyl cellulose (CMHEC), hyaluronic acid, starch phosphate, hydroxypropyl starch, hydroxyethyl starch, agarose, agar, ~~or~~ alginate, or a combination thereof;

(c) the natural protein or derivative thereof ~~comprising~~ which further comprises casein or albumin;

(d) the water-soluble salt ~~comprising~~ which further comprises sodium chloride, sodium bromide, sodium sulfate, sodium phosphate, sodium carbonate, sodium acetate, sodium citrate, potassium chloride, potassium bromide, potassium sulfate, potassium phosphate, potassium carbonate, potassium acetate, ~~or~~ potassium citrate, ~~or~~ saline sodium citrate, or a combination thereof;

(e) the buffer ~~comprising~~ which further comprises an aqueous solution of saline sodium citrate, tris(hydroxymethyl)aminomethane (Tris) optionally with ethylenediaminetetraacetic acid, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (~~H~~REPES), 3-[[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino]-2-hydroxypropane-1-sulfonic acid (TAPSO), N-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine (tricine), 3-(N-morpholino)propanesulfonic acid (MOPS), or 3-(N,N-bis([2-hydroxyethyl]amino)-2-hydroxypropanesulfonic acid (DIPSO);

(f) the water-soluble surfactant ~~comprising~~ which further comprises an anionic or nonionic surfactant, ~~or comprising~~ sodium dodecyl sulfate, an alkyl ethoxylate, an ethoxylated oil, a fat, or a sulfosuccinate; ~~or~~

(g) the sugar, the antioxidant, the glycol, glycerol, or the cyclodextrin;

(h) the chelator ~~comprising~~ which further comprises ethylenediaminetetraacetic acid sodium salt, tris(3-hydroxypropyltriazolylmethyl)amine, (tris(2-carboxyethyl)phosphine), ~~or~~ bathophenanthrolinedisulfonic acid disodium salt; or

(i) a combination thereof.

~~22~~21. The flow cell ~~or method~~ of claim ~~20~~19, wherein the water-soluble protective coating comprises a polyvinyl alcohol/polyethylene glycol graft copolymer, sucrose, dextran, ~~a~~ polyacrylamide, a glycol, tris(hydroxymethyl)aminomethane or a salt thereof, ethylenediaminetetraacetic acid or a salt thereof, (tris(2-carboxyethyl)phosphine), tris(3-hydroxypropyltriazolylmethyl)amine or a salt thereof, bathophenanthrolinedisulfonic acid disodium salt, a hydroxyl functional polymer, glycerol, ~~or~~ saline sodium citrate, or a mixture thereof.

~~23~~22. The flow cell ~~or method~~ of claim ~~20~~19, wherein the water-soluble protective coating comprises a polyvinyl alcohol/polyethylene glycol graft copolymer, sucrose, or a mixture thereof.

~~24~~23. The flow cell ~~or method~~ of any one of ~~the preceding claims~~ claims 19-22, wherein the functionalized polymer coating layer is poly(N-(5-azidoacetamidylpentyl)acrylamide-co-acrylamide (PAZAM).

~~25~~24. The flow cell of any one of ~~the preceding claims~~ claims 19-23, wherein the water-soluble protective coating is present on the flow cell as a mixture with the functionalized polymer coating layer.

~~26~~25. The flow cell ~~or method~~ of any ~~of the preceding claims~~ one of claims 19-24, wherein the chemical group or the first chemical group is optionally substituted alkenyl, optionally substituted cycloalkenyl, optionally substituted norbornenyl, optionally substituted cyclooctynyl, optionally substituted bicyclononynyl, optionally substituted alkynyl, azido, optionally substituted tetrazinyl, hydrazonyl, optionally substituted tetrazolyl, formyl, or hydroxyl.

~~27~~26. The flow cell ~~or method~~ of any ~~of the preceding claims~~ one of claims 19-25, wherein the chemical group or the first chemical group is covalently linked to the exposed surface of the substrate through a silane linker.

~~28~~27. The flow cell ~~or method~~ of claim ~~27~~26, wherein the chemical group or the first chemical group is norbornenyl or substituted norbornenyl.

~~29~~28. The flow cell ~~or method~~ of any ~~of the preceding claims~~ one of claims 19-27, wherein the first reactive group and the second reactive group, where recited, are each independently optionally substituted alkenyl, optionally substituted cycloalkenyl, optionally substituted norbornenyl, optionally substituted cyclooctynyl, optionally substituted bicyclononynyl, optionally substituted alkynyl, azido, optionally substituted tetrazinyl, hydrazonyl, optionally substituted tetrazolyl, formyl, or hydroxyl.

~~30~~29. The flow cell ~~or method~~ of claim ~~29~~28, wherein the first and second reactive groups are both azido.

~~31~~30. The flow cell ~~or method~~ of any ~~of the preceding claims~~ one of claims 19-29, wherein the primer is grafted to the functionalized polymer coating layer due to reaction of the primer with a second reactive group of the functionalized polymer coating layer. and the primer comprises an alkynyl group for reaction with the second reactive group.

31. The flow cell of any one of claims 19-30, wherein the primer is a plurality of primers that includes a population of functional primers and a population of non-functional primers.

32. The method of any one of claims 8-18, wherein the water-soluble protective coating comprises a non-cationic synthetic polymer; a natural polysaccharide or a derivative thereof a natural protein or a derivative thereof a water-soluble salt; or a small molecule compound selected from the group consisting of a water-soluble surfactant, a sugar, an antioxidant, a chelator, a buffer, a glycol, glycerol, and a cyclodextrin; or a combination thereof.

33. The method of claim 32, wherein the water-soluble protective coating comprises:

(a) the non-cationic synthetic polymer which further comprises polyacrylamide, poly(acrylic acid), polyacrylate, poly(methacrylic acid), poly(vinyl pyrrolidone), poly(vinyl alcohol), poly (methacrylamide), a poly(N-alkyl acrylamide), a poly(N-dialkyl acrylamide), poly(N-(2-hydroxypropyl)methacrylamide), poly(divinyl ether-maleic anhydride), a poly(phosphate), a poly(2-alky1-2-oxazoline), poly(hydroxyethyl methacrylate), poly(2-hydroxyethyl acrylate), polyethylene glycol, a polyether, poly(sulfobetaine methacrylate), poly(vinyl ether-maleic acid), a hydroxyl functional polymer, a non-natural polypeptide, a silicone, or a combination thereof;

(b) the natural polysaccharide or derivative thereof which further comprises starch, carboxymethylcellulose, xanthan gum, pectin, dextran, carrageenan, guar gum, cellulose, hydroxypropylmethyl cellulose (HPMC), hydroxypropyl cellulose (HPC), hydroxyethyl cellulose (REC), methyl cellulose, carboxymethylhydroxyethyl cellulose (CMHEC), hyaluronic acid, starch phosphate, hydroxypropyl starch, hydroxyethyl starch, agarose, agar, alginate, or a combination thereof;

(c) the natural protein or derivative thereof which further comprises casein or albumin;

(d) the water-soluble salt which further comprises sodium chloride, sodium bromide, sodium sulfate, sodium phosphate, sodium carbonate, sodium acetate, sodium citrate, potassium chloride, potassium bromide, potassium sulfate, potassium phosphate, potassium carbonate, potassium acetate, potassium citrate, saline sodium citrate, or a combination thereof;

(e) the buffer which further comprises an aqueous solution of saline sodium citrate, tris(hydroxymethyl)aminomethane (Tris) optionally with ethylenediaminetetraacetic acid, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (REPES), 3-[[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino]-2-hydroxypropane-1-sulfonic acid (TAP SO), N-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine (tricine), 3-(Nmorpholino)propanesulfonic acid (MOPS), or 3-(N,N-bis([2-hydroxyethyl]amino)-2-hydroxypropanesulfonic acid (DIPSO);

(f) the water-soluble surfactant which further comprises an anionic or nonionic surfactant, sodium dodecyl sulfate, an alkyl ethoxylate, an ethoxylated oil, a fat, or a sulfosuccinate;

(g) the sugar, the antioxidant, the glycol, glycerol, or the cyclodextrin;

(h) the chelator which further comprises ethylenediaminetetraacetic acid sodium salt, tris(3-hydroxypropyltriazolylmethyl)amine, (tris(2-carboxyethyl)phosphine), bathophenanthrolinedisulfonic acid disodium salt; or

(i) a combination thereof.

34. The method of claim 32, wherein the water-soluble protective coating comprises a polyvinyl alcohol/polyethylene glycol graft copolymer, sucrose, dextran, polyacrylamide, a glycol, tris(hydroxymethyl)aminomethane or a salt thereof, ethylenediaminetetraacetic acid or a salt thereof, (tris(2-carboxyethyl)phosphine), tris(3-hydroxypropyltriazolylmethyl)amine or a salt thereof, bathophenanthrolinedisulfonic acid disodium salt, a hydroxyl functional polymer, glycerol, saline sodium citrate, or a mixture thereof.

35. The method of claim 32, wherein the water-soluble protective coating comprises a polyvinyl alcohol/polyethylene glycol graft copolymer, sucrose, or a mixture thereof.

36. The method of any one of claims 32-35, wherein the functionalized polymer coating layer is poly(N-(5-azidoacetamidylpentyl)acrylamide-co-acrylamide (PAZAM).

37. The method of any one of claims 32-36, wherein the water-soluble protective coating is present on the flow cell as a mixture with the functionalized polymer coating layer.

38. The method of any one of claims 32-37, wherein the chemical group or the first chemical group is optionally substituted alkenyl, optionally substituted cycloalkenyl, optionally substituted norbornenyl, optionally substituted cyclooctynyl, optionally substituted bicyclononynyl, optionally substituted alkynyl, azido, optionally substituted tetrazinyl, hydrazonyl, optionally substituted tetrazolyl, formyl, or hydroxyl.

39. The method of any one of claims 32-38, wherein the chemical group or the first chemical group is covalently linked to the exposed surface of the substrate through a silane linker.

40. The method of claim 39, wherein the chemical group or the first chemical group is norbornenyl or substituted norbornenyl.

41. The method of any one of claims 32-40, wherein the first reactive group and the second reactive group, where recited, are each independently optionally substituted alkenyl, optionally substituted cycloalkenyl, optionally substituted norbornenyl, optionally substituted cyclooctynyl, optionally substituted bicyclononynyl, optionally substituted alkynyl, azido, optionally substituted tetrazinyl, hydrazonyl, optionally substituted tetrazolyl, formyl, or hydroxyl.

42. The method of claim 41, wherein the first and second reactive groups are both azido.

43. The method of any one of claims 32-42, wherein the primer is grafted to the functionalized polymer coating layer due to reaction of the primer with a second reactive group of the functionalized polymer coating layer and the primer comprises an alkynyl group for reaction with the second reactive group.

~~32~~44. The ~~flow cell or method of any of the preceding claims~~ method of any one of claims 32-43, wherein the primer is a plurality of primers that includes a population of functional primers and a population of non-functional primers.

~~33~~45. A method of using the flow cell of any one of claims ~~1-8 or 20-32~~ 1-7 and 19-31, comprising:
inserting the flow cell into a sequencing instrument; and
removing the water-soluble protective coating by exposing the water-soluble protective coating on the flow cell to water.

~~34~~46. The method of claim ~~33~~45, wherein the removing is done by performing a flow through process to remove the water-soluble protective coating.