## CLAIMS

We claim:

1. A method for coupling a ligand to a functionalized composite material, comprising the steps of:

   a. providing a functionalized composite material, wherein the functionalized composite material is arranged in a coplanar stack of coextensive sheets, a tubular configuration, or a spiral wound configuration, comprising:

      i. a support member, comprising a plurality of pores extending through the support member; and

      ii. a macroporous cross-linked gel, wherein the macroporous cross-linked gel comprises a polymer formed from a reaction of one or more polymerizable monomers with one or more cross-linkers; the macroporous cross-linked gel comprises a plurality of pendant reactive functional groups; the macroporous cross-linked gel is located in the pores of the support member; and said macropores of the macroporous cross-linked gel are smaller than the pores of the support member; and

   b. flowing at a first flow rate a first solution substantially through or substantially across the functionalized composite material, wherein the first solution comprises a plurality of first ligands, such that a plurality of covalent bonds forms between the reactive functional groups and the first ligands.

2. The method of claim 1, wherein the pendant reactive functional groups are selected from the group consisting of aldehydes, amines, carbon-carbon double bonds, carbon-carbon triple bonds, epoxides, hydroxyls, thiols, anhydrides, azides, reactive halogens, acid chlorides, and a mixture thereof.

3. The method of claim 1, wherein the pendant reactive functional groups are selected from the group consisting of carbon-carbon double bonds, carbon-carbon triple bonds, and thiols.

4. The method of ~~any one of the preceding claims~~ claim 1, wherein the pendant reactive functional groups are derived from a molecule comprising a thiol functional group or a molecule comprising an unsaturated carbon-carbon bond.

5. The method of claim 4, wherein the pendant reactive functional groups are derived from a molecule comprising a thiol functional group; and the molecule comprising a thiol

functional group is selected from the group consisting of 3-mercaptopropionic acid, 1-mercaptosuccinic acid, a polypeptide comprising a cysteine residue, a protein comprising a cysteine residue, a recombinant protein comprising a cysteine residue, a bacterial immunoglobulin-binding protein comprising a cysteine residue, a recombinant fusion protein comprising a cysteine residue, cysteamine, 1-thiohexitol, poly(ethylene glycol) 2-mercaptoethyl ether acetic acid, poly(ethylene glycol) methyl ether thiol, 1-thioglycerol, 2-naphthalenethiol, biphenyl-4-thiol, 3-amino-1,2,4-triazole-5-thiol, 5-(trifluoromethyl)pyridine-2-thiol, 1-[2-(dimethylamino)ethyl]-1H-tetrazole-5-thiol, 1-propanethiol, 1-butanethiol, 1-pentanethiol, 1-hexanethiol, 1-octanethiol, 8-amino-1-octanethiol hydrochloride, 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-1-octanethiol, 8-mercapto-1-octanol, and γ-Glu-Cys.

6. The method of claim 5, wherein the molecule comprising a thiol functional group is a polypeptide comprising a cysteine residue, a protein comprising a cysteine residue, a recombinant protein comprising a cysteine residue, a bacterial immunoglobulin-binding protein comprising a cysteine residue, and a recombinant fusion protein comprising a cysteine residue.

7. The method of claim 6, wherein the molecule comprising a thiol functional group is a protein comprising a cysteine residue.

8. The method of claim 4, wherein the pendant reactive functional groups are derived from a molecule comprising an unsaturated carbon-carbon bond; and the molecule comprising an unsaturated carbon-carbon bond is selected from the group consisting of 1-octene, 1-hexyne, 4-bromo-1-butene, allyldiphenylphosphine, allylamine, allyl alcohol, 3,4-dihydroxy-1-butene, 7-octene-1,2-diol, 3-allyloxy-1,2-propanediol, 3-butenoic acid, 3,4-dehydro-L-proline, vinyl laurate, 1-vinyl-2-pyrrolidinone, vinyl cinnamate, an acylamide, or an acrylate.

9. The method of claim 1, wherein the pendant reactive functional groups are selected from the group consisting of aldehydes, amines, epoxides, hydroxyls, anhydrides, azides, reactive halogens, and acid chlorides.

10. The method of claim 9, wherein the one or more monomers comprising a pendant reactive functional group are selected from the group consisting of glycidyl methacrylate, acrylamidoxime, acrylic anhydride, azelaic anhydride, maleic anhydride, hydrazide, acryloyl chloride, 2-bromoethyl methacrylate, and vinyl methyl ketone.

11. The method of claim 9, wherein the pendant reactive functional groups are amines.

12. The method of claim 9, wherein the pendant reactive functional groups are epoxides.

13. The method of claim 9, wherein the pendant reactive functional groups are hydroxyls.

14. The method of any one of the preceding claims, wherein the first ligand comprises a first functionality.

15. The method of claim 14, wherein the first ligand further comprises at least one grafting end-group; and the first functionality is selected from the group consisting of cationic, anionic, hydrophobic, hydrophilic, thiophilic, hydrogen bond donating, hydrogen bond accepting, pi-pi bond donating, pi-pi bond accepting, metal chelating, a biological molecule, and a biological ion.

16. The method of claim 15, wherein the first functionality is selected from the group consisting of cationic, anionic, hydrophobic, hydrophilic, thiophilic, hydrogen bond donating, hydrogen bond accepting, pi-pi bond donating, and pi-pi bond accepting.

17. The method of claim 15, wherein a molecule comprises a first functionality, and the molecule is selected from the group consisting of 2-(diethylamino)ethyl methacrylate, 2-aminoethyl methacrylate, 2-carboxyethyl acrylate, 2-(methylthio)ethyl methacrylate, acrylamide, N-acryloxysuccinimide, butyl acrylate or methacrylate, N,N-diethylacrylamide, N,N-dimethylacrylamide, 2-(N,N-dimethylamino)ethyl acrylate or methacrylate, N-[3-(N,N-dimethylamino)propyl]methacryl-amide, N,N-dimethylacrylamide, ethyl acrylate or methacrylate, 2-ethylhexyl methacrylate, hydroxypropyl methacrylate, glycidyl acrylate or methacrylate, ethylene glycol phenyl ether methacrylate, methacrylamide, methacrylic anhydride, propyl acrylate or methacrylate, N-isopropylacrylamide, styrene, 4-vinylpyridine, vinylsulfonic acid, N-vinyl-2-pyrrolidinone (VP), acrylamido-2-methyl-1-propanesulfonic acid, styrenesulfonic acid, alginic acid, (3-acrylamidopropyl)trimethylammonium halide, diallyldimethylammonium halide, 4-vinyl-N-methylpyridinium halide, vinylbenzyl-N-trimethylammonium halide, methacryloxyethyltrimethylammonium halide, 3-sulfopropyl methacrylate, 2-(2-methoxy)ethyl acrylate or methacrylate, hydroxyethyl acrylamide, N-(3-methoxypropyl acrylamide), N-[tris(hydroxymethyl)methyl]acrylamide, N-phenyl acrylamide, N-tert-butyl acrylamide, or diacetone acrylamide.

18. The method of claim 15, wherein the first functionality is a metal chelating functionality.

19. The method of claim 15, wherein the first functionality comprises a metal chelating functionality selected from the group consisting of octadentate, hexadentate, tetradentate, tridentate, bidentate, iminodicarboxylic acid, and iminodiacetic acid.

~~20. The method of claim 15, wherein the first functionality is a biological molecule or a biological ion.~~

~~21~~20. The method of claim 15, wherein the first functionality comprises a biological molecule or a biological ion functionality selected from the group consisting of albumins, lysozyme, viruses, cells, γ-globulins of human and animal origins, immunoglobulins of both human and animal origins, proteins of recombinant or natural origin including, polypeptides of synthetic or natural origin, interleukin-2 and its receptor, enzymes, monoclonal antibodies, antigens, lectins, bacterial immunoglobulin-binding proteins, trypsin and its inhibitor, cytochrome C, myoglobulin, recombinant human interleukin, recombinant fusion protein, Protein A, Protein G, Protein L, Peptide H, nucleic acid derived products, DNA of either synthetic or natural origin, and RNA of either synthetic or natural origin.

~~22. The method of any one of claims 15-21, wherein the at least one grafting end-group is selected from the group consisting of an aldehyde, an amine, a carbon-carbon double bond, a carbon-carbon triple bond, an epoxide, a hydroxyl, a thiol, and a mixture thereof.~~

~~23. The method of any one of claims 15-21, wherein the at least one grafting end-group is an aldehyde.~~

~~24. The method of any one of claims 15-21, wherein the at least one grafting end-group is an amine.~~

~~25. The method of any one of claims 15-21, wherein the at least one grafting end-group is a carbon-carbon double bond or a carbon-carbon triple bond.~~

~~26. The method of any one of claims 15-21, wherein the at least one grafting end-group is an epoxide.~~

~~27. The method of any one of claims 15-21, wherein the at least one grafting end-group is a hydroxyl.~~

~~28. The method of any one of claims 15-21, wherein the at least one grafting end-group is a thiol.~~

~~29. The method of any one of the preceding claims, further comprising the step of:~~

c. flowing at a second flow rate a first wash solution substantially through or substantially across the composite material, thereby removing excess first ligand.

30. The method of any one of the preceding claims, further comprising the step of:

d. flowing at a third flow rate a quenching solution substantially through or substantially across the composite material, wherein the quenching solution comprises a reactive compound to convert any residual pendant reactive functional groups to non-reactive groups; and

e. optionally, flowing at a fourth flow rate a second wash solution substantially through or substantially across the composite material to remove any residual reactive compound.

31. The method of any one of the preceding claims, wherein the first solution of step b. is recirculated through or across the composite material.

32. The method of claim 30 or 31, wherein the quenching solution of step d. is recirculated through or across the composite material.

33. The method of any one of claims 1-28, further comprising the step of:

c. optionally, flowing at a second flow rate a first wash solution substantially through or substantially across the composite material to remove any excess first ligand;

d. flowing at a third flow rate a second solution substantially through or substantially across the functionalized composite material, wherein the second solution comprises a plurality of second ligands comprising at least three reactive groups, wherein the second ligand is a cross-linker, and, optionally, a polymerizable monomer comprising at least two pendant reactive functional groups, thereby forming a plurality of covalent bonds between the reactive functional groups and the second ligands.

34. The method of claim 33, wherein the second ligand is added in at least two portions.

35. The method of claim 33, wherein the polymerizable monomer comprising at least two pendant reactive functional groups is added in at least two portions.

36. The method of any one of claims 33-35, wherein the second ligand comprises a second functionality.

37. The method of claim 36, wherein the second ligand further comprises at least one grafting end-group; and the second functionality is selected from the group consisting of cationic, anionic, hydrophobic, hydrophilic, thiophilic, hydrogen bond donating, hydrogen bond accepting, pi-pi bond donating, pi-pi bond accepting, metal chelating, a biological molecule, and a biological ion.

38. The method of claim 37, wherein the second functionality is selected from the group consisting of cationic, anionic, hydrophobic, hydrophilic, thiophilic, hydrogen bond donating, hydrogen bond accepting, pi-pi bond donating, and pi-pi bond accepting.

39. The method of claim 37, wherein the second ligand comprises a biological molecule or a biological ion comprising at least one grafting end-group selected from the group consisting of an amine, a hydroxyl, and a thiol functional group.

40. The method of claim 39, wherein the biological molecule or the biological ion is selected from the group consisting of albumins, lysozyme, viruses, cells, γ-globulins of human and animal origins, immunoglobulins of both human and animal origins, proteins of recombinant or natural origin including, polypeptides of synthetic or natural origin, interleukin-2 and its receptor, enzymes, monoclonal antibodies, antigens, lectins, bacterial immunoglobulin-binding proteins, trypsin and its inhibitor, cytochrome C, myoglobulin, recombinant human interleukin, recombinant fusion protein, Protein A, Protein G, Protein L, Peptide H, nucleic acid derived products, DNA of either synthetic or natural origin, and RNA of either synthetic or natural origin.

41. The method of claim 39, wherein the biological molecule or the biological ion is selected from the group consisting of γ-globulins of human and animal origins, immunoglobulins of both human and animal origins, proteins of recombinant or natural origin including, polypeptides of synthetic or natural origin, monoclonal antibodies, antigens, bacterial immunoglobulin-binding proteins, recombinant fusion protein, Protein A, Protein G, Protein L, and Peptide H.

42. The method of any one of claims 39-41, wherein the second ligand is a polypeptide, a protein, a recombinant protein, a bacterial immunoglobulin-binding protein, a recombinant fusion protein, Protein A, Protein G, Protein L, and Peptide H.

43. The method of any one of claims 33-42, wherein the polymerizable monomer comprising at least two pendant reactive functional groups is poly(ethylene glycol) divinyl ether.

44. The method of any one of claims 33-43, further comprising the step of:

e. flowing at a fourth flow rate a second wash solution substantially through or substantially across the composite material to remove any excess second ligand and, optionally, any excess polymerizable monomer.

45. The method of any one of claims 33-44, further comprising:

f. flowing at a fifth flow rate a quenching solution substantially through or substantially across the composite material, wherein the quenching solution comprises a reactive compound to convert any residual pendant reactive functional groups to non-reactive groups; and

g. optionally, flowing at a sixth flow rate a third wash solution substantially through or substantially across the composite material to remove any residual reactive compound.

46. The method of any one of the preceding claims, wherein the composite material is arranged in a substantially coplanar stack of substantially coextensive sheets.

47. The method of any one of the preceding claims, wherein the composite material has 2 to 300 separate support members.

48. The method of any one of claims 1-45, wherein the composite material is arranged in a tubular configuration.

49. The method of any one of claims 1-45, wherein the composite material is arranged in a substantially spiral wound configuration.

50. The method of any one of the preceding claims, wherein the support member comprises a polymeric material selected from the group consisting of polysulfones, polyethersulfones, polyphenyleneoxides, polycarbonates, polyesters, cellulose, and cellulose derivatives.

51. The method of any one of the preceding claims, wherein the composite material is a membrane.

52. The method of any one of the preceding claims, wherein the composite material is in contact with one or more interleaf layers.

53. The method of claim 52, wherein the one or more interleaf layers are selected from the group consisting of screen, polypropylene, polyethylene, and paper.

54. The method of claim 52 or 53, wherein one or more interleaf layers are in contact with one or more flow distribution layers.

55. The method of any one of the preceding claims, wherein the cross-linked gel is a neutral hydrogel, a charged hydrogel, a polyelectrolyte gel, a hydrophobic gel, a neutral gel, or a gel comprising functional groups.

56. The method of any one of the preceding claims, wherein the macroporous cross-linked gel comprises macropores having an average size of 10 nm to 3000 nm.

57. The method of any one of the preceding claims, wherein the pores of the support member have an average pore diameter of about 0.1 μm to about 50 μm.

58. The method of any one of the preceding claims, wherein a fluid flow path is substantially through the composite material.

59. The method of any one of the preceding claims, wherein a fluid flow path is substantially across the composite material.

60. The method of any one of the preceding claims, further comprising the step of:

h. flowing at a seventh flow rate a first fluid comprising a substance substantially through or substantially across the composite material, thereby adsorbing or absorbing a portion of the substance onto the composite material.

61. The method of claim 60, wherein the first fluid further comprises a fragmented antibody, aggregated antibodies, a host cell protein, a polynucleotide, an endotoxin, or a virus.

62. The method of claim 60 or 61, wherein the fluid flow path of the first fluid is substantially through the composite material.

63. The method of claim 60 or 61, wherein the fluid flow path of the first fluid is substantially across the composite material.

64. The method of any one of claims 60-63, wherein substantially all of the substance is adsorbed or absorbed onto the composite material after the first fluid flows substantially through or substantially across the composite material.

65. The method of any one of claims 60-64, further comprising the step of:

i. contacting at an eighth flow rate a second fluid with the substance adsorbed or absorbed onto the composite material, thereby releasing a portion of the substance from the composite material.

66. The method of claim 65, wherein the fluid flow path of the second fluid is substantially through the macropores of the composite material.

67. The method of claim 65, wherein the fluid flow path of the second fluid is substantially perpendicular to the macropores of the composite material.

68. The method of any one of claims 60-67, wherein the substance is a biological molecule, biological ion, virus, or virus particle.

69. The method of claim 68, wherein the biological molecule or biological ion is selected from the group consisting of albumins, lysozyme, viruses, cells, γ-globulins of human and animal origins, immunoglobulins of human and animal origins, hIgG, proteins of recombinant and natural origins, polypeptides of synthetic and natural origins, interleukin-2 and its receptor, enzymes, monoclonal antibodies, trypsin and its inhibitor, cytochrome C, myoglobin, myoglobulin, α-chymotrypsinogen, recombinant human interleukin, recombinant fusion protein, nucleic acid derived products, DNA of synthetic and natural origins, and RNA of synthetic and natural origins.

70. The method of claim 69, wherein the biological molecule or biological ion is lysozyme, hIgG, myoglobin, human serum albumin, soy trypsin inhibitor, transferase, enolase, ovalbumin, ribonuclease, egg trypsin inhibitor, cytochrome c, Annexin V, or α-chymotrypsinogen.

71. The method of any one of claims 60-70, wherein the concentration of the substance in the first fluid is about 0.2 mg/mL to about 10 mg/mL.

72. The method of any one of the preceding claims, wherein the first flow rate is about 1 membrane volume (MV)/min to about 75 MV/min.

73. The method of any one of claims 29-72, wherein the second flow rate is about 1 MV/min to about 75 MV/min.

74. The method of any one of claims 30-73, wherein the third flow rate is about 1 MV/min to about 75 MV/min.

~~75. The method of any one of claims 30-74, wherein the fourth flow rate is about 1 MV/min to about 75 MV/min.~~

~~76. The method of any one of claims 45-75, wherein the fifth flow rate is about 1 MV/min to about 75 MV/min.~~

~~77. The method of any one of claims 45-76, wherein the sixth flow rate is about 1 MV/min to about 75 MV/min.~~

~~78. The method of any one of claims 60-77, wherein the seventh flow rate is about 1 MV/min to about 75 MV/min.~~

~~79. The method of any one of claims 65-78, wherein the eighth flow rate is about 1 MV/min to about 75 MV/min.~~