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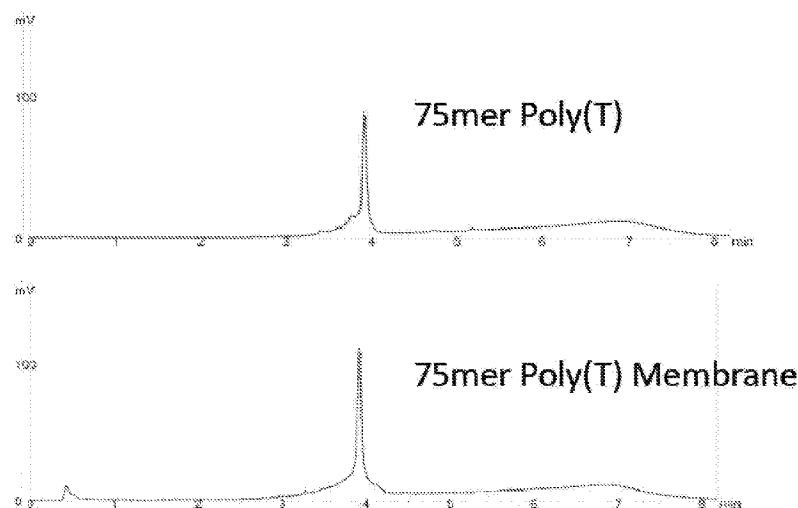


FIG. 1

(57) Abstract: The present disclosure provides a porous solid-phase support for synthesizing biopolymers, and ways of housing the support. The support may be surface activated and comprise a plurality of pores, each of the plurality of pores having a pore diameter greater than about 1 micron.



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METHODS, DEVICES, AND SYSTEMS FOR BIOPOLYMER SYNTHESIS**CROSS-REFERENCE**

[0001] This application claims the benefit of U.S. Provisional Application No. 62/767,377, filed November 14, 2018, which application is incorporated herein by reference in its entirety.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under contract numbers HG006811 and HG000205 awarded by the National Human Genome Research Institute. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] Solid-phase synthesis is a method used to synthesize polymers which relies on attaching one end of a prospective polymer to a solid phase support. This leaves the other end free for controlled extension, and facilitates the removal of excess reagents between extension steps. Due to the many applications of synthesized biopolymers there is constant need for more cost effective synthesis methods.

SUMMARY OF THE INVENTION

[0004] In one aspect, a method is provided for the synthesis of biopolymers, the method comprising synthesizing the biopolymers directly on a surface of a surface-activated solid support, wherein the surface-activated solid support comprises a plurality of pores, each of the plurality of pores having a pore diameter greater than about 1 micron. In some cases, the solid support does not comprise a scaffold or other structure on the surface of the solid support that distances the biopolymer from the surface of the solid support. In some cases, the scaffold is a surface polymer brush phase, a lightly crosslinked polymer phase, a dendrimer phase, a pellicular phase, a fractal polymer phase, and a grafted polymer. In some cases, the solid support comprises a porous sheet or a porous membrane. In some cases, the porous sheet or porous membrane comprises polypropylene. In some cases, the porous sheet or porous membrane is selected from the group consisting of: polyethylene, polypropylene/polyethylene blend, cellulose, cotton, glass, silicon, gold, silver, graphene, paper, and any combination thereof. In some cases, the solid support comprises agarose or dextran. In some cases, the pore diameter is about 2 microns. In some cases, the pore diameter is about 5 microns. In some cases, the pore diameter is about 10 microns. In some cases, the pore diameter is greater than about 10 microns. In some cases, the pore diameter is from about 10-200 microns. In some cases, the solid support

has a thickness from about 50 microns to about 1000 microns. In some cases, the solid support is about 200 microns in thickness. In some cases, the method further comprises activating the surface of the solid support with one or more chemical moieties prior to the synthesizing. In some cases, the activating comprises plasma-treating the surface of the solid support. In some cases, the one or more chemical moieties is selected from the group consisting of: amine groups, hydroxyl groups, carboxyl groups, aldehyde groups, sulfhydryl groups, maleimide groups, and epoxide groups. In some cases, the activating comprises activating the surface of the solid support with allylamine. In some cases, the solid support comprises one or more linker moieties. In some cases, the one or more linker moieties comprises succinyl, oxalyl, disulfide, UnylinkerTM, or derivatives thereof. In some cases, the synthesizing comprises: (i) covalently attaching a first monomer or derivative thereof to the one of more chemical moieties or the one or more linker moieties. In some cases, the synthesizing further comprises: (ii) coupling one or more monomers or derivatives thereof to the first monomer or derivative thereof, thereby generating the biopolymer. In some cases, the biopolymers comprise oligonucleotides. In some cases, the oligonucleotides comprise DNA, RNA, modified DNA, or modified RNA. In some cases, the biopolymers comprise polypeptides. In some cases, the comprise polysaccharides. In some cases, a plurality of the biopolymers are at least 20 monomers in length. In some cases, a plurality of the biopolymers are at least 40 monomers in length. In some cases, a plurality of the biopolymers are at least 60 monomers in length. In some cases, a plurality of the biopolymers are at least 80 monomers in length. In some cases, a plurality of the biopolymers are up to 250 monomers in length. In some cases, the synthesizing further comprises synthesizing the biopolymers with a coupling efficiency of greater than at least 98.5%. In some cases, the synthesizing further comprises synthesizing the biopolymers with a length of at least 20 monomers with a purity of at least 50%. In some cases, the synthesizing further comprises synthesizing the biopolymers with a length of at least 40 monomers with a purity of at least 50%. In some cases, the synthesizing further comprises synthesizing the biopolymers with a length of at least 60 monomers with a purity of at least 50%. In some cases, the synthesizing further comprises synthesizing the biopolymers with a length of at least 80 monomers with a purity of at least 50%. In some cases, the synthesizing further comprises synthesizing the biopolymers with a length of at least 250 monomers with a purity of at least 50%. In some cases, the solid support is free-flowing in a synthesis column. In some cases, the solid support is fitted to a synthesis vessel. In some cases, the solid support is fitted to the synthesis vessel by sonic welding.

[0005] In another aspect, a method for synthesizing an oligonucleotide is provided, the method comprising: a) providing a surface activated solid support comprising a plurality of pores, each

of the plurality of pores having a pore diameter of at least 1 micron; and b) synthesizing the oligonucleotide directly on the surface of the surface activated solid support, wherein the synthesizing comprises sequentially coupling a plurality of nucleosides or derivatives thereof to form the oligonucleotide. In some cases, the surface of the surface activated solid support does not comprise a scaffold or other structure that distances the oligonucleotide from the surface of the surface activated solid support. In some cases, the scaffold is a surface polymer brush phase, a lightly crosslinked polymer phase, a dendrimer phase, a pellicular phase, a fractal polymer phase, and a grafted polymer. In some cases, the synthesizing further comprises synthesizing a plurality of oligonucleotides on the surface activated solid support. In some cases, the synthesizing further comprises synthesizing the oligonucleotide in a pore of the surface activated solid support. In some cases, the oligonucleotide comprises RNA, DNA, modified RNA, or modified DNA. In some cases, the surface activated solid support comprises a porous sheet or a porous membrane. In some cases, the porous sheet or porous membrane comprises polypropylene. In some cases, the porous sheet or porous membrane is selected from the group consisting of: polyethylene, polypropylene/polyethylene blend, cellulose, cotton, glass, silicon, gold, silver, graphene, paper, and any combination thereof. In some cases, the surface activated solid support is plasma treated. In some cases, a surface of the surface activated solid support comprises one or more chemical moieties adsorbed thereon. In some cases, the one or more chemical moieties is selected from the group consisting of: an amine, a hydroxyl, a carboxyl, an aldehyde, a sulfhydryl, a maleimide, and an epoxide. In some cases, a surface of the surface activated solid support further comprises one or more linker moieties. In some cases, the one or more linker moieties comprises succinyl, oxalyl, disulfide, UnylinkerTM, or derivatives thereof. In some cases, the synthesizing of b) further comprises covalently attaching a first nucleoside or derivative thereof to the one or more chemical moieties. In some cases, the synthesizing of b) further comprises coupling a plurality of nucleosides or derivatives thereof to the first nucleoside or derivative thereof. In some cases, the nucleoside or derivative thereof is a nucleoside phosphoramidite. In some cases, each of the plurality of pores has a pore diameter of at least about 2 microns. In some cases, each of the plurality of pores has a pore diameter of at least about 5 microns. In some cases, each of the plurality of pores has a pore diameter of at least about 10 microns. In some cases, each of the plurality of pores has a pore diameter greater than about 10 microns. In some cases, each of the plurality of pores has a pore diameter from about 10 microns to about 200 microns. In some cases, the surface activated solid support has a thickness from about 50 microns to about 1000 microns. In some cases, the surface activated solid support is about 200 microns in thickness. In some cases, the synthesizing further

comprises synthesizing the oligonucleotide with a coupling efficiency of greater than at least 98.5%. In some cases, the synthesizing further comprises synthesizing the oligonucleotide with a length of at least 20 nucleotides with a purity of at least 50%. In some cases, the synthesizing further comprises synthesizing the oligonucleotide with a length of at least 40 nucleotides with a purity of at least 50%. In some cases, the synthesizing further comprises synthesizing the oligonucleotide with a length of at least 60 nucleotides with a purity of at least 50%. In some cases, the synthesizing further comprises synthesizing the oligonucleotide with a length of at least 80 nucleotides with a purity of at least 50%. In some cases, the synthesizing further comprises synthesizing the oligonucleotide with a length of at least 250 nucleotides with a purity of at least 50%. In some cases, the surface activated solid support is free-flowing in a synthesis column. In some cases, the surface activated solid support is fitted to a synthesis vessel. In some cases, the surface activated solid support is fitted to the synthesis vessel by sonic welding.

[0006] In another aspect, a solid support for biopolymer synthesis is provided, wherein the solid support comprises a plurality of pores each having a pore diameter of at least about 1 micron, and wherein the solid support is surface activated such that biopolymer synthesis occurs directly on a surface of the solid support. In some cases, the solid support does not comprise a scaffold. In some cases, the scaffold is a surface polymer brush phase, a lightly crosslinked polymer phase, a dendrimer phase, a pellicular phase, a fractal polymer phase, and a grafted polymer. In some cases, each of the plurality of pores have a pore diameter of at least about 2 microns. In some cases, each of the plurality of pores have a pore diameter of at least about 5 microns. In some cases, each of the plurality of pores have a pore diameter of at least about 10 microns. In some cases, each of the plurality of pores have a pore diameter of greater than about 10 microns. In some cases, a surface of the solid support comprises one or more chemical moieties. In some cases, the one or more chemical moieties is selected from the group consisting of: an amine, a hydroxyl, a carboxyl, an aldehyde, a sulfhydryl, a maleimide, and an epoxide. In some cases, the solid support is a porous sheet or a porous membrane. In some cases, the porous sheet or porous membrane comprises polypropylene. In some cases, the porous sheet or porous membrane is selected from the group consisting of: polyethylene, polypropylene/polyethylene blend, cellulose, glass, silicon, gold, silver, graphene, paper, and any combination thereof. In some cases, the solid support comprises one or more linker moieties attached to a surface thereof. In some cases, the one or more linker moieties comprises succinyl, oxalyl, disulfide, UnylinkerTM, or derivatives thereof. In some cases, the solid support is free-flowing in a synthesis column. In some cases, the solid support is fitted to a synthesis vessel.

[0007] In another aspect, a device for biopolymer synthesis is provided, wherein the device comprises a solid support according to any of the preceding.

[0008] In yet another aspect, a method of producing a surface for biopolymer synthesis is provided, the method comprising: a) providing a solid support comprising a plurality of pores, each having a pore diameter of at least about 1 micron; and b) introducing one or more chemical moieties onto the surface of the solid support, wherein the one or more chemical moieties comprise an amino group.

[0009] In yet another aspect, a method for the synthesis of a plurality of oligonucleotides is provided, the method comprising: a) providing a solid support, wherein the solid support comprises a porous membrane; and b) synthesizing the plurality of oligonucleotides on the solid support, wherein the synthesizing comprises sequentially coupling a plurality of nucleosides or derivatives thereof to form the plurality of oligonucleotides, wherein the plurality of oligonucleotides are synthesized with a coupling efficiency of at least 99%. In some cases, the porous membrane comprises a plurality of pores. In some cases, the plurality of pores each comprise a pore diameter of at least about 1 micron. In some cases, the plurality of pores each comprise a pore diameter of at least about 2 microns. In some cases, the plurality of pores each comprise a pore diameter of at least about 5 microns. In some cases, the plurality of pores each comprise a pore diameter of at least about 10 microns. In some cases, the plurality of pores each comprise a pore diameter of greater than about 10 microns. In some cases, the porous membrane comprises polypropylene. In some cases, the porous membrane is selected from the group consisting of: polyethylene, polypropylene/polyethylene blend, cellulose, glass, silicon, gold, silver, graphene, paper, and any combination thereof.

INCORPORATION BY REFERENCE

[0010] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0012] **FIG. 1** depicts example chromatograms of oligonucleotides synthesized according to an embodiment of the methods as described herein.

[0013] FIG. 2 shows multiple views of an assembled housing containing a solid support.

[0014] FIG. 3 shows cross sections and details of an assembled housing containing a solid support.

[0015] FIG. 4 shows another view of a housing for a solid support and the solid support.

[0016] FIG. 5 shows front and back views of the components of a housing for a solid support and the solid support.

DETAILED DESCRIPTION OF THE INVENTION

[0017] Methods

[0018] This disclosure provides methods for synthesizing biopolymers on a solid support. In one aspect of the disclosure, a method of synthesizing biopolymers is provided comprising synthesizing biopolymers directly on a surface of a surface-activated solid support, wherein the surface-activated solid support comprises a plurality of pores, each having a pore diameter greater than about 1 micron.

[0019] The term “surface activated” when used in reference to a solid support means a solid support that has been modified such that external chemical species become adsorbed onto the surface (including the surfaces within pores) of the solid support. Generally, after activation of the surface, the chemical species adsorbed thereon are capable of covalently linking biopolymers to the surface of the solid support. Non-limiting examples of chemical species include amines, hydroxyls, carboxyls, aldehydes, sulfhydryls, a maleimide, and an epoxide. In preferred embodiments, the chemical species are nucleophiles. A solid support may be surface activated by any method. In some cases, surface activation of a solid support is achieved by wet chemistry treatment or plasma treatment.

[0020] In certain aspects, biopolymer synthesis is performed directly on the surface of a surface activated solid support, meaning that the biopolymer becomes covalently attached to the chemical species adsorbed on the surface of the solid support. In certain aspects, biopolymer synthesis directly on the surface of a surface-activated solid support does not involve the use of a scaffold or other structure that distances the biopolymer from the surface of the solid support.

[0021] The term “about,” as used herein, generally refers to a range that is 15% greater than or less than a stated numerical value within the context of the particular usage. For example, “about 10” would include a range from 8.5 to 11.5.

[0022] In some cases, the solid support comprises a plurality of pores. In some cases, the pores have an average diameter of greater than about 1 micron. In some cases, the pores have an average diameter of greater than about 2 microns. In some cases, the pores have an average diameter of greater than about 3 microns. In some cases, the pores have an average diameter of

greater than about 5 microns. In some cases, the pores have an average diameter of greater than about 10 microns. In some cases, the pores have an average diameter of greater than about 50 microns. In some cases, the pores have an average diameter of greater than about 100 microns. In some cases, the pores have an average diameter of greater than about 150 microns. In some cases, the pores have an average diameter of greater than about 200 microns. In some cases, the diameter of the pores is about 1 micron, about 2 microns, about 5 microns, about 10 microns, about 20 microns, about 50 microns, or about 100 microns. In some instances, the pore diameter is between 1 micron and 500 microns. In some instances, the pore diameter is between 2 microns and 300 microns. In some instances, the pore diameter is between 5 microns and 100 microns. In some instances, the pore diameter is between 10 microns and 200 microns. In some instances, the pore diameter is between 10 microns and 100 microns. In some instances, the pore diameter is between 5 microns and 50 microns. In some instances, the pore diameter is between 5 microns and 20 microns. In some instances, the pore diameter is between 5 microns and 15 microns. In some instances, the pore diameter is between 1 micron and 50 microns. In some cases, the pores have an average diameter greater than about 10,000 angstroms, greater than about 20,000 angstroms, greater than about 50,000 angstroms, greater than about 100,000 angstroms, greater than about 500,000 angstroms, greater than about 1,000,000 angstroms, greater than about 1,500,000 angstroms, or greater than about 2,000,000 angstroms. In some instances, the pore diameter is greater than about 10,000 angstroms, greater than about 20,000 angstroms, greater than about 50,000 angstroms, greater than about 100,000 angstroms, greater than about 500,000 angstroms, greater than about 1,000,000 angstroms, greater than about 1,500,000 angstroms, or greater than about 2,000,000 angstroms.

[0023] In some embodiments, the solid support may have a thickness of between about 10 microns and about 10 millimeters. In some cases, the solid support may have a thickness of between about 50 microns and about 1 millimeter. In some cases, the solid support may have a thickness of between about 100 microns and about 1 millimeter. In some cases, the solid support may have a thickness of between about 150 microns and about 1 millimeter. In some cases, the solid support may have a thickness of between about 200 microns and about 1 millimeter. In some cases, the solid support may have a thickness of between about 100 microns and about 800 microns. The solid support may have a thickness of between about 100 microns and about 500 microns. The solid support may have a thickness of between about 100 microns and about 300 microns. The solid support may have a thickness of between about 150 microns and about 250 microns.

[0024] The solid support may comprise any suitable material. For example, the solid support may comprise, without limitation, polypropylene, polyethylene, polypropylene/polyethylene blend, polystyrene, macroporous polystyrene, cellulose, cotton, glass, controlled pore glass (CPG), silicon, gold, silver, graphene, paper, agarose, dextran, or any combination thereof.

[0025] In some cases, the solid support comprises a sheet or a membrane. In some cases, the sheet is a polymer sheet. In some cases, the membrane is a polymer membrane. In some cases, the sheet or membrane is a porous sheet or porous membrane. In some embodiments, the sheet or membrane is a polypropylene sheet or polypropylene membrane. In some cases, the sheet or membrane may have a thickness of between about 10 microns and about 10 millimeters. In some cases, the sheet or membrane may have a thickness of between about 50 microns and about 1 millimeter. The sheet or membrane may have a thickness of between about 100 microns and about 1 millimeter. The sheet or membrane may have a thickness of between about 150 microns and about 1 millimeter. The sheet or membrane may have a thickness of between about 200 microns and about 1 millimeter. The sheet or membrane may have a thickness of between about 100 microns and about 800 microns. The sheet or membrane may have a thickness of between about 100 microns and about 500 microns. The sheet or membrane may have a thickness of between about 100 microns and about 300 microns. The sheet or membrane may have a thickness of between about 150 microns and about 250 microns.

[0026] A surface of a solid support as disclosed herein may be surface activated. An activated surface may include the surfaces within the pores. In some cases, surface activation may involve treating a solid support such that chemical moieties or species become adsorbed thereon. In some cases, a biopolymer may be synthesized directly on the surface of a surface activated solid support, such that the biopolymers become covalently attached to the chemical moieties or species adsorbed on the surface of the solid support. Non-limiting examples of chemical moieties or species that can be deposited or adsorbed on the surface of a solid support include amines, hydroxyls, carboxyls, aldehydes, sulfhydryls, maleimides, or epoxides. In preferred aspects, the chemical moieties or species adsorbed on a surface activated solid support are nucleophiles. In a non-limiting example, a surface activated solid support is aminated such that the surface comprises amine groups deposited or adsorbed on the surface of the solid support. The chemical moieties or species may be capable of reacting with one or more monomers (or derivatives of monomers) from which a biopolymer is synthesized. In a non-limiting example, the chemical moieties or species are capable of reacting with a nucleoside derivative (e.g., a nucleoside phosphoramidite) such that the nucleoside derivative becomes covalently attached to the chemical moiety or species. In some cases, surface activation is achieved via wet chemistry

treatment. Wet chemistry treatment can be performed with one or more chemicals resulting in one or more chemical moieties or species deposited on the surface of the solid support. In some embodiments, surface activation is achieved via plasma treatment. Methods of plasma treatment include, but are not limited to, corona treatment, atmospheric plasma treatment, flame plasma treatment, vacuum plasma treatment, radiofrequency plasma treatment, and chemical plasma treatment. Various gases can be used for the plasma treatment. In one approach, plasma is produced, which is then used to cause chemical changes in the surface of the solid support. Plasma treatment can be conducted with any suitable gas. For example, plasma treatment can be conducted with, without limitation, oxygen, nitrogen, argon, carbon dioxide (CO₂), ammonia or allylamine. In one non-limiting example, a surface of a solid support is plasma treated with allylamine.

[0027] A surface activated solid support as disclosed herein may further comprise one or more linker moieties. Non-limiting examples of linker moieties include any linker group including, but not limited to, succinyl, oxalyl, disulfide, Unylinker™ (developed at Ionis Pharmaceuticals), or derivatives thereof. In some cases, the linker moiety is cleavable (e.g., by chemical cleavage) such that the biopolymer attached thereto may be released from the solid support.

[0028] In some embodiments, the methods do not require or involve the use of a scaffold or other structure that distances the biopolymer from the surface of the solid support. In preferred aspects, biopolymer synthesis does not involve coupling a monomer (or derivative thereof) to a scaffold. In some aspects, a solid support does not comprise a pellicular structure on the surface of the solid support. In some aspects, a solid support does not comprise a monolith on the surface of the solid support. A scaffold may include, without limitation, a surface polymer brush phase, a lightly crosslinked polymer phase, a dendrimer phase, a pellicular phase, a fractal polymer phase, or a grafted polymer.

[0029] Methods of this disclosure can be used for solid-phase synthesis of a wide range of biopolymers. Biopolymers can be broadly characterized into three groups: polynucleotides, polypeptides, and polysaccharides. Biopolymers may include two or more of these groups, such as glycoproteins which contain both polypeptide and polysaccharide moieties. In some cases, the methods provided herein may be used to synthesize polynucleotides. Polynucleotides can be formed of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or non-naturally occurring nucleotides or nucleotide analogues such as morpholinos, 2'-O-methyl-substituted RNA, locked nucleic acid, bridged nucleic acid, peptide nucleic acid, dideoxynucleotides, unnatural base pairs, d5SICS UBP, dNaM UBP, or fluorescent base analogues. In some cases, polynucleotides can be formed of a mixture of both DNA and RNA nucleotides. In some aspects, the methods provided

herein may be used to synthesize polypeptides. In some cases, the methods provided herein may be used to synthesize polypeptides comprising 1-50, 1-100, or 1-150 residues. Polypeptides synthesized by the methods of this disclosure can contain any sequence of amino acids. Both L and/or D amino acids can be used with the methods of this disclosure. Amino acids can be any one or a combination of naturally occurring amino acids or non-naturally occurring amino acids. Non-naturally occurring amino acids include, for example, beta-amino acids, hmo-amino acids, proline derivatives, pyruvic acid derivatives, 3-substituted alanine derivatives, glycine derivatives, ring-substituted phenylalanine, tyrosine derivatives, linear core amino acids, N-methyl amino acids, as well as derivatives and analogues of any other naturally occurring amino acid. In some aspects, the methods provided herein may be used to synthesize polysaccharides. Polysaccharides are polymers of monosaccharides and can be composed of many different monosaccharides. Polysaccharides can be linear or branched. Monosaccharides include, for example, glucose, dextrose, fructose, levulose, galactose, ribose, and xylose.

[0030] Any chemical reaction for synthesizing nucleic acids, peptides, or polysaccharides on a solid-phase support may be used with the methods of this disclosure. Synthesis of the biopolymer can occur on all surfaces of the solid support, including on the surfaces inside the plurality of pores. In preferred embodiments, synthesis of the biopolymer can occur directly on the surface of a surface activated solid support.

[0031] In some aspects, the methods provide for synthesizing a biopolymer by sequentially coupling a plurality of monomers (or monomer derivatives) to form the biopolymer. For example, synthesis of an oligonucleotide may comprise sequentially coupling a plurality of nucleoside derivatives (e.g., nucleoside phosphoramidites) to form the oligonucleotide. In another example, synthesis of a polypeptide may comprise sequentially coupling a plurality of amino acids to form the polypeptide. In yet another example, synthesis of a polysaccharide may comprise sequentially coupling a plurality of monosaccharides to form the polysaccharide.

[0032] In various aspects, the methods of this disclosure can be used to produce biopolymers of a range of lengths. In some embodiments, the methods of this disclosure can be used to make biopolymers of at least 20 monomers, at least 30 monomers, at least 40 monomers, at least 50 monomers, at least 60 monomers, at least 70 monomers, at least 80 monomers, at least 90 monomers, at least 100 monomers, at least 150 monomers, at least 200 monomers, at least 250 monomers, at least 500 monomers, or at least 1000 monomers. In some cases, the methods of this disclosure can be used to make biopolymers of 10 to 1000 monomers, 20 to 500 monomers, 20 to 250 monomers, 20 to 200 monomers, 20 to 100 monomers, 20 to 60 monomers, 20 to 40 monomers, 40 to 500 monomers, 100 to 500 monomers, or 250 to 500 monomers in length.

[0033] In various aspects, the methods of this disclosure can be used to produce polynucleotides of a range of lengths. In some embodiments, the methods of this disclosure can be used to make polynucleotides of at least 5 nucleotides, at least 10 nucleotides, at least 15 nucleotides, at least 20 nucleotides, at least 25 nucleotides, at least 30 nucleotides, at least 35 nucleotides, at least 40 nucleotides, at least 45 nucleotides, at least 50 nucleotides, at least 55 nucleotides, at least 60 nucleotides, at least 65 nucleotides, at least 70 nucleotides, at least 75 nucleotides, at least 80 nucleotides, at least 85 nucleotides, at least 90 nucleotides, at least 95 nucleotides, at least 100 nucleotides, at least 150 nucleotides, at least 200 nucleotides, at least 250 nucleotides, at least 500 nucleotides, or at least 1000 nucleotides. In some cases, the methods of this disclosure can be used to make polynucleotides of 10 to 1000 nucleotides, 20 to 500 nucleotides, 20 to 250 nucleotides, 20 to 200 nucleotides, 20 to 100 nucleotides, 20 to 60 nucleotides, 20 to 40 nucleotides, 40 to 500 nucleotides, 100 to 500 nucleotides, or 250 to 500 nucleotides in length. In any of these cases, nucleotides can be any natural or non-natural nucleotide.

[0034] In various aspects, the methods of this disclosure can be used to produce polypeptides of a range of lengths. In some embodiments, the methods of this disclosure can be used to make polypeptides of at least 5 amino acids, at least 10 amino acids, at least 15 amino acids, at least 20 amino acids, at least 25 amino acids, at least 30 amino acids, at least 35 amino acids, at least 40 amino acids, at least 45 amino acids, at least 50 amino acids, at least 55 amino acids, at least 60 amino acids, at least 65 amino acids, at least 70 amino acids, at least 75 amino acids, at least 80 amino acids, at least 85 amino acids, at least 90 amino acids, at least 95 amino acids, at least 100 amino acids, or greater than 100 amino acids. For example, the methods of this disclosure can be used to make polypeptides of 5 to 100 amino acids, 5 to 50 amino acids, 5 to 35 amino acids, 10 to 100 amino acids, 10 to 50 amino acids, 10 to 35 amino acids, 15 to 100 amino acids, 15 to 50 amino acids, 15 to 35 amino acids, 20 to 100 amino acids, 20 to 50 amino acids, 20 to 35 amino acids, 25 to 100 amino acids, 25 to 50 amino acids, or 25 to 35 amino acids in length. In any of these cases, amino acids can be any natural or non-natural amino acid.

[0035] In various aspects, the methods of this disclosure can be used to produce polysaccharides of a range of lengths. In some embodiments, the methods of this disclosure can be used to make polysaccharides of at least 2 monosaccharides, at least 5 monosaccharides, at least 10 monosaccharides, at least 15 monosaccharides, 20 monosaccharides, at least 25 monosaccharides, at least 30 monosaccharides, at least 35 monosaccharides, at least 40 monosaccharides, at least 50 monosaccharides, at least 60 monosaccharides, at least 70 monosaccharides, at least 80 monosaccharides, at least 90 monosaccharides, at least 100 monosaccharides, or greater than 100 monosaccharides. For example, the methods of this

disclosure can be used to make polysaccharides of 5 to 100 monosaccharides, 5 to 50 monosaccharides, 5 to 35 monosaccharides, 10 to 100 monosaccharides, 10 to 50 monosaccharides, 10 to 35 monosaccharides, 15 to 100 monosaccharides, 15 to 50 monosaccharides, 15 to 35 monosaccharides, 20 to 100 monosaccharides, 20 to 50 monosaccharides, 20 to 35 monosaccharides, 25 to 100 monosaccharides, 25 to 50 monosaccharides, or 25 to 35 monosaccharides in length. In any of these cases, monosaccharaides can be any natural or non-natural monosaccharide.

[0036] In various aspects, the methods of this disclosure can be used to produce biopolymers with a high coupling efficiency. Coupling efficiency may refer to how efficiently a monomer is added to a growing chain of a polymer. For example, if every available monomer on the growing polymer chain reacted with the next monomer to be added to the chain, the coupling efficiency would be 100%. Generally, the methods described herein generate polymers with a high coupling efficiency (e.g., greater than about 98%).

[0037] The methods of this disclosure can produce polynucleotides or oligonucleotides with a coupling efficiency of greater than 98%, for example, of about 98%-99.9%. For example, the methods of this disclosure can produce polynucleotides or oligonucleotides with a coupling efficiency of greater than 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99.0%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9%. In some cases, the methods herein may be used to produce an oligonucleotide with a length of at least 10 nucleotides, 25 nucleotides, 50 nucleotides, 75 nucleotides, 100 nucleotides, 125 nucleotides, 150 nucleotides, 175 nucleotides, or 200 nucleotides, and a coupling efficiency of at least 98.5%-99.9%.

[0038] The methods of this disclosure can produce polypeptides with a coupling efficiency of greater than 98%, for example, of about 98%-99.9%. For example, the methods of this disclosure can produce polypeptides with a coupling efficiency of greater than 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99.0%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9%. In some cases, the methods of this disclosure can produce polysaccharides with a coupling efficiency of greater than 98%, for example, of about 98%-99.9%. For example, the methods of this disclosure can produce polysaccharides with a coupling efficiency of greater than 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99.0%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9%.

[0039] The methods of this disclosure can be used to produce biopolymers with a high degree of purity. In some examples, purity (P) is calculated by $P = X^y$, where X is the coupling efficiency

and y is the number of couplings. In some aspects, the methods of this disclosure can produce polynucleotides or oligonucleotides with a purity of at least 20%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98%. In some cases, The methods of this disclosure can produce polypeptides with a purity of at least 20%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98%. In some cases, The methods of this disclosure can produce polysaccharides with a purity of at least 20%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98%.

[0040] In some aspects, the methods of this disclosure can produce polynucleotides or oligonucleotides with a purity of greater than 20%, greater than 40%, greater than 50%, greater than 60%, greater than 70%, greater than 80%, greater than 90%, greater than 95%, or greater than 98%. In some aspects, the methods of this disclosure can produce polypeptides with a purity of greater than 20%, greater than 40%, greater than 50%, greater than 60%, greater than 70%, greater than 80%, greater than 90%, greater than 95%, or greater than 98%. In some aspects, the methods of this disclosure can produce polysaccharides with a purity of greater than 20%, greater than 40%, greater than 50%, greater than 60%, greater than 70%, greater than 80%, greater than 90%, greater than 95%, or greater than 98%.

[0041] In various aspects, the methods of this disclosure can be used to synthesize polymers of different lengths with high purity. In some cases, the methods of this disclosure can be used to synthesize a biopolymer with a length of at least 2 monomers and a purity of at least 50%. In some cases, the methods of this disclosure can be used to synthesize a biopolymer with a length of at least 20 monomers and a purity of at least 50%. In some cases, the methods of this disclosure can be used to synthesize a biopolymer with a length of at least 30 monomers and a purity of at least 50%. In some cases, the methods of this disclosure can be used to synthesize a biopolymer with a length of at least 40 monomers and a purity of at least 50%. In some cases, the methods of this disclosure can be used to synthesize a biopolymer with a length of at least 60 monomers and a purity of at least 50%. In some cases, the methods of this disclosure can be used to synthesize a biopolymer with a length of at least 80 monomers and a purity of at least 50%. In some cases, the methods of this disclosure can be used to synthesize a biopolymer with a length of at least 250 monomers and a purity of at least 50%. In some cases, the methods of this disclosure can be used to synthesize a biopolymer with a length of at least 2 monomers, at least 20 monomers, 40 monomers, 60 monomers, 80 monomers, 100 monomers, 150 monomers, 200 monomers, or 250 monomers and a purity of at least 60%. In some cases, the methods of this disclosure can be used to synthesize a biopolymer with a length of at least 2 monomers, at least

20 monomers, 40 monomers, 60 monomers, 80 monomers, 100 monomers, 150 monomers, 200 monomers, or 250 monomers and a purity of at least 70%. In some cases, the methods of this disclosure can be used to synthesize a biopolymer with a length of at least 2 monomers, at least 20 monomers, 40 monomers, 60 monomers, 80 monomers, 100 monomers, 150 monomers, 200 monomers, or 250 monomers and a purity of at least 80%. In some cases, the methods of this disclosure can be used to synthesize a biopolymer with a length of at least 2 monomers, at least 20 monomers, 40 monomers, 60 monomers, 80 monomers, 100 monomers, 150 monomers, 200 monomers, or 250 monomers and a purity of at least 90%. In some cases, the methods of this disclosure can be used to synthesize a biopolymer with a length of at least 2 monomers, at least 20 monomers, 40 monomers, 60 monomers, 80 monomers, 100 monomers, 150 monomers, 200 monomers, or 250 monomers and a purity of at least 95%. In some cases, the methods of the disclosure can be used to synthesize a biopolymer with a length of about 1 to about 20 monomers, about 21 to about 40 monomers, about 41 to about 60 monomers, about 61 to about 100 monomers, about 100 to about 120 monomers, about 120 to about 200 monomers, or at least 200 monomers with a purity of at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or greater than 99%.

[0042] In various aspects, the methods involve synthesizing polynucleotides on a solid support of the disclosure. In some cases, the solid support comprises a plurality of pores, each having a pore diameter of greater than about 1 micron. In some cases, the solid support comprises a thickness from about 50 microns to about 1000 microns. In some cases, the methods involve synthesizing polynucleotides directly on the surface of a surface activated solid support.

[0043] Any method of synthesizing oligonucleotides or polynucleotides may be implemented with the methods described herein. Without being bound by theory, polynucleotide synthesis, such as oligonucleotide synthesis, is the chemical synthesis of fragments of nucleic acids with defined chemical structure or sequence. Synthesized polynucleotides can be DNA or RNA. Synthesis can occur either 5' to 3' or 3' to 5'. Synthesis can occur by liquid phase synthesis or solid phase synthesis. During polynucleotide synthesis, nucleotides can be incorporated into the polynucleotide. Nucleotides can be natural nucleotides, unnatural nucleotides, ribonucleotides, deoxyribonucleotides, nucleotide analogs, nucleotide derivatives, labeled or tagged nucleotides, modified nucleotides, or any combination thereof. Modifications to the phosphodiester backbone can also be incorporated during polynucleotide synthesis. Synthesis can be implemented by solid-phase synthesis. Solid-phase synthesis can comprise using a phosphoramidite method and phosphoramidite building blocks or monomers such as those derived from protected 2'deoxy nucleosides, ribonucleosides, or chemically modified or artificial

nucleosides. In some examples, to obtain a desired polynucleotide, the selected monomers are sequentially coupled to the growing polynucleotide chain in the order required by the desired sequence. Upon completion of the polynucleotide synthesis, the polynucleotide can be released from the solid phase to solution, optionally deprotected, and collected. Subsequent purification or sequence verification can then be performed if desired.

[0044] In some embodiments, protecting groups are added to nucleosides in order to prevent undesired side reactions during polynucleotide synthesis. Suitable protecting groups include, though are not limited to, acid-labile groups, base-labile groups, 4,4'-dimethoxytrityl (DMT), benzoyl group (Bz), isobutyryl group, acetyl group, phenoxyacetyl group (PAC), 4-isopropylphenoxyacetyl (Pr-PAC), dimethylformamidino (dmf), 2-cyanoethyl group, t-butyldimethylsilyl (TBDMS), tri-iso-propylsilyloxymethyl (TOM), or Diisopropylamino (Pr₂N). In some embodiments, polynucleotide synthesis methods comprise a de-blocking step. In some cases, a protection group, such as DMT, is removed, for example, DMT can be removed with trichloroacetic acid (TCA) or dichloroacetic acid (DCA). In some cases, deblocking results in a free 5'-terminal hydroxyl group. In some cases, de-blocking results in a free 3'-terminal phosphoryl group. In some cases, following de-blocking, a wash step is performed to remove the blocking groups, protecting groups, and excess reagents.

[0045] In some embodiments, polynucleotide synthesis methods may further comprise a coupling step. In some cases, monomers, such as phosphoramidites, are activated to allow coupling to a free terminal group of a polynucleotide. In some cases, activated monomers are in molar excess compared to the polynucleotide to which said monomer is to be coupled. The length of time for a coupling step can depend on the monomers, for example, sterically hindered monomers may require a longer coupling time (e.g., 5-15 minutes), while non-sterically hindered monomers may require short coupling times (e.g., 20 seconds). In some cases, following a coupling step, unbound reagents and by-products are removed by washing.

[0046] In some embodiments, polynucleotide synthesis methods may further comprise a capping step. In some cases, a capping step permanently blocks unreacted free terminal ends that did not undergo coupling in a previous coupling step in order to prevent polynucleotide synthesis with an internally missing nucleotide. In some cases, capping removes certain problematic modifications; for example, O⁶ modifications can be removed prior to oxidation which would result in truncated polynucleotides and reduced full-length yields during a final deprotection step.

[0047] In some embodiments, polynucleotide synthesis methods further comprise an oxidation step. In some cases, oxidation converts a tricoordinated phosphite trimer linkage to a

tetracoordinated phosphate trimester linkage. In some cases, oxidation is performed to stabilize a synthesized polynucleotide. In some cases, oxidation is performed in the presence of iodine, water, and a weak base such as pyridine, lutidine, or collidine. In some cases, oxidation is substituted with a sulfurization step. In cases where a sulfurization step replaces an oxidation step, oligonucleotide phosphorothioates can be obtained. In some cases, a sulfurization step is carried out prior to a capping step.

[0048] In various aspects, the methods involve synthesizing polypeptides on a solid support of the disclosure. In some cases, the solid support comprises a plurality of pores, each having a pore diameter of greater than about 1 micron. In some cases, the solid support comprises a thickness from about 50 microns to about 1000 microns. In some cases, the methods involve synthesizing polypeptides directly on the surface of a surface activated solid support.

[0049] Any method of synthesizing polypeptides may be used with the methods described herein. Without being bound by theory, polypeptide synthesis can occur by coupling a carboxyl group (-terminus) of one amino acid to the amino group (N-terminus) of another amino group. Peptide synthesis can occur from the C-terminal end of the peptide or from the N-terminal end, thereby growing a polypeptide from either the C-terminal end or the N-terminal end, respectively. During polypeptide synthesis, amino acids can be incorporated into the polypeptide. Amino acids can be natural amino acids, unnatural amino acids, D-amino acids, L-amino acids, amino acid analogs, amino acid derivatives, or any combination thereof. Modifications to the peptide backbone can also be incorporated during polypeptide synthesis. An amino group or peptide can be covalently attached to a solid support. Attachment can occur at the N-terminus. Attachment can occur at the C-terminus. The free end can be protected by a protection group, such as Fmoc or Boc. In some cases, polypeptide synthesis occurs by repeated cycles of deprotection, wash, coupling, and wash.

[0050] A protecting group can be a N-terminal protecting group. N-terminal protecting groups include, but are not limited to, tert-butyloxycarbonyl (t-Boc or Boc), and 9-fluorenylmethyloxycarbonyl (Fmoc). N-terminal protecting groups can comprise a carbamate group which readily releases carbon dioxide for an irreversible decoupling. A protecting group can be a side-chain protecting group. Non-limiting examples of a side chain protecting group include benzyloxy-carbonyl (Z) group, and other carbamate-based groups. Other examples of protecting groups include, but are not limited to, allyloxycarbonyl (alloc) groups, and lithographic groups. Protecting groups can be removed during a deprotection step. The specific deprotection solvent depends on the chosen protection group. Following deprotection, a wash step can occur to remove released protection groups and excess deprotection solvents. Following deprotection

and washing, a coupling reaction can occur. For coupling an amino acid, the carboxyl group may be activated. In some cases, the amino group is activated. Activating groups can include, but are not limited to carbodiimides, triazolols, FDPP, PFPOH, and BOP-Cl. Carbodiimide activating groups can include, but are not limited to, dicyclohexylcarbodiimide (DCC), and diisopropylcarbodiimide (DIC). Triazole activating groups can include, but are not limited to, 1-hydroxy-benzotriazole (HOBt) and 1-hydroxy-7-aza-benzotriazole (HOAt).

[0051] Solid Supports

[0052] The disclosure herein further provides solid supports. In various aspects, the solid supports may be used to perform the methods disclosed herein. In various aspects, the solid supports may be used with a device of the disclosure.

[0053] In some cases, the solid support comprises a plurality of pores. In some cases, the pores have an average diameter of greater than about 1 micron. In some cases, the pores have an average diameter of greater than about 2 microns. In some cases, the pores have an average diameter of greater than about 3 microns. In some cases, the pores have an average diameter of greater than about 5 microns. In some cases, the pores have an average diameter of greater than about 10 microns. In some cases, the pores have an average diameter of greater than about 50 microns. In some cases, the pores have an average diameter of greater than about 100 microns. In some cases, the pores have an average diameter of greater than about 150 microns. In some cases, the pores have an average diameter of greater than about 200 microns. In some cases, the diameter of the pores is about 1 micron, about 2 microns, about 5 microns, about 10 microns, about 20 microns, about 50 microns, or about 100 microns. In some instances, the pore diameter is between 1 micron and 500 microns. In some instances, the pore diameter is between 2 microns and 300 microns. In some instances, the pore diameter is between 5 microns and 100 microns. In some instances, the pore diameter is between 10 microns and 200 microns. In some instances, the pore diameter is between 10 microns and 100 microns. In some instances, the pore diameter is between 5 microns and 50 microns. In some instances, the pore diameter is between 5 microns and 20 microns. In some instances, the pore diameter is between 5 microns and 15 microns. In some instances, the pore diameter is between 1 micron and 50 microns. In some cases, the pores have an average diameter greater than about 10,000 angstroms, greater than about 20,000 angstroms, greater than about 50,000 angstroms, greater than about 100,000 angstroms, greater than about 500,000 angstroms, greater than about 1,000,000 angstroms, greater than about 1,500,000 angstroms, or greater than about 2,000,000 angstroms. In some instances, the pore diameter is greater than about 10,000 angstroms, greater than about 20,000 angstroms, greater than about 50,000 angstroms, greater than about 100,000 angstroms, greater

than about 500,000 angstroms, greater than about 1,000,000 angstroms, greater than about 1,500,000 angstroms, or greater than about 2,000,000 angstroms.

[0054] In some embodiments, the solid support may have a thickness of between about 10 microns and about 10 millimeters. In some cases, the solid support may have a thickness of between about 50 microns and about 1 millimeter. In some cases, the solid support may have a thickness of between about 100 microns and about 1 millimeter. In some cases, the solid support may have a thickness of between about 150 microns and about 1 millimeter. In some cases, the solid support may have a thickness of between about 200 microns and about 1 millimeter. In some cases, the solid support may have a thickness of between about 100 microns and about 800 microns. The solid support may have a thickness of between about 100 microns and about 500 microns. The solid support may have a thickness of between about 100 microns and about 300 microns. The solid support may have a thickness of between about 150 microns and about 250 microns.

[0055] The solid support may comprise any suitable material. For example, the solid support may comprise, without limitation, polypropylene, polyethylene, polypropylene/polyethylene blend, polystyrene, macroporous polystyrene, cellulose, cotton, glass, controlled pore glass (CPG), silicon, gold, silver, graphene, paper, agarose, dextran, or any combination thereof.

[0056] In some cases, the solid support comprises a sheet or a membrane. In some cases, the sheet is a polymer sheet. In some cases, the membrane is a polymer membrane. In some cases, the sheet or membrane is a porous sheet or porous membrane. In some embodiments, the sheet or membrane is a polypropylene sheet or polypropylene membrane. In some cases, the sheet or membrane may have a thickness of between about 10 microns and about 10 millimeters. In some cases, the sheet or membrane may have a thickness of between about 50 microns and about 1 millimeter. The sheet or membrane may have a thickness of between about 100 microns and about 1 millimeter. The sheet or membrane may have a thickness of between about 150 microns and about 1 millimeter. The sheet or membrane may have a thickness of between about 200 microns and about 1 millimeter. The sheet or membrane may have a thickness of between about 100 microns and about 800 microns. The sheet or membrane may have a thickness of between about 100 microns and about 500 microns. The sheet or membrane may have a thickness of between about 100 microns and about 300 microns. The sheet or membrane may have a thickness of between about 150 microns and about 250 microns.

[0057] A surface of a solid support as disclosed herein may be surface activated. An activated surface may include the surfaces within the pores. In some cases, surface activation may involve treating a solid support such that chemical moieties or species become adsorbed thereon. In

some cases, a biopolymer may be synthesized directly on the surface of a surface activated solid support, such that the biopolymers become covalently attached to the chemical moieties or species absorbed on the surface of the solid support. Non-limiting examples of chemical moieties or species that can be deposited or adsorbed on the surface of a solid support include amines, hydroxyls, carboxyls, aldehydes, sulfhydryls, maleimides, or epoxides. In preferred aspects, the chemical moieties or species adsorbed on a surface activated solid support are nucleophiles. In a non-limiting example, a surface activated solid support is aminated such that the surface comprises amine groups deposited or adsorbed on the surface of the solid support. The chemical moieties or species may be capable of reacting with one or more monomers (or derivatives of monomers) from which a biopolymer is synthesized. In a non-limiting example, the chemical moieties or species are capable of reacting with a nucleoside derivative (e.g., a nucleoside phosphoramidite) such that the nucleoside derivative becomes covalently attached to the chemical moiety or species. In some cases, surface activation is achieved via wet chemistry treatment. Wet chemistry treatment can be performed with one or more chemicals resulting in one or more chemical moieties or species deposited on the surface of the solid support. In some embodiments, surface activation is achieved via plasma treatment. Methods of plasma treatment include, but are not limited to, corona treatment, atmospheric plasma treatment, flame plasma treatment, vacuum plasma treatment, radiofrequency plasma treatment, and chemical plasma treatment. Various gases can be used for the plasma treatment. In one approach, plasma is produced, which is then used to cause chemical changes in the surface of the solid support. Plasma treatment can be conducted with any suitable gas. For example, plasma treatment can be conducted with, without limitation, oxygen, nitrogen, argon, carbon dioxide (CO₂), ammonia or allylamine. In one non-limiting example, a surface of a solid support is plasma treated with allylamine.

[0058] A surface activated solid support as disclosed herein may further comprise one or more linker moieties. Non-limiting examples of linker moieties include any linker group including, but not limited to, succinyl, oxalyl, disulfide, Unylinker™ (developed at Isis Pharmaceuticals), or derivatives thereof. In some cases, the linker moiety is cleavable (e.g., by chemical cleavage) such that the biopolymer attached thereto may be released from the solid support.

[0059] In some embodiments, the methods do not require or involve the use of a scaffold or other structure that distances the biopolymer from the surface of the solid support. In preferred aspects, biopolymer synthesis does not involve coupling a monomer (or derivative thereof) to a scaffold. In some aspects, a solid support does not comprise a pellicular structure on the surface of the solid support. In some aspects, a solid support does not comprise a monolith on the

surface of the solid support. A scaffold may include, without limitation, a surface polymer brush phase, a lightly crosslinked polymer phase, a dendrimer phase, a pellicular phase, a fractal polymer phase, or a grafted polymer.

[0060] Devices

[0061] Further provided herein are devices. In some cases, the devices may be used to perform the methods provided herein. In some cases, the devices may comprise a solid support as described herein. In some cases, the device is a synthesis vessel. In some cases, a solid support as disclosed herein may be contained in the synthesis vessel. In some cases, a solid support may be free-floating in the synthesis vessel, or may be fitted to the synthesis vessel. In some cases, a solid support may be fitted to the synthesis vessel by sonic welding. In some cases, a solid support may be a porous membrane and fitted to a synthesis vessel in such a way that reagents can be passed through the membrane. In some cases, a synthesis vessel may be part of an array of many such vessels. For example, the synthesis vessel may be contained in a plate containing an array of vessels such as, e.g., a 12-by-8 array of vessels, or a 16-by-16 array of vessels.

[0062] A synthesis vessel or vessels as disclosed herein may also be in the form of a device for solid-phase polymer synthesis. Such a device may comprise a solid support of this disclosure. In some embodiments, the solid support of the device comprises a plurality of pores, each of the plurality of pores having a pore diameter of at least 1 micron, at least 5 microns, at least 10 microns, at least 40 microns or at least 100 microns. In some cases, the solid support has a thickness according to the disclosure, such as from about 200 microns to about 1000 microns. In some cases, the device contains a surface activated solid support as described herein. In a particular example, the solid support may be a plasma-treated porous polypropylene membrane with a pore size of about 20 microns. The device may further comprise a plurality of synthesis vessels each containing a solid support.

[0063] A device as disclosed herein may have a geometric shape. The geometric shape can be determined by an array of synthesis vessels. For example, an array of 12-by-8 vessels can have a rectangular shape, and an array of 16-by-16 vessels can have a square shape. A device as disclosed herein can have any depth. Depth can be the depth of vessels within an array of vessels. Depth can be determined based on the parameters of a downstream machine within which the device will be placed.

[0064] A device as disclosed herein can be a single component. Alternatively, a device can be comprised of two or more components. In some examples, a device is comprised of two components, for example, a top piece and a bottom piece. In some examples, a device is

comprised of three components, for example, a top piece, a solid support (e.g., a membrane as described herein), and a bottom piece.

[0065] In cases where a device as disclosed herein comprises a top piece and a bottom piece, the top piece and the bottom piece can be configured such that they fit together. In some cases, a top piece and a bottom piece fit together when the device is in an assembled configuration. Fitting together can comprise locking, snapping, or other mechanical mechanisms that maintain the interaction between the top and the bottom piece when they are in an assembled configuration. In some cases, there is no locking mechanism and the top piece is placed on top of the bottom piece, or the top piece is placed within the bottom piece.

[0066] In cases where a device as disclosed herein comprises a top piece and a bottom piece, the top piece and the bottom piece can be configured such that a pore of the top piece aligns with a pore of the bottom piece. In preferred embodiments, when the device is in an assembled configuration, the majority or all of the pores of the top piece may align with a corresponding pore of the bottom piece. The device can have an alignment mechanism that ensures proper alignment of the pores of the top piece with the pores of the bottom piece. Such an alignment mechanism can comprise an alignment feature on the top piece that interacts with a corresponding alignment feature on the bottom piece. When the device is in an assembled configuration, the alignment feature of the top piece can come into contact with or engage with the alignment feature of the bottom piece such that the majority or all of the pores of the top piece are aligned with a corresponding pore of the bottom piece. A corresponding pore can be a pore that has the same location within an array of pores. Aligned can mean that the outer circumference of the top pore is perfectly or nearly perfectly aligned with the outer circumference of the corresponding pore of the bottom piece.

[0067] In some examples, a top piece of a device as disclosed herein comprises one or more edges. In preferred embodiments, the top piece comprises four edges. One or more of the four edges can extend below an array of pores. In such cases, the height of the edges is greater than the height or depth of the pores of the top piece. In some examples, the top piece comprises a protrusion from an inner surface of one or more of the edges of the top piece. Such protrusions can be vertical ridges. One or more such vertical ridges can be comprised on the inner surface of one or more edges of the top piece. In some examples, the bottom piece comprises an indentation in one or more of the edges of the bottom piece. Such indentations can extend the entire height of the edge. One or more such indentations can be comprised along the outside of one or more edges of the bottom piece.

[0068] In some examples, the ridges of the top pieces fit into the indentations of the bottom piece such that they restrain and guide the top and bottom pieces into a desired orientation when the device is in an assembled configuration. In some examples, the ridges of the top piece and the indentations of the bottom piece function as an alignment mechanism to ensure the array of pores of the top piece align with the array of pores of the bottom piece.

[0069] In some examples, the top piece comprises a protrusion from an inner surface of one or more of the edges of the top piece. Such a protrusion can extend along the entire length of the edge, or only a portion of the length of the edge. In some cases, multiple protrusions are comprised on the inner surface of an edge. In any of these cases, the one or more protrusions can form a ledge.

[0070] In some examples, a bottom piece of a device as disclosed herein comprises one or more edges. In preferred embodiments, the bottom piece comprises four edges. One or more of the four edges can extend beyond an array of pores, such that the one or more edges forms a lip on the bottom piece. In some examples, the lip extends along the entire length of an edge, or in some cases, a lip can extend along only a portion of the length of the edge. In some cases, multiple lips are comprised on an edge.

[0071] In some examples, when a device as disclosed herein is in an assembled configuration, one or more lips of the bottom piece can sit on top of one or more protrusions or ledges of the top piece. In some cases, the interaction between one or more protrusions of the top piece and one or more lips of the bottom piece functions as a locking mechanism that maintains the interaction between the top piece and the bottom piece. In some cases, the interaction between one or more protrusions of the top piece and one or more lips of the bottom piece functions as an alignment mechanism that maintains the alignment between an array of pores of the top piece and an array of pores of the bottom piece.

[0072] It should be appreciated that a top piece and a bottom piece as disclosed herein may be designed such that the top piece comprises one or more lips as disclosed herein and the bottom piece comprises one or more protrusions or ledges as disclosed herein, thereby reversing the direction of the locking mechanism or the alignment mechanism, but still resulting in the same locking function or alignment function.

[0073] In cases where a device as disclosed herein comprises a membrane as disclosed herein, such as a porous membrane, the porous membrane can be housed between a top piece and a bottom piece. In such cases, a membrane can be held in place by the top and the bottom piece when the device is in an assembled configuration.

[0074] An example of such a device as disclosed herein is shown in **FIGS. 2 - 5**. **FIG. 2** shows multiple views of an assembled synthesis device comprising multiple reaction vessels. In this example, the device is a three-part assembly including a top part, the membrane, and a bottom. The top has ribs on the underside which match notches on the bottom, so that it may be assembled in only one way. The assembled chip may also be keyed with a notch in the A1 position so that a receiving fixture may be designed to hold it such that the device is always oriented correctly. In this example, the device is 41.75 mm x 41.57 mm square. Well A1 is offset from both the top and left edge by 3.175 mm. Well pitch is 2.25 mm (vertically and horizontally). The opening of the wells at the top is 1.35 mm x 1.35 mm. The diameter of the well where it meets the membrane is 1.25 mm.

[0075] **FIG. 3** shows a cross section of an example synthesis device showing the location of the solid support. In this example, the skirt of the assembled device sits just above the bottom of drip directors, so that the devices can be stacked on top of one another. The drip directors may drop slightly into the top hole of a plate that they are set on top of. In some aspects, the device may be designed to be assembled via sonic welding. In such cases, the energy directors may be designed according to Branson's ultrasonic assembly guidelines. In some cases, the energy directors may be thin and may melt with about the same acoustic energy as the membrane melts, so that they co-melt and create a uniform seal. In other examples, a device of the disclosure may be produced by other methods, including, but not limited to, laser welding and heat. In some aspects, the device may be square and smaller than a standard titer plate format such that the acoustic horn that is used to produce the acoustic energy can be kept small and may maintain greater uniformity across the surface. In some cases, this may allow for uniform sealing across the plate.

[0076] **FIG. 4** shows the components of an example device, comprising a top piece, a solid support membrane and a bottom piece, the top and bottom pieces bonded together around the solid support membrane. **FIG. 5** shows a front and back view of the components of an example device as they would be stacked together. In this exploded view, the top section has square holes and the bottom section has round holes. This allows the top (where reagents are delivered) to have closer to a maximum surface area to minimize splashing of reagent while maintaining the amount of material between wells that keeps the device structurally stable. In some cases, tapering from square to round may be optional.

EXAMPLES

[0077] The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion. The present examples, along with the methods described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses which are encompassed within the spirit of the invention as defined by the scope of the claims will occur to those skilled in the art.

Example 1. Oligonucleotide synthesis

[0078] Polypropylene membrane with a thickness of 200 microns and pores of about 10 microns was plasma treated with allylamine. An oligonucleotide 75 nucleotides in length was produced in parallel on the treated membrane and on a standard controlled pore size glass support.

[0079] Oligonucleotide purity was obtained by HPLC analysis. Samples were run DMT-off on a reverse phase column using a 15 minute gradient optimized for separation of n-1 species of long oligonucleotides of about 50 nucleotides in length. The resulting chromatograms are shown in **FIG. 1**.

Example 2. Oligonucleotide synthesis

[0080] Polypropylene membrane with a thickness of 200 microns and pores of about 10 microns was plasma treated with allylamine. Treated membrane was cut into pieces of 5-10 mm² and placed in a conventional synthesizer column. Oligonucleotides of 20, 40, 60 and 80 nucleotides in length were produced in parallel with oligonucleotides of the same length on standard controlled pore size glass supports.

[0081] Oligonucleotide purity was obtained by HPLC analysis. Samples were run DMT-off on a reverse phase column using a 15 minute gradient optimized for separation of n-1 species of long oligonucleotides of about 50 nucleotides in length. Data is shown in **Table 1**.

Table 1. Oligonucleotide synthesis

Substrate	Oligo length (nt)	Purity (%)	Coupling Efficiency (%)
Controlled Pore Glass (CPG)	20	86	99.2
	40	76	99.3
	60	62	99.2
	80	57	99.3
Small Pore Membrane (0.45 micron pore size)	20	69	98.2
	40	51	98.3
	60	34	98.2
	80	26	98.3
Large Pore Membrane (10 micron pore size)	25	91	99.6
	50	78	99.5
	75	68	99.5
	100	61	99.5

[0082] Purity was estimated by calculating the area under the peak of the major product (with the assumption the major peak was full length product) and compared to the total area under the chromatogram. The purity could be used to back calculate the coupling efficiency, which is an industry standard for evaluating the degree of success of DNA synthesis. Coupling efficiency was calculated by the following calculation: $P = X^y$, where P is the final purity, X is the coupling efficiency, y is the number of coupling steps.

Example 3. Custom fitted support membrane

[0083] To investigate the hypothesis that porous solid support material is restricting flow in an open column design and thus decreasing synthesis efficacy a porous sheet is custom fitted to a synthesis vessel. A vessel as shown in **Figures 2-5** is used such that reagents can flow through the membrane. Using this vessel and solid support configuration the biopolymer synthesis shows comparable or higher purity and coupling efficacy when compared with controlled pore size glass supports.

Example 4. Synthesis conditions on the membrane

[0084] Oligonucleotide synthesis was performed using the phosphoramidite approach on a custom oligonucleotide synthesizer and substrate. The synthesizer dispensed reagents onto the membrane, which was secured into the custom substrate, the “S-chip”. Example synthesis cycles are as follows, where “n” is the number of bases being synthesized on top of the universal or first base support. Ranges of times and volumes represent successful combinations used on the membrane.

[0085] Non-cycled preparation steps:

- 1) Wash, 2-8 μ L
- 2) Drain, 5-30 seconds

[0086] Cycle “n” times:

- 3) Deblock, 1-8 μ L, hold 5-20 seconds
- 4) Wash, 2-8 μ L
- 5) Drain, 5-30 seconds
- 6) Wash, 2-8 μ L
- 7) Drain 5-30 seconds
- 8) Couple, phosphoramidite 1-4 μ L and activator 1-4 μ L, hold 20-60 seconds
- 9) Drain 15-30 seconds
- 10) Oxidize, 2-5 μ L, 5-60 seconds
- 11) Drain, 5-30 seconds
- 12) Capping, CapA and CapB solutions, 1-4 μ L each, hold 5-60 seconds)
- 13) Drain 5-30 seconds

[0087] Non-cycled final steps:

- 14) Deblock, 1-8 μ L, hold 5-20 seconds
- 15) Drain, 5-30 seconds
- 16) Deblock, 1-8 μ L, hold 5-20 seconds
- 17) Drain, 5-30 seconds
- 18) Wash, 2-8 μ L
- 19) Drain, 5-30 seconds
- 20) Wash, 8 μ L
- 21) Drain, 5-30 seconds
- 22) Wash, 2-8 μ L
- 23) Drain, 5-30 seconds

[0088] Post synthesis treatment: Cleavage and deprotection was performed in liquid phase using ammonium hydroxide (28-30%) in water, or ammonia (4M or 7N) in methanol. Other alkali treatments commonly used including, but not limited to, ammonium hydroxide methylamine (AMA), butylamine, and propylamine, should be compatible.

[0089] Reagent formulations were used as follows: Wash cycles were typically performed in synthesis grade acetonitrile with 10 ppm or less water content. Alternatively, acetone, methyl acetate, and ethyl acetate were successfully used. All drain steps were performed in high purity, low moisture argon, nitrogen, or helium gasses. Activator used in the coupling steps were either

0.25M 5-ethylthio-1H-Tetrazole, however, 0.4M 1-H-Tetrazole, 0.25M 4,5-Dicyanoimidazole, 0.25M 5-Benzylthio-1H-Tetrazole, Activator 42, and other activators may be substituted. Phosphoramidites were purchased as a dry powder and resuspended in dry acetonitrile to a concentration of 0.03-0.1M using anhydrous acetonitrile. Several nucleobase protection groups were used in the validation experiments including Phosphoramidite A with Benzoyl or phenoxyacetyl nucleobase protection, Phosphoramidite G with isobutyryl, dimethylformamidine, or phenoxyacetyl nucleobase protection, Phosphoramidite C with benzoyl or acetyl nucleobase protection, and Phosphoramidite T (unprotected). Other standard phosphoramidites should also be compatible. Oxidizing solutions containing 0.02M-0.05M Iodine in THF/Pyridine/Water were used for all synthesis experiments interchangeably, and several other standard formulations have been validated in phosphoramidite chemistry and are compatible with the synthesis chemistry on membranes. The preferred CapA formulation was Acetic Anhydride/Pyridine/Tetrahydrofuran (10%, 10%, 80%) and CapB was 16% 1-Methylimidazole in Tetrahydrofuran, however there other capping solutions such as 6.5% Dimethylaminopyridine in Tetrahydrofuran and capping phosphoramidites which may be compatible. Deblocking was performed using either 2%-5% Trichloroacetic Acid in Dichloromethane or Toluene, or 3%-10% Dichloroacetic Acid in Dichloromethane or Toluene.

Example 5. Preparation of the Membrane for Synthesis

[0090] Membranes were prepared for synthesis by creating radicals on the membrane by introducing a nitrogen group into a radio frequency plasma. Nitrogen groups were introduced into the plasma as either ammonia or allylamine. This produced amine functionalities on the membrane which are reactive to certain reagents.

[0091] The first base on the 3' end of the oligonucleotide, either an A, G, C, or T, were attached to the membrane by dispensing a phosphoramidite, herein referred to as "firstbase" amidites. There is a unique firstbase amidite for each of the four natural DNA bases: Thymidine-succinyl hexamide CED phosphoramidite, Adenosine-succinyl hexamide CED phosphoramidite, Guanosine-succinyl hexamide CED phosphoramidite, and Cytidine-succinyl hexamide CED phosphoramidite. In another embodiment, a universal linker or universal phosphoramidite can be used instead of the firstbase amidites, and may provide added convenience to the end user since they do not require any well-specific addition to the substrate to produce the desired 3' base. Coupling of this linker was either carried out in a well-specific manner on the synthesizer by dispensing 1-4 μ L of the firstbase amidite, diluted to 0.03M-0.1M, and 1-4 μ L of activator, and reacting on the synthesizer for up to 15 minutes, followed by 1-3 washes with acetonitrile.

Alternatively, the entire membrane sheet could be coupled to a single firstbase amidite by submerging in a reaction vessel with activator if well-specific sequence differences aren't required. This would also be a useful approach if a universal linker were substituted for the firstbase amidite(s). Once the firstbase amidite was attached to the membrane, they were either used immediately on the synthesizer or stored in a desiccator for later use.

[0092] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for the synthesis of biopolymers, the method comprising synthesizing the biopolymers directly on a surface of a surface-activated solid support, wherein the surface-activated solid support comprises a plurality of pores, each of the plurality of pores having a pore diameter greater than about 1 micron.
2. The method of claim 1, wherein the solid support does not comprise a scaffold or other structure on the surface of the solid support that distances the biopolymer from the surface of the solid support.
3. The method of claim 2, wherein the scaffold is a surface polymer brush phase, a lightly crosslinked polymer phase, a dendrimer phase, a pellicular phase, a fractal polymer phase, and a grafted polymer.
4. The method of any one of claims 1-3, wherein the solid support comprises a porous sheet or a porous membrane.
5. The method of claim 4, wherein the porous sheet or porous membrane comprises polypropylene.
6. The method of claim 4 or 5, wherein the porous sheet or porous membrane is selected from the group consisting of: polyethylene, polypropylene/polyethylene blend, cellulose, cotton, glass, silicon, gold, silver, graphene, paper, and any combination thereof.
7. The method of any one of claims 1-6, wherein the solid support comprises agarose or dextran.
8. The method of any one of claims 1-7, wherein the pore diameter is about 2 microns.
9. The method of any one of claims 1-7, wherein the pore diameter is about 5 microns.
10. The method of any one of claims 1-7, wherein the pore diameter is about 10 microns.
11. The method of any one of claims 1-7, wherein the pore diameter is greater than about 10 microns.
12. The method of any one of claims 1-7, wherein the pore diameter is from about 10-200 microns.
13. The method of any one of claims 1-12, wherein the solid support has a thickness from about 50 microns to about 1000 microns.
14. The method of any one of claims 1-13, wherein the solid support is about 200 microns in thickness.

15. The method of any one of claims 1-14, further comprising activating the surface of the solid support with one or more chemical moieties prior to the synthesizing.

16. The method of claim 15, wherein the activating comprises plasma-treating the surface of the solid support.

17. The method of claim 15 or 16, wherein the one or more chemical moieties is selected from the group consisting of: amine groups, hydroxyl groups, carboxyl groups, aldehyde groups, sulfhydryl groups, maleimide groups, and epoxide groups.

18. The method of claim 15 or 16, wherein the activating comprises activating the surface of the solid support with allylamine.

19. The method of any one of claims 1-18, wherein the solid support comprises one or more linker moieties.

20. The method of claim 19, wherein the one or more linker moieties comprises succinyl, oxalyl, disulfide, UnylinkerTM, or derivatives thereof.

21. The method of any one of claims 15-20, wherein the synthesizing comprises: (i) covalently attaching a first monomer or derivative thereof to the one or more chemical moieties or the one or more linker moieties.

22. The method of claim 21, wherein the synthesizing further comprises: (ii) coupling one or more monomers or derivatives thereof to the first monomer or derivative thereof, thereby generating the biopolymer.

23. The method of any one of claims 1-22, wherein the biopolymers comprise oligonucleotides.

24. The method of claim 23, wherein the oligonucleotides comprise DNA, RNA, modified DNA, or modified RNA.

25. The method of any one of claims 1-22, wherein the biopolymers comprise polypeptides.

26. The method of any one of claims 1-22, wherein the biopolymers comprise polysaccharides.

27. The method of any one of claims 1-26, wherein a plurality of the biopolymers are at least 20 monomers in length.

28. The method of any one of claims 1-27, wherein a plurality of the biopolymers are at least 40 monomers in length.

29. The method of any one of claims 1-28, wherein a plurality of the biopolymers are at least 60 monomers in length.

30. The method of any one of claims 1-29, wherein a plurality of the biopolymers are at least 80 monomers in length.

31. The method of any one of claims 1-30, wherein a plurality of the biopolymers are up to 250 monomers in length.

32. The method of any one of claims 1-31, wherein the synthesizing further comprises synthesizing the biopolymers with a coupling efficiency of greater than at least 98.5%.

33. The method of any one of claims 1-32, wherein the synthesizing further comprises synthesizing the biopolymers with a length of at least 20 monomers with a purity of at least 50%.

34. The method of any one of claims 1-33, wherein the synthesizing further comprises synthesizing the biopolymers with a length of at least 40 monomers with a purity of at least 50%.

35. The method of any one of claims 1-34, wherein the synthesizing further comprises synthesizing the biopolymers with a length of at least 60 monomers with a purity of at least 50%.

36. The method of any one of claims 1-35, wherein the synthesizing further comprises synthesizing the biopolymers with a length of at least 80 monomers with a purity of at least 50%.

37. The method of any one of claims 1-36, wherein the synthesizing further comprises synthesizing the biopolymers with a length of at least 250 monomers with a purity of at least 50%.

38. The method of any one of claims 1-37, wherein the solid support is free-flowing in a synthesis column.

39. The method of any one of claims 1-38, wherein the solid support is fitted to a synthesis vessel.

40. The method of claim 39, wherein the solid support is fitted to the synthesis vessel by sonic welding.

41. A method for synthesizing an oligonucleotide, the method comprising:

a) providing a surface activated solid support comprising a plurality of pores, each of the plurality of pores having a pore diameter of at least 1 micron; and

b) synthesizing the oligonucleotide directly on the surface of the surface activated solid support, wherein the synthesizing comprises sequentially coupling a plurality of nucleosides or derivatives thereof to form the oligonucleotide.

42. The method of claim 41, wherein the surface of the surface activated solid support does not comprise a scaffold or other structure that distances the oligonucleotide from the surface of the surface activated solid support.

43. The method of claim 42, wherein the scaffold is a surface polymer brush phase, a lightly crosslinked polymer phase, a dendrimer phase, a pellicular phase, a fractal polymer phase, and a grafted polymer.

44. The method of any one of claims 41-43, wherein the synthesizing further comprises synthesizing a plurality of oligonucleotides on the surface activated solid support.

45. The method of any one of claims 41-44, wherein the synthesizing further comprises synthesizing the oligonucleotide in a pore of the surface activated solid support.

46. The method of any one of claims 41-45, wherein the oligonucleotide comprises RNA, DNA, modified RNA, or modified DNA.

47. The method of any one of claims 41-46, wherein the surface activated solid support comprises a porous sheet or a porous membrane.

48. The method of claim 47, wherein the porous sheet or porous membrane comprises polypropylene.

49. The method of claim 47, wherein the porous sheet or porous membrane is selected from the group consisting of: polyethylene, polypropylene/polyethylene blend, cellulose, cotton, glass, silicon, gold, silver, graphene, paper, and any combination thereof.

50. The method of any one of claims 41-49, wherein the surface activated solid support is plasma treated.

51. The method of any one of claims 41-50, wherein a surface of the surface activated solid support comprises one or more chemical moieties adsorbed thereon.

52. The method of claim 51, wherein the one or more chemical moieties is selected from the group consisting of: an amine, a hydroxyl, a carboxyl, an aldehyde, a sulfhydryl, a maleimide, and an epoxide.

53. The method of any one of claims 41-52, wherein a surface of the surface activated solid support further comprises one or more linker moieties.

54. The method of claim 53, wherein the one or more linker moieties comprises succinyl, oxalyl, disulfide, UnylinkerTM, or derivatives thereof.

55. The method of any one of claims 51-54, wherein the synthesizing of b) further comprises covalently attaching a first nucleoside or derivative thereof to the one or more chemical moieties.

56. The method of claim 55, wherein the synthesizing of b) further comprises coupling a plurality of nucleosides or derivatives thereof to the first nucleoside or derivative thereof.

57. The method of any one of claims 41-56, wherein the nucleoside or derivative thereof is a nucleoside phosphoramidite.

58. The method of any one of claims 41-57, wherein each of the plurality of pores has a pore diameter of at least about 2 microns.

59. The method of any one of claims 41-58, wherein each of the plurality of pores has a pore diameter of at least about 5 microns.

60. The method of any one of claims 41-59, wherein each of the plurality of pores has a pore diameter of at least about 10 microns.

61. The method of any one of claims 41-60, wherein each of the plurality of pores has a pore diameter greater than about 10 microns.

62. The method of any one of claims 41-57, wherein each of the plurality of pores has a pore diameter from about 10 microns to about 200 microns.

63. The method of any one of claims 41-62, wherein the surface activated solid support has a thickness from about 50 microns to about 1000 microns.

64. The method of any one of claims 41-63, wherein the surface activated solid support is about 200 microns in thickness.

65. The method of any one of claims 41-64, wherein the synthesizing further comprises synthesizing the oligonucleotide with a coupling efficiency of greater than at least 98.5%.

66. The method of any one of claims 41-65, wherein the synthesizing further comprises synthesizing the oligonucleotide with a length of at least 20 nucleotides with a purity of at least 50%.

67. The method of any one of claims 41-66, wherein the synthesizing further comprises synthesizing the oligonucleotide with a length of at least 40 nucleotides with a purity of at least 50%.

68. The method of any one of claims 41-67, wherein the synthesizing further comprises synthesizing the oligonucleotide with a length of at least 60 nucleotides with a purity of at least 50%.

69. The method of any one of claims 41-68, wherein the synthesizing further comprises synthesizing the oligonucleotide with a length of at least 80 nucleotides with a purity of at least 50%.

70. The method of any one of claims 41-69, wherein the synthesizing further comprises synthesizing the oligonucleotide with a length of at least 250 nucleotides with a purity of at least 50%.

71. The method of any one of claims 41-70, wherein the surface activated solid support is free-flowing in a synthesis column.

72. The method of any one of claims 41-70, wherein the surface activated solid support is fitted to a synthesis vessel.

73. The method of claim 72, wherein the surface activated solid support is fitted to the synthesis vessel by sonic welding.

74. A solid support for biopolymer synthesis, wherein the solid support comprises a plurality of pores each having a pore diameter of at least about 1 micron, and wherein the solid support is surface activated such that biopolymer synthesis occurs directly on a surface of the solid support.

75. The solid support of claim 74, wherein the solid support does not comprise a scaffold.

76. The solid support of claim 75, wherein the scaffold is a surface polymer brush phase, a lightly crosslinked polymer phase, a dendrimer phase, a pellicular phase, a fractal polymer phase, and a grafted polymer.

77. The solid support of any one of claims 74-76, wherein each of the plurality of pores have a pore diameter of at least about 2 microns.

78. The solid support of any one of claims 74-77, wherein each of the plurality of pores have a pore diameter of at least about 5 microns.

79. The solid support of any one of claims 74-78, wherein each of the plurality of pores have a pore diameter of at least about 10 microns.

80. The solid support of any one of claims 74-79, wherein each of the plurality of pores have a pore diameter of greater than about 10 microns.

81. The solid support of any one of claims 74-80, wherein a surface of the solid support comprises one or more chemical moieties.

82. The solid support of claim 81, wherein the one or more chemical moieties is selected from the group consisting of: an amine, a hydroxyl, a carboxyl, an aldehyde, a sulfhydryl, a maleimide, and an epoxide.

83. The solid support of any one of claims 74-82, wherein the solid support is a porous sheet or a porous membrane.

84. The solid support of claim 83, wherein the porous sheet or porous membrane comprises polypropylene.

85. The solid support of claim 83, wherein the porous sheet or porous membrane is selected from the group consisting of: polyethylene, polypropylene/polyethylene blend, cellulose, glass, silicon, gold, silver, graphene, paper, and any combination thereof.

86. The solid support of any one of claims 74-85, wherein the solid support comprises one or more linker moieties attached to a surface thereof.

87. The solid support of claim 86, wherein the one or more linker moieties comprises succinyl, oxalyl, disulfide, Unylinker™, or derivatives thereof.

88. The solid support of any one of claims 74-87, wherein the solid support is free-flowing in a synthesis column.

89. The solid support of any one of claims 74-87, wherein the solid support is fitted to a synthesis vessel.

90. A device for biopolymer synthesis, wherein the device comprises a solid support according to any one of claims 74-89.

91. A method of producing a surface for biopolymer synthesis, the method comprising:

(a) providing a solid support comprising a plurality of pores, each having a pore diameter of at least about 1 micron; and

(b) introducing one or more chemical moieties onto the surface of the solid support, wherein the one or more chemical moieties comprise an amino group.

92. A method for the synthesis of a plurality of oligonucleotides, the method comprising:

(a) providing a solid support, wherein the solid support comprises a porous membrane; and

(b) synthesizing the plurality of oligonucleotides on the solid support, wherein the synthesizing comprises sequentially coupling a plurality of nucleosides or derivatives thereof to form the plurality of oligonucleotides,

wherein the plurality of oligonucleotides are synthesized with a coupling efficiency of at least 99%.

93. The method of claim 92, wherein the porous membrane comprises a plurality of pores.

94. The method of claim 93, wherein the plurality of pores each comprise a pore diameter of at least about 1 micron.

95. The method of claim 93 or 94, wherein the plurality of pores each comprise a pore diameter of at least about 2 microns.

96. The method of any one of claims 92-95, wherein the plurality of pores each comprise a pore diameter of at least about 5 microns.

97. The method of any one of claims 92-96, wherein the plurality of pores each comprise a pore diameter of at least about 10 microns.

98. The method of any one of claims 92-97, wherein the plurality of pores each comprise a pore diameter of greater than about 10 microns.

99. The method of any one of claims 92-98, wherein the porous membrane comprises polypropylene.

100. The method of any one of claims 92-98, wherein the porous membrane is selected from the group consisting of: polyethylene, polypropylene/polyethylene blend, cellulose, glass, silicon, gold, silver, graphene, paper, and any combination thereof.

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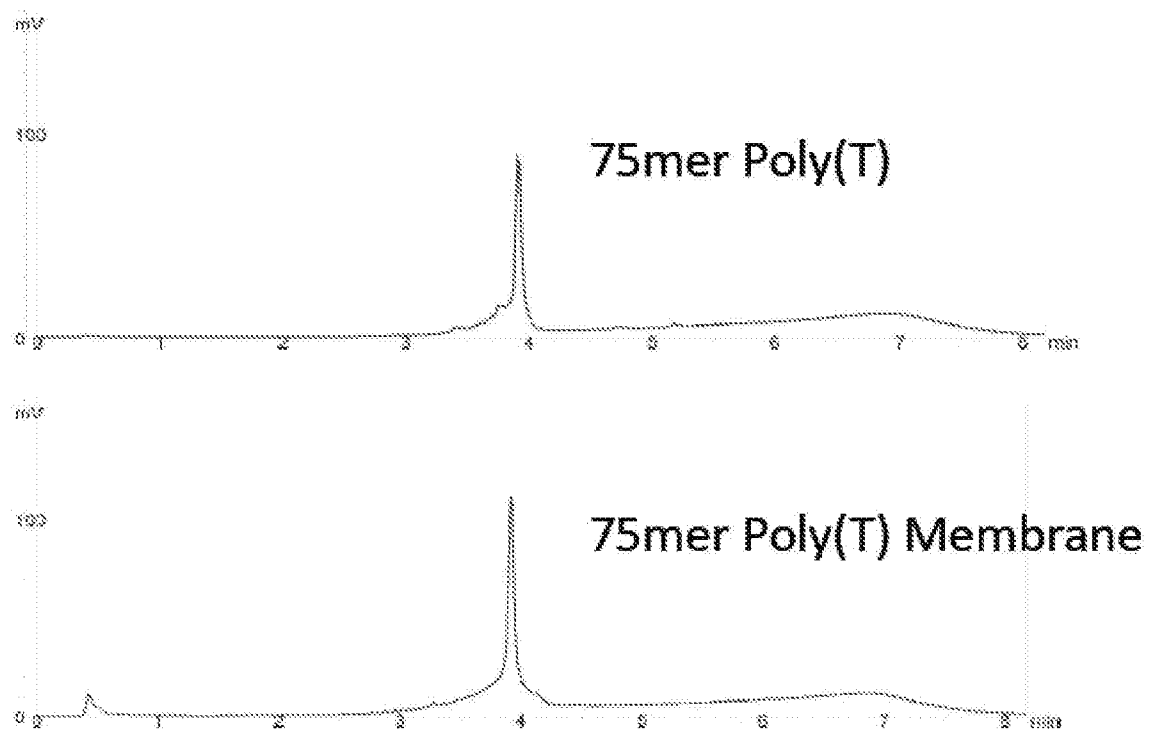


FIG. 1

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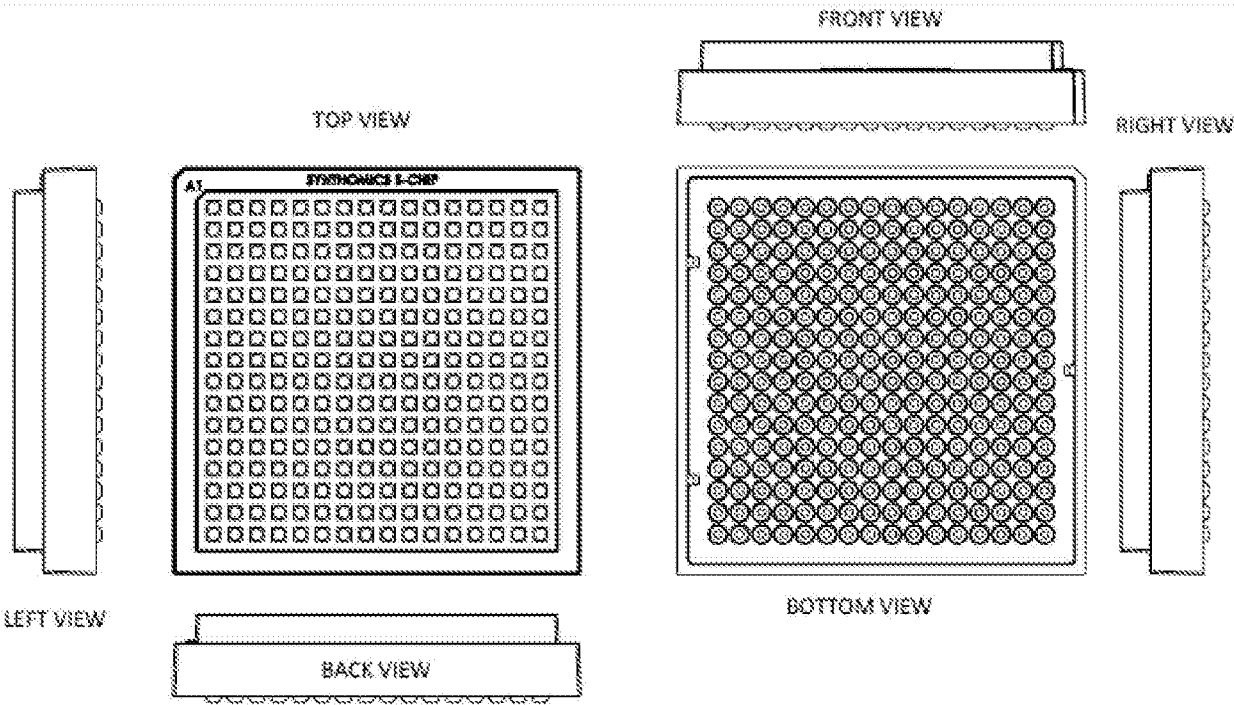


FIG. 2

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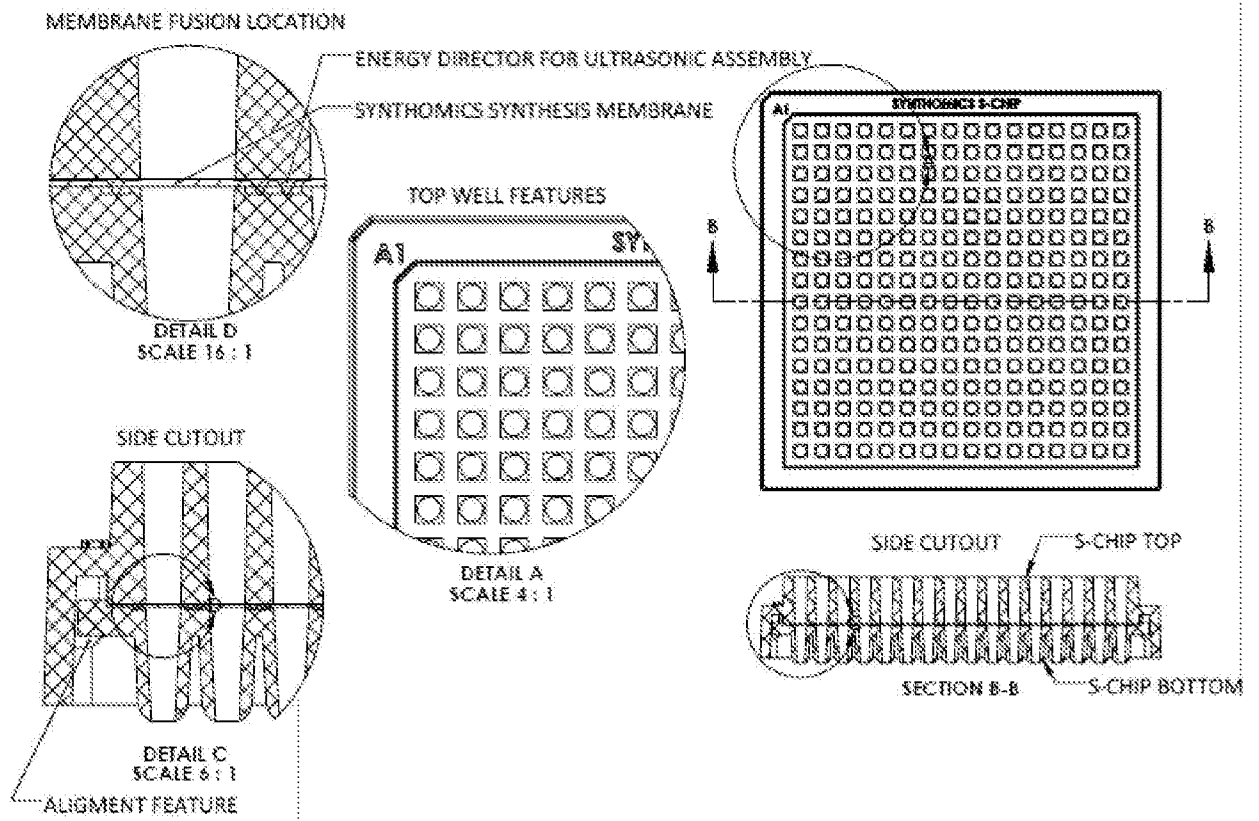


FIG. 3

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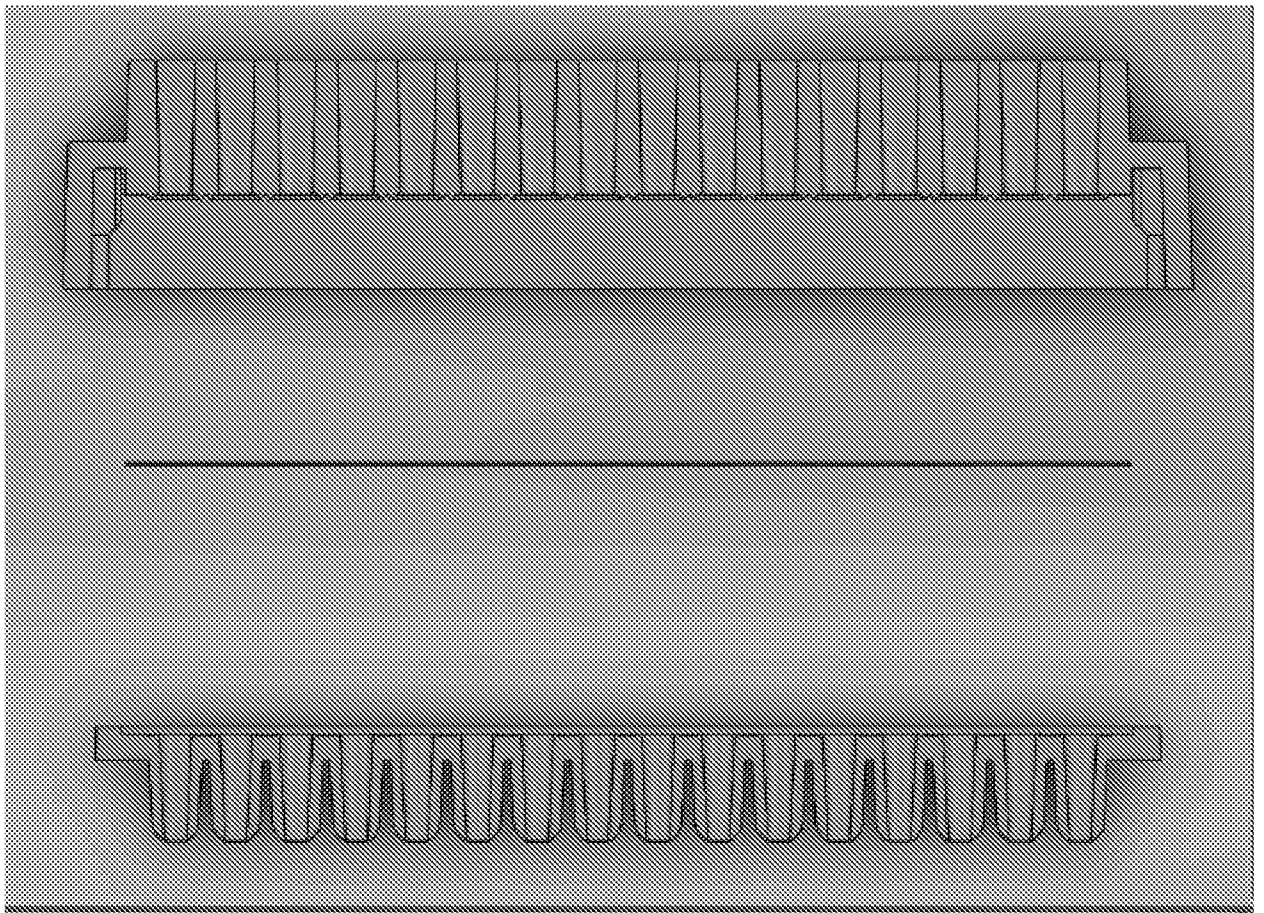


FIG. 4

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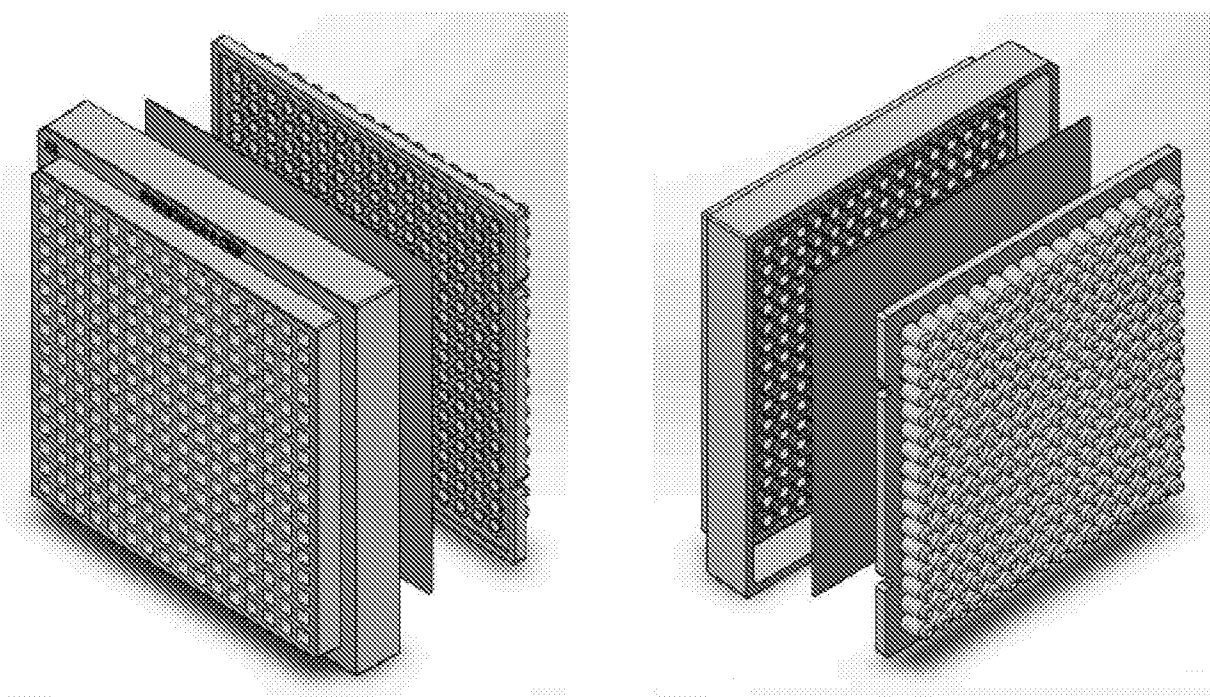


FIG. 5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/061541

A. CLASSIFICATION OF SUBJECT MATTER

B01J 19/00 (2006.01) C40B 50/18 (2006.01) C40B 60/14 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases: PATENW, BIOSIS, BIOTECHNO, CAPLUS, EMBASE, ESBIODBASE, GENBANK, IFIALL, IMSRESEARCH, MEDLINE, PQSCITECH, SCISEARCH, TEMA, USGENE, WPIX, Google.

IPC/CPC symbols: B01J19/0046, C40B50/[14,18].

Keywords: solid support, synthesis support, porous, pore, micron, micromet[er], angstrom, dna, rna, nucleo[s,t]ide, synthesi[s,z]er.

Applicant & Inventor search was conducted in BIOSIS, BIOTECHNO, CAPLUS, EMBASE, ESBIODBASE, GENBANK, IFIALL, IMSRESEARCH, MEDLINE, PQSCITECH, SCISEARCH, TEMA, USGENE, WPIX and Google.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

☒ Further documents are listed in the continuation of Box C☒ See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"D" document cited by the applicant in the international application	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

13 February 2020

Date of mailing of the international search report

13 February 2020

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INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/US2019/061541
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2005/0106606 A1 (PARKER et al.) 19 May 2005 see Examples 2 & 6; Fig. 12G; [0087], [0089], [0256] & Fig. 5D; [0261], [0327], [0374], [0335], [0379]	1-91
A	see [0087], [0089]	94-99
X	JENSEN, M. <i>et al.</i> , 'Next generation 1536-well oligonucleotide synthesizer with on-the-fly dispense', Journal of Biotechnology, 2014, vol. 171, pp. 76-81 see §§ 2.4, 2.6, 3.2, 3.7	92-100
A	US 2015/0038373 A1 (BANYAI et al.) 05 February 2015 see [0212]	13, 14, 63, 64
A	US 6,410,643 B1 (SWANSON) 25 June 2002 see Abstract	25, 26

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Supplemental Box**Continuation of: Box III**

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- Claims 1-91 are directed to a method, solid support, or device for synthesising biopolymers, such as oligonucleotides, wherein the method, solid support or device comprises a surface activated solid support comprising a plurality of pores, each of the pores having a pore diameter greater than about 1 micron, wherein the surface of the activated solid support may comprise one or more chemical moieties comprising an amino group. The feature of an activated solid support comprising a plurality of pores, each of the pores having a pore diameter greater than about 1 micron is specific to this group of claims.
- Claims 92-100 are directed to a method comprising: (a) providing a solid support, wherein the solid support comprises a porous membrane; and (b) synthesising the plurality of oligonucleotides on the solid support, wherein the synthesising comprises sequentially coupling a plurality of nucleosides or derivatives thereof to form the plurality of nucleotides, wherein the plurality of nucleotides are synthesised with a coupling efficiency of at least 99 %. The feature of synthesising the plurality of oligonucleotides on the solid support, wherein the synthesising comprises sequentially coupling a plurality of nucleosides or derivatives thereof to form the plurality of nucleotides, wherein the plurality of nucleotides are synthesised with a coupling efficiency of at least 99 % is specific to this group of claims.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

The technical feature, common to all of the claims is *a porous solid support for biopolymer synthesis*. However, this is not considered to be a special technical feature because it is common general knowledge in the art.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a priori*.

INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/US2019/061541	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

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INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/US2019/061541	
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