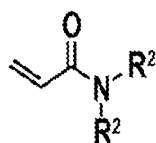


## WHAT IS CLAIMED IS:

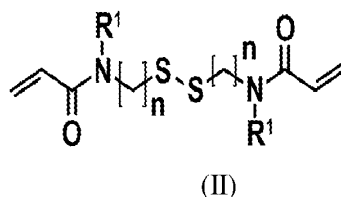
1. A method for making on-flow cell three-dimensional polymer structures, comprising:  
loading a polymer precursor solution onto a flow cell,  
wherein the polymer precursor solution includes a monomer, a crosslinker, and a photoinitiator,  
wherein the flow cell includes at least one channel for receiving the polymer precursor solution, and  
wherein the at least one channel has an upper interior surface and a lower interior surface; and  
illuminating the polymer precursor solution through a patterned photomask using a light at a wavelength sufficient to activate the photoinitiator, wherein activation of the photoinitiator polymerizes at least some of the polymer precursor solution underneath apertures in the patterned photomask and forms three-dimensional polymer structures that extend from the upper interior surface to the lower interior surface of the at least one channel.
2. The method of claim 1, further comprising a light source, wherein the light source is an ultraviolet light source.
3. The method of any of claims 1-2, further comprising cleaving at least some of the three-dimensional polymer structures from the flow cell using heat, cleaving chemicals, or a combination of heat and cleaving chemicals.
4. The method of any of claims 1-3, wherein the monomer is the compound of formula I:



(I)

wherein each R<sup>2</sup> is independently hydrogen or (C<sub>1-6</sub>) alkyl.

5. The method of any of claims 1-4, wherein the crosslinker is a compound of formula II:



wherein:

each n is independently an integer from 1-6; and

each R¹ is independently hydrogen or (C<sub>1-6</sub>) alkyl.

6. The method of any of claims 1-5, wherein the photoinitiator is a diazosulfonate initiator; a monoacylphosphineoxide (MAPO) salt; a bisacylphosphineoxide (BAPO) salt; or combinations thereof.
7. The method of any of claims 1-6, wherein the monomer is acrylamide, the crosslinker is N,N'-Bis(acryloyl)cystamine (BACy), and the photoinitiator is lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP).
8. The method of any of claims 1-7, wherein the polymer precursor solution includes polyethylene glycol (PEG)-thiol, PEG-acrylate, acrylamide, N,N'-Bis(acryloyl)cystamine (BACy), PEG, polypropylene oxide (PPO), polyacrylic acid, poly(hydroxyethyl methacrylate) (HEMA), poly(methyl methacrylate) (PMMA), poly(N-isopropylacrylamide) (PNIPAAm), poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL), poly(vinylsulfonic acid) (PVSA), poly(L-aspartic acid), poly(L-glutamic acid), polylysine, agar, agarose, alginate, heparin, alginate sulfate, dextran sulfate, hyaluronan, pectin, carrageenan, gelatin, chitosan, cellulose, collagen, bisacrylamide, diacrylate, diallylamine, triallylamine, divinyl sulfone, diethyleneglycol diallyl ether, ethyleneglycol diacrylate, polymethyleneglycol diacrylate, polyethyleneglycol diacrylate, trimethylopropane trimethacrylate, ethoxylated trimethylol triacrylate, ethoxylated pentaerythritol tetracrylate, or combinations thereof.
9. The method of any of claims 1-8, wherein the polymer precursor solution includes polyethylene glycol (PEG)-thiol/PEG-acrylate; acrylamide/N,N'-bis(acryloyl)cystamine (BACy); PEG/polypropylene oxide (PPO), or combinations thereof.

10. The method of any of claims 1-9, wherein the photomask comprises polyethylene terephthalate, carbon ink, a chemically etched metal film, or combinations thereof.
11. The method of any of claims 1-10, wherein the three-dimensional polymer structures are cylindrical.
12. The method of any of claims 1-11, wherein the three-dimensional polymer structures are reverse C-shaped.
13. The method of any of claims 1-12, further comprising reacting a bi-functional linker, having a first end and a second end, with the functionalized polymer, wherein the first end of the bi-functional linker is chemically or enzymatically attached to the functionalized polymer, and wherein the second end of the bi-functional linker selectively binds predetermined types of molecules.
14. The method of claim 13, wherein the functionalized polymer is poly(N-(5-azidoacetamidylpentyl) acrylamide-co-acrylamide (PAZAM) containing azide moieties and wherein the bi-functional linker is a biotin-PEG-alkyne complex, and the method further comprising reacting the biotin-PEG-alkyne complex with the azide moieties in the PAZAM using an azide-alkyne click reaction.
15. The method of claim 14, further comprising binding a streptavidin to a biotin in the biotin-PEG-alkyne complex.
16. The method of claim 15, further comprising binding a biotinylated capture oligonucleotide to the streptavidin, wherein the biotinylated capture oligonucleotide is specific for a target of interest in a sequencing library.
17. A method for sequencing in three-dimensions using an on flow-cell three-dimensional sequencing matrix, comprising:  
  
loading a polymer precursor solution into a flow cell, wherein the polymer precursor solution includes monomers and oligonucleotides;  
  
polymerizing the polymer precursor solution to create a permeable three-dimensional matrix within the flow cell;

diffusing a sequencing library into the permeable three-dimensional polymer matrix, wherein the sequencing library includes nucleic acid fragments;

diffusing enzymes and reagents into the permeable three-dimensional polymer matrix;

hybridizing the nucleic acid fragments to the oligonucleotides in the permeable three-dimensional polymer matrix to produce hybridized nucleic acid fragments;

clonally amplifying the hybridized nucleic acid fragments to create clusters for sequencing within the permeable three-dimensional polymer matrix;

sequencing the clusters within the permeable three-dimensional polymer matrix; and

optically imaging the sequenced clusters within three-dimensional matrix in multiple, discrete two-dimensional slices to characterize the sequencing library, wherein the multiple, discrete two-dimensional slices represent the entire three-dimensional internal volume of the flow cell.

18. The method of claim 17, further comprising fragmenting the released nucleic acid and ligating adapters to the ends of the nucleic acid fragments.
19. The method of claim 18, further comprising seeding the nucleic acid fragments on a upper and lower surfaces of the at least one sequencing channel by:

introducing a diffusion barrier into the at least one channel,

heating the flow cell to a temperature that cleaves the polymer structures and releases the nucleic acid fragments therefrom,

hybridizing the nucleic acid fragments to the oligonucleotides on the upper and lower surfaces of the at least one channel, and

washing the cleaved polymer structures out of the flow cell.

20. A flow cell, comprising:
- a channel, wherein the channel includes an upper interior surface having primers coated thereon and a lower interior surface having primers coated thereon; and
- reversible, permeable, three-dimensional polymer structures in the channel from a polymer precursor solution, wherein the three-dimensional polymer structures extend from the upper interior surface of the channel to the lower interior surface of the channel.