METHODS AND COMPOSITIONS FOR GENERATING BIOACTIVE ASSEMBLIES OF INCREASED COMPLEXITY AND USES

A. DDD2:
CGMOIOPPOLTELLOGYTVEVLROO
PDLDVEFAVEYTRLEKARlSEQ ID NO: 1)

B. AD2:
CGOIEYLAOKOVDNAIQOAGC (SEQ ID NO:2)

C. DDD3:
SLRECELYVQKNQALLKSIVQCLTPRPEMFLREYFRLECE
AK (SEQ ID NO:3)

D. DDD3C:
MSCGGSRLRECELYVQKNQALLKSIVQCLTPRPEMFLREYFRLE
EAK (SEQ ID NO:4)

E. AD3:
CGFEELAWKIAKMIWSDVFQOGC (SEQ ID NO:5)

Abstract: The present invention concerns methods and compositions for making and using bioactive assemblies of defined compositions, which may have multiple functionalities and/or binding specificities. In particular embodiments, the bioactive assembly is formed using dock-and-lock (DNL) methodology, which takes advantage of the specific binding interaction between dimerization and docking domains (DDD) and anchoring domains (AD) to form the assembly. In various embodiments, one or more effectors may be attached to a DDD or AD sequence. Complementary AD or DDD sequences may be attached to an adaptor module that forms the core of the bioactive assembly, allowing formation of the assembly through the specific DDD/AD binding interactions. Such assemblies may be attached to a wide variety of effector moieties for treatment, detection and/or diagnosis of a disease, pathogen infection or other medical or veterinary condition.

Published: — without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.
METHODS AND COMPOSITIONS FOR GENERATING BIOACTIVE ASSEMBLIES OF INCREASED COMPLEXITY AND USES

Related Applications


Field of the Invention

[0002] Various embodiments of the present invention concern methods and compositions for making and using multivalent, multispecific and/or multifunctional complexes. Such complexes find use in a wide variety of applications, particularly in the field of treatment, detection and/or diagnosis of infections, diseases and other health-related conditions, including but not limited to cancer, autoimmune disease, cardiovascular disease, metabolic diseases, degenerative diseases, including such neurologic disorders as Alzheimer's, and organ transplant rejection.

Background of the Invention

[0003] Man-made agents that incorporate multiple copies of both targeting and effector moieties are highly desirable, as they should provide more avid binding and confer enhanced potency. Although recombinant technologies are commonly applied for making fusion proteins with both targeting and effector domains, multimeric structures that comprise the same or different monomeric components to acquire multivalency or multifunctionality may be obtained only with judicious applications of conjugation chemistries.

[0004] For agents generated by recombinant engineering, problems may include high manufacturing cost, low expression yields, instability in serum, instability in solution resulting in formation of aggregates or dissociated subunits, undefined batch composition due to the presence of multiple product forms, contaminating side-products, reduced functional
activities or binding affinity/avidity attributed to steric factors or altered conformations, etc. For agents generated by various methods of chemical cross-linking, high manufacturing cost and heterogeneity of the purified product are two major limitations.

[0005] Thus, there remains a need in the art for a general method of making multivalent structures of multiple specificities or functionalities, which are of defined composition, homogeneous purity, and unaltered affinity, and can be produced in high yields without the requirement of extensive purification. Furthermore, such structures must also be sufficiently stable in serum to allow in vivo applications. A need also exists for stable, multivalent structures of multiple specificities or functionalities that are easy to construct and/or obtain in relatively purified form.

Summary

[0006] The present invention discloses a platform technology for generating bioactive assemblies of increased complexity that are suitable for in vitro as well as in vivo applications. The assemblies are built by site-specific conjugation of at least two different proteins or nonproteins using a strategy that is based on the Dock and Lock (DNL) method as discussed in U.S. provisional patent applications 60/728,292, filed October 19, 2005; 60/751,196, filed December 16, 2005; 60/782,332, filed March 14, 2006; and U.S. patent applications 11/389,358, filed March 24, 2006 and 11/391,584, filed March 28, 2006, (each of which is incorporated herein by reference in its entirety); and reported recently (Rossi et al, Proc Natl Acad Sci USA, 2006, 103: 6841-6846).

[0007] Methods of use of bioactive assemblies may include detection, diagnosis and/or treatment of a disease or other medical condition. Such conditions may include, but are not limited to, cancer, hyperplasia, diabetic retinopathy, macular degeneration, inflammatory bowel disease, Crohn's disease, ulcerative colitis, rheumatoid arthritis, diabetes, sarcoidosis, asthma, edema, pulmonary hypertension, psoriasis, corneal graft rejection, neovascular glaucoma, Osier-Webber Syndrome, myocardial angiogenesis, plaque neovascularization, restenosis, neointima formation after vascular trauma, telangiectasia, hemophilic joints, angiofibroma, fibrosis associated with chronic inflammation, lung fibrosis, amyloidosis, Alzheimer's disease, organ transplant rejection, deep venous thrombosis or wound granulation.
In particular embodiments, the disclosed methods and compositions may be of use to treat autoimmune disease, such as acute idiopathic thrombocytopenic purpura, chronic idiopathic thrombocytopenic purpura, dermatomyositis, Sydenham's chorea, myasthenia gravis, systemic lupus erythematosus, lupus nephritis, rheumatic fever, polyglandular syndromes, bullous pemphigid, juvenile diabetes mellitus, Henoch-Schönlein purpura, post-streptococcal nephritis, erythema nodosum, Takayasu's arteritis, Addison's disease, rheumatoid arthritis, multiple sclerosis, sarcoidosis, ulcerative colitis, erythema multiforme, IgA nephropathy, polyarteritis nodosa, ankylosing spondylitis, Goodpasture's syndrome, thromboangiitis obliterans, Sjogren's syndrome, primary biliary cirrhosis, Hashimoto's thyroiditis, thyrotoxicosis (i.e., Graves' disease), scleroderma, chronic active hepatitis, polymyositis/dermatomyositis, polychondritis, pemphigus vulgaris, Wegener's granulomatosis, membranous nephropathy, amyotrophic lateral sclerosis, tabes dorsalis, giant cell arteritis/polymyalgia, pernicious anemia, rapidly progressive glomerulonephritis, psoriasis or fibrosing alveolitis.

In certain embodiments, the bioactive assemblies may be of use for therapeutic treatment of cancer. It is anticipated that any type of tumor and any type of tumor antigen may be targeted. Exemplary types of tumors that may be targeted include acute lymphocytic leukemia, acute myelogenous leukemia, biliary cancer, breast cancer, cervical cancer, chronic lymphocytic leukemia, chronic myelogenous leukemia, colorectal cancer, endometrial cancer, esophageal cancer, gastric cancer, head and neck cancers, Hodgkin's lymphoma, lung cancer, medullary thyroid cancer, non-Hodgkin's lymphoma, multiple myeloma, renal cancer, ovarian cancer, pancreatic cancer, melanoma, liver cancer, prostate cancer, glioblastoma and other brain and spinal cord tumors, and urinary bladder cancer.

Tumor-associated antigens that may be targeted include, but are not limited to, carbonic anhydrase IX, A3, antigen specific for A33 antibody, BrE3-antigen, CD1, CD1a, CD3, CD5, CD15, CD16, CD19, CD20, CD21, CD22, CD23, CD25, CD30, CD45, CD74, CD79a, CD80, HLA-DR, NCA 95, NCA90, HCG and its subunits, CEA (CEACAM-5), CEACAM-6, CSAp, EGFR, EGP-I, EGP-2, Ep-CAM, Ba 733, HER2/neu, hypoxia inducible factor (HIF), KC4-antigen, KS-1-antigen, KS1-4, Le-Y, macrophage inhibition factor (MIF), MAGE, MUC1, MUC2, MUC3, MUC4, MUC16, PAM-4-antigen, PSA, PSMA, RS5, SIOO, TAG-72, p53, tenascin, IL-6, IL-8, insulin growth factor-1 (IGF-I), TN antigen, Thomson-Friedenreich antigens, tumor necrosis antigens, VEGF, placenta growth factor (PIGF), 17-

[0011] In other embodiments, the bioactive assemblies may be of use to treat infection with pathogenic organisms, such as bacteria, viruses, fungi, or unicellular parasites. Exemplary fungi that may be treated include Microsporum, Trichophyton, Epidermophyton, Sporothrix schenckii, Cryptococcus neoformans, Coccidioides immitis, Histoplasma capsulatum, Blastomyces dermatitidis or Candida albican. Exemplary viruses include human immunodeficiency virus (HIV), herpes virus, cytomegalovirus, rabies virus, influenza virus, human papilloma virus, hepatitis B virus, hepatitis C virus, Sendai virus, feline leukemia virus, Reo virus, polio virus, human serum parvo-like virus, simian virus 40, respiratory syncytial virus, mouse mammary tumor virus, Varicella-Zoster virus, Dengue virus, rubella virus, measles virus, adenovirus, human T-cell leukemia viruses, Epstein-Barr virus, murine leukemia virus, mumps virus, vesicular stomatitis virus, Sindbis virus, lymphocytic choriomeningitis virus or blue tongue virus. Exemplary bacteria include Bacillus anthracis, Streptococcus agalactiae, Legionella pneumophila, Streptococcus pyogenes, Escherichia coli, Neisseria gonorrhoeae, Neisseria meningitidis, Pneumococcus spp., Hemophilis influenzae B, Treponema pallidum, Lyme disease spirochetes, Pseudomonas aeruginosa, Mycobacterium leprae, Brucella abortus, Mycobacterium tuberculosis or a Mycoplasma. Exemplary parasites include Giardia lamblia, Giardia spp., Pneumocystis carinii, Toxoplasma gondii, Cryptosporidium spp., Acanthamoeba spp., Naegleria spp., Leishmania spp., Balantidium coli, Trypanosoma evansi, Trypanosoma spp., Dientamoeba fragilis, Trichomonas vaginalis, Trichomonas spp. Entamoeba spp. Dientamoeba spp. Babesia spp., Plasmodium falciparum, Isospora spp., Toxoplasma spp. Enterocytozoon spp., Pneumocystis spp. and Balantidium spp.

[0012] Although not limiting, in various embodiments, one or more protein or peptide therapeutic or diagnostic agents may be attached to or incorporated into a bioactive assembly, such as a bacterial toxin, a plant toxin, ricin, abrin, a ribonuclease (RNase), DNase I, Staphyloccocal enterotoxin-A, pokeweed antiviral protein, gelonin, diphertherin toxin,
Pseudomonas exotoxin, Pseudomonas endotoxin, Ranpimase (Rap), Rap (N69Q), PE38, dgA, DT390, PLC, tPA, a cytokine, a growth factor, a soluble receptor component, surfactant protein D, IL-4, sIL-4R, sIL-13R, VEGF, TPO, EPO, a clot-dissolving agent, an enzyme, a fluorescent protein, sTNFα-R, an avimer, a scFv, a dsFv or a nanobody.

[0013] In other embodiments, an anti-angiogenic agent may form part of or may be attached to a bioactive assembly. Exemplary anti-angiogenic agents of use include angiostatin, baculostatin, canstatin, maspin, anti-VEGF antibodies or peptides, anti-placental growth factor antibodies or peptides, anti-Flk-1 antibodies, anti-Fit-1 antibodies or peptides, lamminin peptides, fibronectin peptides, plasminogen activator inhibitors, tissue metalloproteinase inhibitors, interferons, interleukin 12, IP-IO, Gro-β, thrombospondin, 2-methoxyoestradiol, proliferin-related protein, carboxiamidotriazole, CMI01, Marimastat, pentosan polysulphate, angiopoietin 2, interferon-alpha, herbimycin A, PNU145156E, 16K prolactin fragment, Linomide, thalidomide, pentoxifylline, genistein, TNP-470, endostatin, paclitaxel, accutin, angiostatin, cidofovir, vincristine, bleomycin, AGM-1470, platelet factor 4 or minocycline.

[0014] In still other embodiments, one or more therapeutic agents, such as aplidin, azaribine, anastrozole, azacytidine, bleomycin, bortezomib, bryostatin-1, busulfan, calicheamycin, camptothecin, 10-hydroxycamptothecin, carmustine, celebrex, chlorambucil, cisplatin, irinotecan (CPT-II), SN-38, carboplatin, cladribine, cyclophosphamide, cytarabine, dacarbazine, docetaxel, dactinomycin, daunomycin glucuronide, daunorubicin, dexamethasone, diethylstilbestrol, doxorubicin, 2-pyrrolidinodoxorubicine (2P-DOX), cyanomorpholino doxorubicin, doxorubicin glucuronide, epirubicin glucuronide, ethynyl estradiol, estramustine, etoposide, etoposide glucuronide, etoposide phosphate, floxuridine (FUDR), 3',5'-O-dioleoyl-FudR (FUDR-dO), fludarabine, flutamide, fluorouracil, fluoroxymesterone, gemcitabine, hydroxyprogesterone caproate, hydroxyurea, idarubicin, ifosfamide, L-asparaginase, leucovorin, lomustine, mechloretamine, medroprogesterone acetate, megestrol acetate, melphalan, mercaptopurine, 6-mercaptopurine, methotrexate, mitoxantrone, mithydroxyurea, mithramycin, mitomycin, mitotane, phenyl butyrate, prednisone, procarbazine, paclitaxel, pentostatin, PSI-341, semustine streptozocin, tamoxifen, taxanes, taxol, testosterone propionate, thalidomide, thioguanine, thiopeta, teniposide, topotecan, uracil mustard, velcade, vinblastine, vinorelbine, vincristine, ricin, abrin, ribonuclease, onconase, rapLRL1, DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, Pseudomonas endotoxin, an antisense oligonucleotide, an
interference RNA, or a combination thereof, may be conjugated to or incorporated into a bioactive assembly.

[0015] In various embodiments, one or more effectors, such as a diagnostic agent, a therapeutic agent, a chemotherapeutic agent, a radioisotope, an imaging agent, an anti-angiogenic agent, a cytokine, a chemokine, a growth factor, a drug, a prodrug, an enzyme, a binding molecule, a ligand for a cell surface receptor, a chelator, an immunomodulator, an oligonucleotide, an interference RNA, an aptamer, a hormone, a photodetectable label, a dye, a peptide, a toxin, a contrast agent, a paramagnetic label, an ultrasound label, a pro-apoptotic agent, a liposome, a nanoparticle or a combination thereof, may be attached to a bioactive assembly.

[0016] Various embodiments may concern bioactive assemblies and methods of use of same that are of use to induce apoptosis of diseased cells. Further details may be found in U.S. Patent Application Publication No. 20050079184, the entire text of which is incorporated herein by reference. Such structures may comprise a first and/or second binding moiety, such as an antibody or antibody fragment, with affinity for an antigen selected from the group consisting of CD2, CD3, CD8, CD10, CD21, CD23, CD24, CD25, CD30, CD33, CD37, CD38, CD40, CD48, CD52, CD55, CD59, CD70, CD74, CD80, CD86, CD138, CD147, HLA-DR, CEA, CSAp, CA-125, TAG-72, EFG, HER2, HER3, HER4, IGF-IR, c-Met, PDGFR, MUC1, MUC2, MUC3, MUC4, MUC16, TNFR1, TNFR2, NGFR, Fas (CD95), DR3, DR4, DR5, DR6, VEGF, PIGF, ED-B fibronectin, tenascin, PSMA, PSA, carbonic anhydrase DC, and IL-6. In more particular embodiments, a bioactive assembly of use to induce apoptosis may comprise monoclonal antibodies, Fab fragments, chimeric, humanized or human antibodies or fragments. In preferred embodiments, the bioactive assembly may comprise combinations of anti-CD74 X anti-CD20, anti-CD74 X anti-CD22, anti-CD22 X anti-CD20, anti-CD20 X anti-HLA-DR, anti-CD19 X anti-CD20, anti-CD19 x anti-CD22, anti-CD20 X anti-CD80, anti-CD2 X anti-CD25, anti-CD8 X anti-CD25, and anti-CD2 X anti-CD147. In more preferred embodiments, the chimeric, humanized or human antibodies or antibody fragments may be derived from the variable domains of LL2 (anti-CD22), LL1 (anti-CD74) and A20 (anti-CD20).

[0017] In certain embodiments, any therapeutic protein or peptide known in the art may be attached to an AD or DDD sequence and used as an effector in the claimed methods and compositions. A large number of such therapeutic proteins or peptides are known, and are
described for example, in U.S. Patent Application Publication No. 20060084794, "Albumin fusion proteins," filed November 2, 2005, incorporated herein by reference in its entirety. Table 1 of 20060084794, which lists various known exemplary therapeutic proteins or peptides of use, including exemplary identifiers, patent reference numbers and preferred indications, is specifically incorporated herein by reference in its entirety. Additional therapeutic proteins or peptides of use are disclosed, for example, in U.S. Patent No. 6,309,633, incorporated herein by reference in its entirety, and may include but are not limited to adrenocorticotropic hormone, ebratide, angiotensin, angiotensin II, asparaginase, atrial natriuretic peptides, atrial sodium diuretic peptides, bacitracin, beta-endorphins, blood coagulation factors VII, VIII and IX, blood thymic factor, bone morphogenic factor, bone morphogenetic protein, bradykinin, caerulein, calcitonin gene related polypeptide, calcitonins, CCK-8, cell growth factors, EGF, TGF-alpha, TGF-beta, acidic FGF, basic FGF, chemokines, cholecystokinin, cholecystokinin-8, cholecystokinin-pancreozymin, colistin, colony-stimulating factors, GMCSF, MCSF, corticotropin-releasing factor, cytokines, desmopressin, dipeptide, dismutase, dynorphin, eledoisin, endorphins, endothelin, endothelin-antagonistic peptides, endotherins, enkephalins, epidermal growth factor, erythropoietin, follicle-stimulating hormone, gallanin, gastric inhibitory polypeptide, gastrin-releasing polypeptide, gastrins, G-CSF, glucagon, glutathione peroxidase, glutathio-peroxidase, gonadotropin, gramicidin, gramicidines, growth factor, growth hormone-releasing factor, growth hormones, h-ANP, hormone releasing hormone, human chorionic gonadotrophin, human chorionic gonadotrophin .beta.-chain, human placent al lactogen, insulin, insulin-like growth factors, IGF-I, IGF-II, interferons, interleukins, intestinal polypeptide, kallikrein, kyotorphin, luliberin, luteinizing hormone, luteinizing hormone-releasing hormone, lysozyme chloride, melanocyte-stimulating hormone, melanophore stimulating hormone, mellitin, motilin, muramyl, muramyldipeptide, nerve growth factor, nerve nutrition factors, NT-3, NT-4, CNTF, GDNF, BDNF, neuropeptide Y, neurotensin, oxytocin, pancreastatin, pancreatic polypeptide, pancreozymin, parathyroid hormone, pentagastrin, polypeptide YY, pituitary adrenyl cyclase-activating polypeptides, platelet derived growth factor, polymixin B, prolactin, protein synthesis stimulating polypeptide, PTH-related protein, relaxin, renin, secretin, serum thyic factor, somatomedins, somatostatins, substance P, superoxide, superoxide dismutase, taftsin, tetragastrin, thrombopoeitin, thymic humoral factor, thymopoietin, thymosin, thymostimulin, thyroid hormone releasing hormone, thyroid-stimulating hormone, thyrotropin releasing hormone TRH, trypsin, tuftsin, tumor growth
factor, tumor necrosis factor, tyrocidin, urogastrone, urokinase, vasoactive intestinal polypeptide, vasopressins, and functional equivalents.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[0018] FIG. 1 shows exemplary peptide sequences of use in the formation of bioactive assemblies, including DDD2 (SEQ ID NO:1); AD2 (SEQ ID NO:2); DDD3 (SEQ ID NO:3); DDD3C (SEQ ID NO:4); and AD3 (SEQ ID NO:5). Compositions and methods of use of such sequences for formation of bioactive assemblies are discussed below.

[0019] FIG. 2 shows a schematic diagram for an X$_2$(Ma)$_2$Y bioactive assembly, based on a type-a adaptor molecule (Ma), for example attached to one molecule each of AD2 and AD3. The AD2 and AD3 serve as binding sites for DDD2 and DDD3C, for example. Those dimerization and docking domains may in turn be attached to a variety of effectors or binding molecules (X and Y). The result is a heterotetramer comprised of two different homodimers.

[0020] FIG. 3 shows a schematic diagram for an X(Mb)$_2$Y bioactive assembly, based on a type-b adaptor molecule (Mb), for example attached to one molecule each of DDD2 and DDD3. Addition of appropriate anchoring domains, for example AD2 and AD3, that are attached to two different effector moieties results in dimerization and formation of the X(Mb)$_2$Y assembly.

[0021] FIG. 4 shows a schematic diagram for an X$_2$(Mc)$_2$X$_2$ bioactive assembly, based on a type-c adaptor molecule (Mc), for example attached to one molecule each of DDD3 and AD2. Dimerization of the DDD3 sequences attached to different Mc molecules provides two anchoring sites (AD2) for binding of homodimers, each comprised of, for example, DDD2 attached to an effector X. The resulting bioactive assembly is a homotetramer comprising four copies of effector X.

[0022] FIG. 5 shows a schematic diagram for an X$_2$(Md)$_2$Y bioactive assembly, based on a type-d adaptor molecule (Md). The difference with the assembly shown in FIG. 4 is that a DDD3C dimerization and docking domain is used, allowing binding of an AD3 anchor domain attached to effector Y.

[0023] FIG. 6 shows the complete amino acid sequence of an exemplary DDD3C-CH2-CH3-AD2 construct (see Example 7), comprising DDD3C (SEQ ID NO:4), a first linker (SEQ ID
NO:6), CH2 (SEQ ID NO:7), CH3 (SEQ ID NO:8), a second linker (SEQ ID NO:9), and AD2 (SEQ ID NO:2).

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0024] All documents, or portions of documents, cited in this application, including but not limited to patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference in their entirety.

Definitions

[0025] As used herein, "a" or "an" may mean one or more than one of an item.

[0026] As used herein, the terms "and" and "or" may be used to mean either the conjunctive or disjunctive. That is, both terms should be understood as equivalent to "and/or" unless otherwise stated.

[0027] An antibody, as described herein, refers to a full-length (i.e., naturally occurring or formed by normal immunoglobulin gene fragment recombinatorial processes) immunoglobulin molecule (e.g., an IgG antibody) or an immunologically active (i.e., specifically binding) portion or analog of an immunoglobulin molecule, like an antibody fragment.

[0028] An antibody fragment is a portion of an antibody such as F(ab)2, F(ab')2, Fab, Fv, sFv and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. The term "antibody fragment" also includes any synthetic or genetically engineered protein that acts like an antibody by binding to a specific antigen to form a complex. For example, antibody fragments include isolated fragments consisting of the variable regions, such as the "Fv" fragments consisting of the variable regions of the heavy and light chains, recombinant single chain polypeptide molecules in which light and heavy variable regions are connected by a peptide linker ("scFv proteins"), and minimal recognition units (CDR) consisting of the amino acid residues that mimic the hypervariable region.

[0029] An effector is an atom, molecule, or compound that brings about a chosen result. An effector may include a therapeutic agent and/or a diagnostic agent.
[0030] A therapeutic agent is an atom, molecule, or compound that is useful in the treatment of a disease. Examples of therapeutic agents include antibodies, antibody fragments, drugs, toxins, enzymes, nucleases, hormones, immunomodulators, antisense oligonucleotides, small interfering RNA (siRNA), aptamers, chelators, boron compounds, photoactive agents, dyes, and radioisotopes. Other exemplary therapeutic agents and methods of use are disclosed in U.S. Patent Publication Nos. 2005002945, 20040018557, 20030148409 and 20050014207, each incorporated herein by reference.

[0031] A diagnostic agent is an atom, molecule, or compound that is useful in diagnosing a disease, either by in vitro or in vivo tests. Useful diagnostic agents include, but are not limited to, radioisotopes, dyes (such as with the biotin-streptavidin complex), contrast agents, fluorescent compounds or molecules, and enhancing agents (e.g., paramagnetic ions) for magnetic resonance imaging (MRI).

[0032] An immunoconjugate is a conjugate of a binding molecule (e.g., an antibody component) with an atom, molecule, or a higher-ordered structure (e.g., with a carrier, a therapeutic agent, or a diagnostic agent).

[0033] A naked antibody is an antibody that is not conjugated to any other agent.

[0034] A carrier is an atom, molecule, or higher-ordered structure that is capable of associating with a therapeutic or diagnostic agent to facilitate delivery of such agent to a targeted cell. Carriers may include proteins, peptides, lipids (e.g., amphiphilic lipids that are capable of forming higher-ordered structures), polysaccharides (such as dextran), or other higher-ordered structures, such as micelles, liposomes, or nanoparticles.

[0035] As used herein, the term antibody fusion protein refers to a recombinantly produced antigen-binding molecule in which two or more of the same or different scFv or antibody fragments with the same or different specificities are linked. Valency of the fusion protein indicates how many binding arms or sites the fusion protein has to a single antigen or epitope; i.e., monovalent, bivalent, trivalent or multivalent. The multivalency of the antibody fusion protein means that it can take advantage of multiple interactions in binding to an antigen, thus increasing the avidity of binding to the antigen. Specificity indicates how many antigens or epitopes an antibody fusion protein is able to bind; i.e., monospecific, bispecific, trispecific, multispecific. Using these definitions, a natural antibody, e.g., an IgG, is bivalent because it has two binding arms but is monospecific because it binds to one epitope. Monospecific,
multivalent fusion proteins have more than one binding site for an epitope but only binds to
one such epitope, for example a diabody with two binding site reactive with the same antigen.
The fusion protein may comprise a single antibody component, a multivalent or multispecific
combination of different antibody components, or multiple copies of the same antibody
component. The fusion protein may additionally comprise an antibody or an antibody
fragment and a therapeutic agent. Examples of therapeutic agents suitable for such fusion
proteins include immunomodulators ("antibody-immunomodulator fusion protein") and
toxins ("antibody-toxin fusion protein"). One preferred toxin comprises a ribonuclease (RNase), preferably a recombinant RNase.

[0036] An antibody or immunoconjugate preparation, or a composition described herein, is
said to be administered in a "therapeutically effective amount" if the amount administered is
physiologically significant. An agent is physiologically significant if its presence results in a
detectable change in the physiology of a recipient mammal. In particular, an antibody
preparation is physiologically significant if its presence invokes an antitumor response or
mitigates the signs and symptoms of an autoimmune disease state. A physiologically
significant effect could also be the evocation of a humoral and/or cellular immune response in
the recipient mammal leading to modulation, growth inhibition or death of target cells.

DNL Based Bioactive Assemblies

[0037] Certain embodiments of the invention may concern bioactive assemblies that are built
by a site-specific conjugation strategy based on the Dock-and-Lock (DNL) method. The
DNL method exploits α-helical peptides that are found in nature to bind specifically with
each other. The α-helical peptides are the dimerization and docking domain (DDD) in the
regulatory (R) subunits of cAMP-dependent protein kinase (PKA) and the anchoring domain
(AD) in various A-kinase anchoring proteins (AKAPs). By recombinantly fusing or
chemically attaching each peptide to an entity of interest, these helices provide an excellent
linker module for "docking" the two modified entities into a quasi-stable structure, which is
further "locked" into a stable complex via the disulfide linkages formed from cysteine
residues introduced into these helices. Two types of R subunits (RI and RII) are identified in
PKA and each has α and β isoforms. Because the R subunits have been isolated only as stable
dimers and AKAPs bind only to dimeric R subunits, a unique feature of the DNL method is
that the entity derivatized with a peptide derived from the DDD always forms a homodimer, resulting in two copies of that entity in the final complex.

[0038] Two pairs of interacting DDD and AD peptides are of particular interest as the linker modules. The first pair consists of DDD2 (Figure 1A, SEQ ID NO:1), derived from the 44-amino terminal residues of human RIIa, and AD2 (Figure 1B, SEQ ID NO:2), derived from AKAP-ZS, a synthetic peptide optimized for RIIα-selective binding (Alto et al., Proc Natl Acad Sci USA, 2003, 100: 4445-4450). The second pair consists of DDD3 (Figure 1C, SEQ ID NO:3) or DDD3C (Figure 1D, SEQ ID NO:4), derived from the peptide fragment (residues 12-61) of human RIIa (Leon et al., J Biol Chem, 1997, 272: 28431-28437), and AD3 (Figure 1E, SEQ ID NO:5), derived from PV-38, a mutant peptide of D-AKAP2 that specifically binds to RIIa (Burns-Hamuro et al, Proc Natl Acad Sci USA, 2003, 100: 4072-4077).

[0039] In one embodiment, a biological entity, referred to hereafter as the type-a adaptor module (Ma), containing two distinct AD peptides, one reacting preferentially with the DDD of RIIa (for example, AD2 with DDD2), and the other reacting preferentially with the DDD of RIIa (for example, AD3 with DDD3C), is produced and used to complex with two other biological entities, referred to hereafter as the peripheral modules, one comprising a homodimer (designated as X₂) with each monomeric subunit linked to the DDD of RIIa and the other comprising a different homodimer (designated as Y₂) with each monomeric subunit linked to the DDD of RIIa, resulting in an assembly OfX₂(Ma)Y₂ that contains five individual components, as illustrated in Figure 2.

[0040] In another embodiment, a biological entity, referred to hereafter as the type-b adaptor module (Mb), which contains two distinct DDD peptides, one reacting preferentially with AD2 (for example, DDD2), and the other reacting preferentially with AD3 (for example DDD3C), is produced as a homodimer, referred to as (Mb)₂ hereafter, and used to complex with two peripheral modules, one comprising a monomeric subunit linked to AD2 (designated as X) and the other comprising a different monomeric subunit linked to AD3 (designated as Y), resulting in an assembly OfX(Mb)₂Y that contains four individual components, as illustrated in Figure 3.

[0041] In yet another embodiment, a biological entity, referred to hereafter as the type-c adaptor module (Mc), containing both AD2 and DDD3, is produced as a homodimer, referred
to hereafter as \((\text{Mc})_2\), and used to complex with two identical peripheral modules, each comprising a homodimer (designated as \(X_2\)) with individual monomelic subunit linked to DDD2, resulting in an assembly of \(X_2(\text{Mc})_2X_2\) that contains six individual components as illustrated in Figure 4.

[0042] In a further embodiment, a biological entity, referred to as type-d adaptor module (Md) hereafter, which contains AD2 and DDD3C (instead of DDD3 as in type-c), is produced as a homodimer, referred to hereafter as (Md)_2, and used to complex three peripheral modules, two of which are identical homodimers (designated as \(X_2\)) with individual monomelic subunit linked to DDD2 and the third one consisting of a monomeric subunit linked to AD3 (designated as \(Y\)), resulting in an assembly \(\text{Of}X_2(\text{Md})_2YX_2\) that contains seven individual components, as illustrated in Figure 5.

[0043] In other embodiments, the bioactive assemblies produced by the present invention may be further conjugated with effectors and carriers to acquire additional functions enabled by such modifications. In addition, bioactive assemblies can be constructed to contain components capable of forming complexes with DNA or RNA, or synthetic oligodeoxynucleotides (ODN) containing the immunostimulatory CpG motifs (Klinman, Nat Rev Immunol, 2004, 4: 1-10; Krieg, Nat Rev Drug Discov, 2006, 5: 471-484).

[0044] Numerous bioactive assemblies can be designed and produced by the disclosed methods and compositions, with wide applications depending on which type of the adaptor module is selected and what peripheral modules are linked to the adaptor module. Bioactive entities that are of particular interest as the adaptor modules include the Fc of human IgGl, human serum albumin (HSA), various heat shock proteins (HSPs), bioluminescent proteins, human transferrin (hTf), and human protamines. Bioactive entities that may be derivatized to serve as peripheral modules include cytokines, chemokines, growth factors, soluble receptors, antibody fragments, fluorescent proteins, \(^\sim\)-peptides, \(^\wedge\)-peptides, peptides containing unnatural amino acids, peptoids, peptomimetics, DNA sequences, synthetic CpG ODN, small interfering RNAs, human protamine 1, DNA-binding peptides derived from protamines, protein transduction domains, nuclear localization signals, peptides that facilitate transdermal delivery or membrane penetration, DNA or RNA aptamers, peptide aptamers, cholera toxin subunit B monomer, enzymes, polyethylene glycol, nanoparticles, drug-containing polymers, chelates, quantum dots, and various scaffold-based binding proteins such as Nanobody, Evibody, Ankyrin repeat protein, Trans-body, Anticalin, Microbody, AdNectin, Domain
antibody, Affibody, Maxibody, Tetrannectin, Affilin molecule, iMabs, and Monobody (Hey et al., Trends Biotechnol, 2005, 23: 514-522; Binz et al., Nat Biotechnol, 2005, 23: 1257-1268). Specific compositions of selected assemblies based on type-a, -b, -c, and -d adaptor modules are listed in Tables 1, 2, 3, and 4, respectively.

[0045] Adaptor modules based on HSPs. Subunit vaccines that consist of well-characterized molecules, although highly desirable due to their superior safety profile and ease of manufacturing, are hampered by their poor immunogenicity and limited stability, which may be remedied by the development of improved delivery vehicles as well as more efficacious yet nontoxic adjuvants. The present compositions and methods can be applied to generate subunit vaccines that (1) contain defined antigenic molecules, (2) have built-in adjuvants to enhance the immune response, and (3) are able to elicit an antigen-specific T cell immunity.

[0046] One approach is to generate type-a adaptor modules based on HSPs for linking to peripheral modules derived from target antigens and immune enhancers that are proteins or peptides, resulting in protein- or peptide-based vaccines for direct immunization or ex vivo priming of dendritic cells to achieve both MHC-I and MHC-II presentations (Srivastava, Nat Rev Immunol, 2002, 2: 185-194). Alternatively, the HSP-based adaptor modules are linked to peripheral modules derived from DNA-binding proteins, for example, human protamines (Song et al, Nat Biotechnol, 2005, 23: 709-717), or DNA-binding peptides containing clustered arginine residues, for example, RRRRRGGRRRRRR (SEQ ID NO: 10) (Brewer et al., J Biol Chem, 2003, 278: 42403-42406), and targeting molecules, for example, antibody fragments, resulting in multifunctional assemblies useful as target-specific DNA vaccines upon complexation with plasmids that encode the genes for target antigens or both target antigens and immune enhancers. Further expansion of the spectrum for broader protection by such vaccines is also feasible due to the ability of HSPs to noncovalently associate with a variety of antigenic peptides (U.S. Pat. No. 5,935,576; U.S. Pat. No. 5,750,119). Example 1 describes the generation and use of type-a adaptor modules based on HSPs.

[0047] Adaptor modules based on human protamines. Bioactive assemblies that use adaptors modules based on human protamines are particularly suitable for delivery of DNA vaccines, siRNAs, or therapeutic genes to specific cells. A fusion protein (F105-P) consisting of anti-gpl20 Fab and human protamine 1 (hPl) has been shown to be effective in delivering plasmids encoding Pesudomonas exotoxin A (Chen et al., Gene Ther, 1995, 2: 116-123) or
siRNAs to HIV-infected cells or HIV envelope-expressing tumor cells (Song et al, Nat Biotechnol, 2005, 23: 709-717). Type-b adaptor modules based on hPl can be generated for linking to peripheral modules derived from different biological entities, such as target specific binding proteins, and the resulting assembly is used as a carrier for target specific delivery of plasmids or siRNAs, which are bound to hPl by complexation. Alternatively, type-c adaptor modules based on hPl can be generated for linking to peripheral modules derived from the same biological entity, such as target specific binding proteins, and the resulting assembly, which contains four copies of the target specific binding proteins, is used as a carrier for target specific delivery of plasmids or siRNAs, which are bound to hPl by complexation. A further embodiment is to generate type-d adaptor modules based on hPl for linking to peripheral modules derived from two different biological entities and the resulting assembly, which contains four copies of one entity and one copy of the other entity is used as a carrier for plasmids or siRNAs, which are bound to hPl by complexation. Examples 2 and 3 describe the generation and use of type-b and -c adaptor modules based on hPl, respectively.

[0048] **Adaptor modules based on the Fc of human immunoglobulins.** Fusion proteins containing the Fc of human IgG have many advantages conferred by the innate properties of the Fc. For example, binding of the Fc to the neonatal receptor (FcRn) expressed on lung and intestine epithelium facilitates transport of Fc-fusion proteins across the mucosal barriers (Spiekermann et al., J Exp Med, 2002, 196: 303-310), thus making pulmonary or oral delivery feasible (Dumont et al., J Aerosol Med, 2005, 18: 294-303; Bitonti et al., Proc Natl Acad Sci USA, 2004, 101: 9763-9768; Low et al., Hum Reprod, 2005, 20: 1805-1813). The pH-dependent binding of the Fc to the FcRn expressed in continuous capillary endothelium also prolongs the serum half-lives of IgG antibodies or Fc-containing fusion proteins. IgG or Fc mutants with higher affinity for the FcRn were shown to substantially increase the serum half-lives of such engineered constructs (Hinton et al., J Immunol, 2006, 176: 346-356; Hinton et al., J Biol Chem, 2004, 279: 6213-6216). On the other hand, IgG or Fc mutants with lower affinity for the FcRn exhibited shorter serum half-lives compared to the corresponding wild types (Kenanova et al., Cancer Res, 2005, 65: 622-631). The ability to tailor the pharmacokinetics of a biological entity containing Fc is very attractive for drug design. The generation and use of type-b, -c, and - d adaptor modules based on the Fc are outlined in Examples 4, 5, and 6, respectively. Detailed methods of constructing the expression vectors for **DDD3-CH2-CH3-AD2** and **DDD3C-CH2-CH3-AD2** are described in Example 7.
Conjugates of Bioactive Assemblies

[0049] Additional moieties can be conjugated to the bioactive assemblies described above. For example, drugs, toxins, radioactive compounds, enzymes, hormones, cytotoxic proteins, chelates, cytokines, and other functional agents may be conjugated to the bioactive assemblies. Conjugation can be via, for example, covalent attachments to amino acid residues containing amine, carboxyl, thiol or hydroxyl groups in the side-chains. Various conventional linkers may be used for this purpose, for example, diisocyanates, diisothiocyanates, bis(hydroxysuccinimide) esters, carbodiimides, maleimide-hydroxysuccinimide esters, glutaraldehyde and the like. Conjugation of agents to the bioactive assemblies preferably does not significantly affect the activity of each subunit contained in the unmodified structures. Conjugation can be carried out separately to the different peripheral modules and the resulting conjugates used for preparing the bioactive assemblies. In addition, cytotoxic agents may be first coupled to a polymeric carrier, which is then conjugated to a bioactive assembly. For this method, see Ryser et al., Proc. Natl. Acad. Sci. USA, 75:3867-3870, 1978; U.S. 4,699,784 and U.S. 4,046,722, which are incorporated herein by reference. As discussed below, one or more effectors may also be conjugated to a carrier moiety, which may then be targeted to a bioactive assembly by incorporation into the assembly of, for example, a monoclonal antibody or fragment that binds specifically to the carrier moiety. An exemplary use of carrier moieties for delivery of effector molecules to bioactive assemblies localized to a targeted cell, tissue or pathogenic organism is described below in the pre-targeting section.

[0050] The conjugates described herein can be prepared by various methods known in the art. For example, a bioactive assembly can be radiolabeled with ¹³¹I and conjugated to a lipid, such that the resulting conjugate can form a liposome. The liposome may incorporate one or more therapeutic (e.g., a drug such as FUdR-dO) or diagnostic agents. Alternatively, in addition to the carrier, a bioactive assembly may be conjugated to ¹³¹I (e.g., at a tyrosine residue) and a drug (e.g., at the epsilon amino group of a lysine residue), and the carrier may incorporate an additional therapeutic or diagnostic agent. Therapeutic and diagnostic agents may be covalently associated with one or more than one subunit of the bioactive assemblies.


[0053] A wide variety of diagnostic and therapeutic agents can be advantageously used to form the conjugates of the bioactive assemblies, or may be linked to haptens that bind to a recognition site on the bioactive assemblies. Diagnostic agents may include radioisotopes, enhancing agents for use in MRI or contrast agents for ultrasound imaging, and fluorescent compounds. Many appropriate imaging agents are known in the art, as are methods for their attachment to proteins or peptides (see, e.g., U.S. patents 5,021,236 and 4,472,509, both incorporated herein by reference). Certain attachment methods involve the use of a metal chelate complex employing, for example, an organic chelating agent such a DTPA attached to the protein or peptide (U.S. Patent 4,472,509).

[0054] In order to load a bioactive assembly with radioactive metals or paramagnetic ions, it may be necessary to first react it with a carrier to which multiple copies of a chelating group for binding the radioactive metals or paramagnetic ions have been attached. Such a carrier can be a polylsine, polysaccharide, or a derivatized or derivatizable polymeric substance having pendant groups to which can be bound chelating groups such as, e.g., ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), porphyrins, polyamines, crown ethers, bis-thiosemicarbazones, polyoximes, and the like known to be useful for this purpose. Carriers containing chelates are coupled to the bioactive
assembly using standard chemistries in a way to minimize aggregation and loss of immunoreactivity.

[0055] Other methods and reagents that may be applied for preparing such conjugates are disclosed in U.S. 4,824,659, which is incorporated herein in its entirety by reference. Particularly useful metal-chelate combinations include 2-benzyl-DTPA and its monomethyl and cyclohexyl analogs, used with diagnostic isotopes in the general energy range of 60 to 4,000 keV. Some useful diagnostic nuclides may include $^{124}$I, $^{18}$F, $^{52}$Fe, $^{52}$Cu, $^{64}$Cu, $^{67}$Cu, $^{67}$Ga, $^{68}$Ga, $^{86}$Y, $^{90}$Zr, $^{94}$Tc, $^{94m}$Tc, $^{99m}$Tc, or $^{111}$In. The same chelates complexed with non-radioactive metals, such as manganese, iron and gadolinium, are useful for MRI, when used along with the bioactive assemblies and carriers described herein. Macrocyclic chelates such as NOTA, DOTA, and TETA are of use with a variety of metals and radiometals, most particularly with radionuclides of gallium, yttrium and copper, respectively. Such metal-chelate complexes can be made very stable by tailoring the ring size to the metal of interest. Other ring-type chelates, such as macrocyclic polyethers for complexing $^{223}$Ra, may be used.

[0056] Therapeutic agents include, for example, chemotherapeutic drugs such as vinca alkaloids, anthracyclines, epipodophyllotoxins, taxanes, antimetabolites, alkylating agents, antibiotics, Cox-2 inhibitors, antimitotics, antiangiogenic and proapoptotic agents, particularly doxorubicin, methotrexate, taxol, CPT-11, SN-38, camptothecans, and others from these and other classes of anticancer agents, and the like. Other cancer chemotherapeutic drugs include nitrogen mustards, alkyl sulfonates, nitrosoureas, triazenes, folic acid analogs, pyrimidine analogs, purine analogs, platinum coordination complexes, hormones, and the like. Suitable chemotherapeutic agents are described in REMINGTON'S PHARMACEUTICAL SCIENCES, 19th Ed. (Mack Publishing Co. 1995), and in GOODMAN AND GILMAN'S THE PHARMACOLOGICAL BASIS OF THERAPEUTICS, 7th Ed. (MacMillan Publishing Co. 1985), as well as revised editions of these publications. Other suitable chemotherapeutic agents, such as experimental drugs, are known to those of skill in the art, and may be conjugated to the bioactive assemblies described herein using methods that are known in the art.

[0057] Another class of therapeutic agents consists of radionuclides that emit $\alpha$-particles (such as $^{212}$Pb, $^{212}$Bi, $^{212}$Bi, $^{211}$At, $^{223}$Ra, $^{223}$Ac), $\beta$-particles (such as $^{32}$P, $^{33}$P, $^{47}$Sc, $^{67}$Cu, $^{67}$Ga, $^{89}$Sr, $^{90}$Y, $^{111}$Ag, $^{125}$I, $^{131}$I, $^{142}$Pr, $^{153}$Sm, $^{161}$Tb, $^{166}$Ho, $^{166}$Dy, $^{177}$Lu, $^{186}$Re, $^{188}$Re, $^{189}$Re), or Auger electrons (such as $^{111}$In, $^{125}$I, $^{67}$Ga, $^{191}$Os, $^{193m}$Pt, $^{195m}$Pt, $^{195}$Hg). The bioactive
assemblies may be labeled with one or more of the above radionuclides using methods as
described for the diagnostic agents.

[0058] Exemplary therapeutic peptides or proteins of use as effectors are disclosed in U.S.
Patent Serial No. 6,309,633 (incorporated herein by reference) and may include, for example:
adrenocorticotropic hormone (ACTH); adrenocorticotropic hormone derivatives (e.g.,
ebiratide); angiotensin; angiotensin II; asparaginase; atrial natriuretic peptides; atrial sodium
diuretic peptides; bacitracin; beta-endorphins; blood coagulation factors VII, VIII and IX;
blood thymic factor (FTS); blood thymic factor derivatives (see U.S. Pat. No. 4,229,438);
bombesin; bone morphogenic factor (BMP); bone morphogenic protein; bradykinin;
caeruline; calcitonin gene related polypeptide (CGRP); calcitonins; CCK-8; cell growth
factors (e.g., EGF; TGF-alpha; TGF-beta; PDGF; acidic FGF; basic FGF); cerulein;
chemokines; cholecystokinin; cholecystokinine-8; cholecystokinin-pancreozymin (CCK-PZ);
colistin; colony-stimulating factors (e.g. CSF; GCSF; GMCSF; MCSF); corticotropin-
releasing factor (CRF); cytokines; desmopressin; dinorphin; dipeptide; dismutase; dynorphin;
eldeoisin; endorphins; endothelin; endothelin-antagonistic peptides (see European Patent
Publication Nos. 436189; 457195 and 496452 and Japanese Patent Unexamined Publication
Nos. 94692/1991 and 130299/1991); endotherins; enkephalins; enkephalin derivatives (see
U.S. Pat. No. 4,277,394 and European Patent Publication No. 31567); epidermal growth
factor (EGF); erythropoietin (EPO); follicle-stimulating hormone (FSH); gallanin; gastric
inhibitory polypeptide; gastrin-releasing polypeptide (GRP); gastrins; G-CSF; glucagon;
glutathione peroxidase; glutathio-peroxidase; gonadotropins (e.g., human chorionic
gonadotrophin and .alpha., and .beta., subunits thereof); gramicidin; gramicidines; growth
factor (EGF); growth hormone-releasing factor (GRF); growth hormones; hormone releasing
hormone (LHRH); human atrial natriuretic polypeptide (h-ANP); human placental lactogen;
insulin; insulin-like growth factors (IGF-I; IGF-II); interferon; interferons (e.g., alpha- beta-
and gamma-interferons); interleukins (e.g. 1; 2; 3; 4; 5; 6; 7; 8; 9; 10; 11 and 12); intestinal
polypeptide (VIP); kallikrein; kytoporhin; luliberin ; luteinizing hormone (LH); luteinizing
hormone-releasing hormone (LH-RH); lysozyme chloride; melanocyte-stimulating hormone
(MSH); melanophore stimulating hormone; mellitin; motilin; muramyl; muramyldipeptide;
nerve growth factor (NGF); nerve nutrition factors (e.g. NT-3; NT-4; CNTF; GDNF; BDNF);
neuropeptide Y; neurotensin; oxytocin; pancreastatin; pancreatic polypeptide; pancreozymin;
parathyroid hormone (PTH); pentagastrin; polypeptide YY; pituitary adenyl cyclase-
activating polypeptides (PACAPs); platelet-derived growth factor; polymixin B; prolactin;
protein synthesis stimulating polypeptide; PTH-related protein; relaxin; renin; secretin; serum thymic factor; somatomedins; somatostatins derivatives (Sandostatin; see U.S. Pat. Nos. 4,087,390; 4,093,574; 4,100,117 and 4,253,998); substance P; superoxide dismutase; taftsin; tetragastrin; throni bopoietin (TPO); thymic humoral factor (THF); thymopoietin; thymosin; thymostimulin; thyroid hormone releasing hormone; thyroid-stimulating hormone (TSH); thyrotropin releasing hormone TRH; trypsin ; tuftsin; tumor growth factor (TGF-alpha); tumor necrosis factor (TNF); tyrocidin; urogastrone; urokinase; vasoactive intestinal polypeptide; vasopressins, and functional equivalents of such polypeptides.

[0059] A suitable peptide containing a detectable label (e.g., a fluorescent molecule), or a cytotoxic agent, (e.g., a radioiodine), can be covalently, non-covalently, or otherwise associated with the bioactive assemblies. For example, a therapeutically useful conjugate can be obtained by incorporating a photoactive agent or dye onto the bioactive assemblies. Fluorescent compositions, such as fluochrome, and other chromogens, or dyes, such as porphyrins sensitive to visible light, have been used to detect and to treat lesions by directing the suitable light to the lesion. In therapy, this has been termed photoradiation, phototherapy, or photodynamic therapy. See Jori et al. (eds.), PHOTODYNAMIC THERAPY OF TUMORS AND OTHER DISEASES (Libreria Progetto 1985); van den Bergh, Chem. Britain (1986), 22:430. Moreover, monoclonal antibodies have been coupled with photoactivated dyes for achieving phototherapy. See Mew et al., J. Immunol. (1983),130:1473; idem., Cancer Res. (1985), 45:4380; Oseroff et al., Proc. Natl. Acad. Sci. USA (1986), 83:8744; idem., Photochem. Photobiol. (1987), 46:83; Hasan et al., Prog. Clin. Biol. Res. (1989), 288:471; Tatsuta et al., Lasers Surg. Med. (1989), 9:422; Pelegrin et al., Cancer (1991), 67:2529. Endoscopic applications are also contemplated. Endoscopic methods of detection and therapy are described in U.S. 4,932,412; U.S. 5,525,338; U.S. 5,716,595; U.S. 5,736,119; U.S. 5,922,302; U.S. 6,096,289; and U.S. 6,387,350, which are incorporated herein by reference in their entirety.

[0060] In certain embodiments, the novel constructs and methods disclosed herein are useful for targeted delivery of RNAi for therapeutic intervention. The delivery vehicle can be a bioactive assembly with an internalizing antibody binding domain fused to human protamine (peptide of ~ 50 amino acid residues). An example would be an assembly comprising human protamine 1 (hPl) and/or human protamine 2 (hP2), both capable of forming stable DNA or RNA complexes such as RNAi for in vivo applications (Nat Biotechnol. 23: 709-717, 2005;
Gene Therapy. 13: 194-195, 2006). The multivalent complex will facilitate the binding to and receptor-mediated internalization into target cells, where the noncovalently bound RNAi is dissociated in the endosomes and released into cytoplasm. In addition to delivery of RNAi, these constructs may also be of use for targeted delivery of therapeutic genes or DNA vaccines. Another area of use is to apply the technology for producing intrabodies, which is the protein analog of RNAi in terms of function.

**Peptide Administration**

[0061] Various embodiments of the claimed methods and/or compositions may concern one or more peptide based bioactive assemblies to be administered to a subject. Administration may occur by any route known in the art, including but not limited to oral, nasal, buccal, inhalational, rectal, vaginal, topical, orthotopic, intradermal, subcutaneous, intramuscular, intraperitoneal, intraarterial, intrathecal or intravenous injection.

[0062] Unmodified peptides administered orally to a subject can be degraded in the digestive tract and depending on sequence and structure may exhibit poor absorption across the intestinal lining. However, methods for chemically modifying peptides to render them less susceptible to degradation by endogenous proteases or more absorbable through the alimentary tract are well known (see, for example, Blondelle et al., 1995, Biophys. J. 69:604-11; Ecker and Crooke, 1995, Biotechnology 13:351-69; Goodman and Ro, 1995, BURGER'S MEDICINAL CHEMISTRY AND DRUG DISCOVERY, VOL. 1, ed. Wollf, John Wiley & Sons; Goodman and Shao, 1996, Pure & Appl. Chem. 68:1303-08). Methods for preparing libraries of peptide analogs, such as peptides containing D-amino acids; peptidomimetics consisting of organic molecules that mimic the structure of a peptide; or peptoids such as vinylogous peptoids, have also been described and may be used to construct peptide based bioactive assemblies suitable for oral administration to a subject.

[0063] In certain embodiments, the standard peptide bond linkage may be replaced by one or more alternative linking groups, such as CH₂-NH, CH₂-S, CH₂-CH₂, CH=CH, CO-CH₂, CHOH-CH₂ and the like. Methods for preparing peptide mimetics are well known (for example, Hruby, 1982, Life Sci 31:189-99; Holladay et al., 1983, Tetrahedron Lett. 24:4401-04; Jennings-White et al., 1982, Tetrahedron Lett. 23:2533; Almquist et al., 1980, J. Med. Chem. 23:1392-98; Hudson et al., 1979, Int. J. Pept. Res. 14:177-185; Spatola et al., 1986, Life Sci 38:1243-49; U.S. Patent Nos. 5,169,862; 5,539,085; 5,576,423, 5,051,448, 5,559,103,
each incorporated herein by reference.) Peptide mimetics may exhibit enhanced stability and/or absorption in vivo compared to their peptide analogs.

[0064] Alternatively, peptides may be administered by oral delivery using N-terminal and/or C-terminal capping to prevent exopeptidase activity. For example, the C-terminus may be capped using amide peptides and the N-terminus may be capped by acetylation of the peptide. Peptides may also be cyclized to block exopeptidases, for example by formation of cyclic amides, disulfides, ethers, sulfides and the like.

[0065] Peptide stabilization may also occur by substitution of D-amino acids for naturally occurring L-amino acids, particularly at locations where endopeptidases are known to act. Endopeptidase binding and cleavage sequences are known in the art and methods for making and using peptides incorporating D-amino acids have been described (e.g., U.S. Patent Application Publication No. 20050025709, McBride et al., filed June 14, 2004, incorporated herein by reference). In certain embodiments, peptides and/or proteins may be orally administered by co-formulation with proteinase- and/or peptidase-inhibitors.

[0066] Other methods for oral delivery of therapeutic peptides are disclosed in Mehta ("Oral delivery and recombinant production of peptide hormones," June 2004, BioPharm International). The peptides are administered in an enteric-coated solid dosage form with excipients that modulate intestinal proteolytic activity and enhance peptide transport across the intestinal wall. Relative bioavailability of intact peptides using this technique ranged from 1% to 10% of the administered dosage. Insulin has been successfully administered in dogs using enteric-coated microcapsules with sodium cholate and a protease inhibitor (Ziv et al., 1994, J. Bone Miner. Res. 18 (Suppl. 2):792-94). Oral administration of peptides has been performed using acylcarnitine as a permeation enhancer and an enteric coating (Eudragit L30D-55, Rohm Pharma Polymers, see Mehta, 2004). Excipients of use for orally administered peptides may generally include one or more inhibitors of intestinal proteases/peptidases along with detergents or other agents to improve solubility or absorption of the peptide, which may be packaged within an enteric-coated capsule or tablet (Mehta, 2004). Organic acids may be included in the capsule to acidify the intestine and inhibit intestinal protease activity once the capsule dissolves in the intestine (Mehta, 2004). Another alternative for oral delivery of peptides would include conjugation to polyethylene glycol (PEG)-based amphiphilic oligomers, increasing absorption and resistance to enzymatic degradation (Soltero and Ekwuribe, 2001, Pharm. Technol. 6:110).
In still other embodiments, peptides may be modified for oral or inhalational administration by conjugation to certain proteins, such as the Fc region of IgG1 (see Examples 3-7). Methods for preparation and use of peptide-Fc conjugates are disclosed, for example, in Low et al. (2005, *Hum. Reprod.* 20:1805-13) and Dumont et al. (2005, *J. Aerosol. Med.* 18:294-303), each incorporated herein by reference. Low et al. (2005) disclose the conjugation of the alpha and beta subunits of FSH to the Fc region of IgG1 in single chain or heterodimer form, using recombinant expression in CHO cells. The Fc conjugated peptides were absorbed through epithelial cells in the lung or intestine by the neonatal Fc receptor mediated transport system. The Fc conjugated peptides exhibited improved stability and absorption *in vivo* compared to the native peptides. It was also observed that the heterodimer conjugate was more active than the single chain form.

**Proteins and Peptides**

A variety of polypeptides or proteins may be used within the scope of the claimed methods and compositions. In certain embodiments, the proteins may comprise antibodies or fragments of antibodies containing an antigen-binding site. As used herein, a protein, polypeptide or peptide generally refers, but is not limited to, a protein of greater than about 200 amino acids, up to a full length sequence translated from a gene; a polypeptide of greater than about 100 amino acids; and/or a peptide of from about 3 to about 100 amino acids. For convenience, the terms "protein," "polypeptide" and "peptide" are used interchangeably herein. Accordingly, the term "protein or peptide" encompasses amino acid sequences comprising at least one of the 20 common amino acids found in naturally occurring proteins, or at least one modified or unusual amino acid.

As used herein, an "amino acid residue" refers to any naturally occurring amino acid, any amino acid derivative or any amino acid mimic known in the art. In certain embodiments, the residues of the protein or peptide are sequential, without any non-amino acid interrupting the sequence of amino acid residues. In other embodiments, the sequence may comprise one or more non-amino acid moieties. In particular embodiments, the sequence of residues of the protein or peptide may be interrupted by one or more non-amino acid moieties.

Accordingly, the term "protein or peptide" encompasses amino acid sequences comprising at least one of the 20 common amino acids found in naturally occurring proteins,
or at least one modified or unusual amino acid, including but not limited to those shown below.

<table>
<thead>
<tr>
<th>Modified and Unusual Amino Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abbr.</strong></td>
</tr>
<tr>
<td>Aad</td>
</tr>
<tr>
<td>Baad</td>
</tr>
<tr>
<td>Bala</td>
</tr>
<tr>
<td>Abu</td>
</tr>
<tr>
<td>4Abu</td>
</tr>
<tr>
<td>Acp</td>
</tr>
<tr>
<td>Ahe</td>
</tr>
<tr>
<td>Aib</td>
</tr>
<tr>
<td>Baib</td>
</tr>
<tr>
<td>Apm</td>
</tr>
<tr>
<td>Dbu</td>
</tr>
<tr>
<td>Des</td>
</tr>
<tr>
<td>Dpm</td>
</tr>
<tr>
<td>Dpr</td>
</tr>
<tr>
<td>EtGly</td>
</tr>
</tbody>
</table>

[0071] Proteins or peptides may be made by any technique known to those of skill in the art, including the expression of proteins, polypeptides or peptides through standard molecular biological techniques, the isolation of proteins or peptides from natural sources, or the chemical synthesis of proteins or peptides. The nucleotide and protein, polypeptide and peptide sequences corresponding to various genes have been previously disclosed and may be found at computerized databases known to those of ordinary skill in the art. One such database is the National Center for Biotechnology Information's Genbank and GenPept databases (www.ncbi.nlm.nih.gov). The coding regions for known genes may be amplified and/or expressed using the techniques disclosed herein or as would be known to those of ordinary skill in the art. Alternatively, various commercial preparations of proteins, polypeptides, and peptides are known to those of skill in the art.
Peptide mimetics

[0072] Another embodiment for the preparation of polypeptides is the use of peptide mimetics. Mimetics are peptide-containing molecules that mimic elements of protein secondary structure. See, for example, Johnson et al, "Peptide Turn Mimetics" in BIOTECHNOLOGY AND PHARMACY, Pezzuto et al, Eds., Chapman and Hall, New York (1993), incorporated herein by reference. The rationale behind the use of peptide mimetics is that the peptide backbone of proteins exists chiefly to orient amino acid side chains so as to facilitate molecular interactions, such as those of antibody and antigen. A peptide mimic is expected to permit molecular interactions similar to the natural molecule.

Fusion proteins

[0073] Various embodiments may concern fusion proteins. These molecules generally have all or a substantial portion of a peptide, linked at the N- or C-terminus, to all or a portion of a second polypeptide or protein. Methods of generating fusion proteins are well known to those of skill in the art. Such proteins may be produced, for example, by chemical attachment using bifunctional cross-linking reagents, by de novo synthesis of the complete fusion protein, or by attachment of a DNA sequence encoding a first protein or peptide to a DNA sequence encoding a second peptide or protein, followed by expression of the intact fusion protein.

Synthetic Peptides

[0074] Proteins or peptides may be synthesized, in whole or in part, in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. See, for example, Stewart and Young, (1984, Solid Phase Peptide Synthesis, 2d. ed., Pierce Chemical Co.); Tam et al, (1983, J. Am. Chem. Soc, 105:6442); Merrifield, (1986, Science, 232: 341-347); and Barany and Merrifield (1979, The Peptides, Gross and Meienhofer, eds., Academic Press, New York, pp. 1-284). Short peptide sequences, usually from about 6 up to about 35 to 50 amino acids, can be readily synthesized by such methods. Alternatively, recombinant DNA technology may be employed wherein a nucleotide sequence which encodes a peptide of interest is inserted into an expression vector, transformed or transfected into an appropriate host cell, and cultivated under conditions suitable for expression.
Antibodies

Various embodiments may concern antibodies for a target. The term "antibody" is used herein to refer to any antibody-like molecule that has an antigen binding region, and includes antibody fragments such as Fab', Fab, F(ab')₂, single domain antibodies (DABs), Fv, scFv (single chain Fv), and the like. Techniques for preparing and using various antibody-based constructs and fragments are well known in the art. Means for preparing and characterizing antibodies are also well known in the art (See, e.g., Harlowe and Lane, 1988, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory). Antibodies of use may also be commercially obtained from a wide variety of known sources. For example, a variety of antibody secreting hybridoma lines are available from the American Type Culture Collection (ATCC, Manassas, VA). A large number of antibodies against various disease targets, including but not limited to tumor-associated antigens, have been deposited at the ATCC and are available for use in the claimed methods and compositions. (See, for example, U.S. Patent Nos. 7,060,802; 7,056,509; 7,049,060; 7,045,132; 7,041,803; 7,041,802; 7,041,293; 7,038,018; 7,037,498; 7,012,133; 7,001,598; 6,998,468; 6,994,976; 6,994,852; 6,989,241; 6,974,863; 6,965,018; 6,964,854; 6,962,981; 6,962,813; 6,956,107; 6,951,924; 6,949,244; 6,946,129; 6,943,020; 6,939,547; 6,921,645; 6,921,645; 6,921,533; 6,919,433; 6,919,078; 6,916,475; 6,905,681; 6,897,468; 6,887,466; 6,884,594; 6,881,405; 6,878,812; 6,875,580; 6,872,568; 6,867,006; 6,864,062; 6,861,511; 6,861,227; 6,861,226; 6,838,282; 6,835,549; 6,835,370; 6,824,780; 6,824,778; 6,812,206; 6,793,924; 8,783,758; 6,770,450; 6,767,711; 6,764,681; 6,764,679; 6,743,898; 6,733,981; 6,730,307; 6,720,15; 6,716,966; 6,709,653; 6,693,176; 6,692,908; 6,689,607; 6,689,362; 6,689,355; 6,682,737; 6,682,736; 6,682,734; 6,673,344; 6,652,852; 6,635,482; 6,630,144; 6,610,833; 6,610,294; 6,605,441; 6,605,279; 6,596,852; 6,592,868; 6,576,745; 6,572,856; 6,566,076; 6,562,618; 6,545,130; 6,544,749; 6,534,058; 6,528,625; 6,528,269; 6,521,227; 6,518,404; 6,511,665; 6,491,915; 6,488,930; 6,482,598; 6,482,408; 6,479,247; 6,468,531; 6,468,529; 6,465,173; 6,461,823; 6,458,356; 6,455,044; 6,455,040; 6,451,310; 6,444,206; 6,441,143; 6,432,404; 6,432,402; 6,419,928; 6,413,726; 6,406,694; 6,403,770; 6,403,091; 6,395,274; 6,383,759; 6,383,484; 6,376,654; 6,372,215; 6,359,126; 6,355,481; 6,355,444; 6,355,245; 6,355,244; 6,346,246; 6,344,198; 6,340,571; 6,340,459 each incorporated herein by reference with respect to the ATCC deposit number for the antibody-secreting hybridoma cell lines and the associated target antigens for the antibodies or fragments thereof.) These are exemplary only and a wide variety of other antibody-secreting hybridomas are known in the art. The
skilled artisan will realize that antibody-secreting hybridomas against almost any disease-associated antigen may be obtained by a simple search of the ATCC, PubMed and/or USPTO databases for antibodies against the selected disease-associated target of interest.

Production of Antibody Fragments

[0076] Some embodiments of the claimed methods and/or compositions may concern antibody fragments. Such antibody fragments may be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antibody fragments may be produced by enzymatic cleavage of antibodies with pepsin to provide F(ab')2 fragments. This fragment may be further cleaved using a thiol reducing agent and, optionally, followed by a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce Fab' monovalent fragments. Alternatively, an enzymatic cleavage using papain produces two monovalent Fab fragments and an Fc fragment. Exemplary methods for producing antibody fragments are disclosed in U.S. Pat. No. 4,036,945; U.S. Pat. No. 4,331,647; Nisonoff et al., 1960, Arch. Biochem. Biophys., 89:230; Porter, 1959, Biochem. J., 73:19; Edelman et al., 1967, METHODS IN ENZYMOLOGY, page 422 (Academic Press), and Coligan et al. (eds.), 1991, CURRENT PROTOCOLS IN IMMUNOLOGY, (John Wiley & Sons).

[0077] Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments or other enzymatic, chemical or genetic techniques also may be used, so long as the fragments bind to the antigen that is recognized by the intact antibody. For example, Fv fragments comprise an association of V_H and V_L chains. This association can be noncovalent, as described in Inbar et al., 1972, Proc. Nat'l. Acad. Sci. USA, 69:2659. Alternatively, the variable chains may be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde. See Sandhu, 1992, Crit. Rev. Biotech., 12:437.

[0078] Preferably, the Fv fragments comprise V_H and V_L chains connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising DNA sequences encoding the V_H and V_L domains, connected by an oligonucleotides linker sequence. The structural gene is inserted into an expression vector that is subsequently introduced into a host cell, such as E. coli. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains.

[0079] Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells. See Larrick et al., 1991, Methods: A Companion to Methods in Enzymology 2:106; Ritter et al. (eds.), 1995, MONOClonal Antibodies: Production, Engineering and Clinical Application, pages 166-179 (Cambridge University Press); Birch et al., (eds.), 1995, Monoclonal Antibodies: Principles and Applications, pages 137-185 (Wiley-Liss, Inc.). Where an antibody-secreting hybridoma cell line is publicly available, the CDR sequences encoding antigen-binding specificity may be obtained, incorporated into chimeric or humanized antibodies, and used.

Chimeric and Humanized Antibodies

[0080] A chimeric antibody is a recombinant protein in which the variable regions of a human antibody have been replaced by the variable regions of, for example, a mouse antibody, including the complementarity-determining regions (CDRs) of the mouse antibody. Chimeric antibodies exhibit decreased immunogenicity and increased stability when administered to a subject. Methods for constructing chimeric antibodies are well known in the art (e.g., Leung et al., 1994, Hybridoma 13:469).

[0081] A chimeric monoclonal antibody may be humanized by transferring the mouse CDRs from the heavy and light variable chains of the mouse immunoglobulin into the corresponding variable domains of a human antibody. The mouse framework regions (FR) in the chimeric monoclonal antibody are also replaced with human FR sequences. To preserve the stability and antigen specificity of the humanized monoclonal, one or more human FR residues may be replaced by the mouse counterpart residues. Humanized monoclonal antibodies may be used for therapeutic treatment of subjects. The affinity of humanized antibodies for a target may also be increased by selected modification of the CDR sequences

Other embodiments may concern non-human primate antibodies. General techniques for raising therapeutically useful antibodies in baboons may be found, for example, in Goldenberg et al., WO 91/1 465 (1991), and in Losman et al., Int. J. Cancer 46: 310 (1990).

Human Antibodies

Methods for producing fully human antibodies using either combinatorial approaches or transgenic animals transformed with human immunoglobulin loci are known in the art (e.g., Mancini et al., 2004, New Microbiol. 27:315-28; Conrad and Scheller, 2005, Comb. Chem. High Throughput Screen. 8:117-26; Brekke and Loset, 2003, Curr. Opin. Phamacol. 3:544-50; each incorporated herein by reference). Such fully human antibodies are expected to exhibit even fewer side effects than chimeric or humanized antibodies and to function in vivo as essentially endogenous human antibodies. In certain embodiments, the claimed methods and procedures may utilize human antibodies produced by such techniques.

In one alternative, the phage display technique may be used to generate human antibodies (e.g., Dantas-Barbosa et al., 2005, Genet. Mol. Res. 4:126-40, incorporated herein by reference). Human antibodies may be generated from normal humans or from humans that exhibit a particular disease state, such as cancer (Dantas-Barbosa et al., 2005). The advantage to constructing human antibodies from a diseased individual is that the circulating antibody repertoire may be biased towards antibodies against disease-associated antigens.

In one non-limiting example of this methodology, Dantas-Barbosa et al. (2005) constructed a phage display library of human Fab antibody fragments from osteosarcoma patients. Generally, total RNA was obtained from circulating blood lymphocytes (Id.). Recombinant Fab were cloned from the µ, γ and κ chain antibody repertoires and inserted into a phage display library (Id.). RNAs were converted to cDNAs and used to make Fab cDNA libraries using specific primers against the heavy and light chain immunoglobulin sequences (Marks et al., 1991, J. Mol. Biol. 222:581-97, incorporated herein by reference).
Library construction was performed according to Andris-Widhopf et al. (2000, In: Phage Display Laboratory Manual, Barbas et al. (eds), 1st edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY pp. 9.1 to 9.22, incorporated herein by reference). The final Fab fragments were digested with restriction endonucleases and inserted into the bacteriophage genome to make the phage display library. Such libraries may be screened by standard phage display methods, as known in the art. The skilled artisan will realize that this technique is exemplary only and any known method for making and screening human antibodies or antibody fragments by phage display may be utilized.

[0086] In another alternative, transgenic animals that have been genetically engineered to produce human antibodies may be used to generate antibodies against essentially any immunogenic target, using standard immunization protocols. A non-limiting example of such a system is the XenoMouse® (e.g., Green et al., 1999, J. Immunol. Methods 231:11-23, incorporated herein by reference) from Abgenix (Fremont, CA). In the XenoMouse® and similar animals, the mouse antibody genes have been inactivated and replaced by functional human antibody genes, while the remainder of the mouse immune system remains intact.

[0087] The XenoMouse® was transformed with germline-configured YACs (yeast artificial chromosomes) that contained portions of the human IgH and Igkappa loci, including the majority of the variable region sequences, along accessory genes and regulatory sequences. The human variable region repertoire may be used to generate antibody producing B cells, which may be processed into hybridomas by known techniques. A XenoMouse® immunized with a target antigen will produce human antibodies by the normal immune response, which may be harvested and/or produced by standard techniques discussed above. A variety of strains of XenoMouse® are available, each of which is capable of producing a different class of antibody. Such human antibodies may be coupled to other molecules by chemical cross-linking or other known methodologies. Transgenically produced human antibodies have been shown to have therapeutic potential, while retaining the pharmacokinetic properties of normal human antibodies (Green et al., 1999). The skilled artisan will realize that the claimed compositions and methods are not limited to use of the XenoMouse® system but may utilize any transgenic animal that has been genetically engineered to produce human antibodies.
Pre-Targeting

[0088] One strategy for use of bi-specific bioactive assemblies includes pre-targeting methodologies, in which an effector molecule is administered to a subject after a bi-specific assembly has been administered. The bi-specific assembly, which would include a binding site for an effector, hapten or carrier and one for the diseased tissue, localizes to the diseased tissue and increases the specificity of localization of the effector to the diseased tissue (U.S. Patent Application No. 20050002945). Because the effector molecule may be cleared from circulation much more rapidly than the bi-specific assembly, normal tissues may have a decreased exposure to the effector molecule when a pre-targeting strategy is used than when the effector molecule is directly linked to the disease targeting antibody.


[0090] In certain embodiments, bi-specific assemblies and targetable constructs may be of use in treating and/or imaging normal or diseased tissue and organs, for example using the methods described in U.S. Pat. Nos. 6,126,916; 6,077,499; 6,010,680; 5,776,095; 5,776,094; 5,776,093; 5,772,981; 5,753,206; 5,746,996; 5,697,902; 5,328,679; 5,128,119; 5,101,827; and 4,735,210, each incorporated herein by reference. Additional methods are described in
Aptamers

[0091] In certain embodiments, a precursor for bioactive assembly formation may comprise an aptamer. Methods of constructing and determining the binding characteristics of aptamers are well known in the art. For example, such techniques are described in U.S. Patent Nos. 5,582,981, 5,595,877 and 5,637,459, each incorporated herein by reference.

[0092] Aptamers may be prepared by any known method, including synthetic, recombinant, and purification methods, and may be used alone or in combination with other ligands specific for the same target. In general, a minimum of approximately 3 nucleotides, preferably at least 5 nucleotides, are necessary to effect specific binding. Aptamers of sequences shorter than 10 bases may be feasible, although aptamers of 10, 20, 30 or 40 nucleotides may be preferred.

[0093] Aptamers need to contain the sequence that confers binding specificity, but may be extended with flanking regions and otherwise derivatized. In preferred embodiments, the binding sequences of aptamers may be flanked by primer-binding sequences, facilitating the amplification of the aptamers by PCR or other amplification techniques. In a further embodiment, the flanking sequence may comprise a specific sequence that preferentially recognizes or binds a moiety to enhance the immobilization of the aptamer to a substrate.

[0094] Aptamers may be isolated, sequenced, and/or amplified or synthesized as conventional DNA or RNA molecules. Alternatively, aptamers of interest may comprise modified oligomers. Any of the hydroxyl groups ordinarily present in aptamers may be replaced by phosphonate groups, phosphate groups, protected by a standard protecting group, or activated to prepare additional linkages to other nucleotides, or may be conjugated to solid supports. One or more phosphodiester linkages may be replaced by alternative linking groups, such as P(O)O replaced by P(O)S, P(O)NR₂, P(O)R, P(O)OR₁, CO, or CNR₂, wherein R is H or alkyl (1-20C) and R’ is alkyl (1-20C); in addition, this group may be attached to adjacent nucleotides through O or S. Not all linkages in an oligomer need to be identical.

[0095] Methods for preparation and screening of aptamers that bind to particular targets of interest are well known, for example U.S. Pat. No. 5,475,096 and U.S. Pat. No. 5,270,163,
each incorporated by reference. The technique generally involves selection from a mixture of candidate aptamers and step-wise iterations of binding, separation of bound from unbound aptamers and amplification. Because only a small number of sequences (possibly only one molecule of aptamer) corresponding to the highest affinity aptamers exist in the mixture, it is generally desirable to set the partitioning criteria so that a significant amount of aptamers in the mixture (approximately 5-50%) is retained during separation. Each cycle results in an enrichment of aptamers with high affinity for the target. Repetition for between three to six selection and amplification cycles may be used to generate aptamers that bind with high affinity and specificity to the target.

Avimers

[0096] In certain embodiments, the peripheral modules and/or assemblies described herein may comprise one or more avimer sequences. Avimers are a class of binding proteins somewhat similar to antibodies in their affinities and specificities for various target molecules. They were developed from human extracellular receptor domains by in vitro exon shuffling and phage display. (Silverman et al., 2005, Nat. Biotechnol. 23:1493-94; Silverman et al., 2006, Nat. Biotechnol. 24:220.) The resulting multidomain proteins may comprise multiple independent binding domains that may exhibit improved affinity (in some cases sub-nanomolar) and specificity compared with single-epitope binding proteins. (Id.) In various embodiments, avimers may be attached to, for example, DDD and/or AD sequences for use in the claimed methods and compositions. Additional details concerning methods of construction and use of avimers are disclosed, for example, in U.S. Patent Application Publication Nos. 20040175756, 20050048512, 20050053973, 20050089932 and 20050221384, the Examples section of each of which is incorporated herein by reference.

Methods of Disease Tissue Detection, Diagnosis and Imaging

Protein-Based In Vitro Diagnosis

[0097] The present invention contemplates the use of bioactive assemblies to screen biological samples in vitro and/or in vivo for the presence of the disease-associated antigens. In exemplary immunoassays, a bioactive assembly comprising an antibody, fusion protein, or fragment thereof may be utilized in liquid phase or bound to a solid-phase carrier, as described below. Preferred embodiments, particularly those involving in vivo administration, the antibody or fragment thereof is humanized. Also preferred, the antibody
or fragment thereof is fully human. Still more preferred, the fusion protein comprises a humanized or fully human antibody. The skilled artisan will realize that a wide variety of techniques are known for determining levels of expression of a particular gene and any such known method, such as immunoassay, RT-PCR, mRNA purification and/or cDNA preparation followed by hybridization to a gene expression assay chip may be utilized to determine levels of expression in individual subjects and/or tissues. Exemplary in vitro assays of use include RIA, ELISA, sandwich ELISA, Western blot, slot blot, dot blot, and the like. Although such techniques were developed using intact antibodies, bioactive assemblies that incorporate antibodies, antibody fragments or other binding moieties may be used.

[0098] Bioactive assemblies incorporating antibodies, fusion proteins, antibody fragments and/or other binding moieties may also be used to detect the presence of a target antigen in tissue sections prepared from a histological specimen. Such in situ detection can be used to determine the presence of the antigen and to determine the distribution of the antigen in the examined tissue. In situ detection can be accomplished by applying a detectably-labeled assembly to frozen or paraffin-embedded tissue sections. General techniques of in situ detection are well-known to those of ordinary skill. See, for example, Ponder, "Cell Marking Techniques and Their Application," in MAMMALIAN DEVELOPMENT: A PRACTICAL APPROACH 113-38 Monk (ed.) (IRL Press 1987), and Coligan at pages 5.8.1-5.8.8.

[0099] Bioactive assemblies can be detectably labeled with any appropriate marker moiety, for example, a radioisotope, an enzyme, a fluorescent label, a dye, a chromogen, a chemiluminescent label, a bioluminescent label or a paramagnetic label.

[0100] The marker moiety may be a radioisotope that is detected by such means as the use of a gamma counter or a beta-scintillation counter or by autoradiography. In a preferred embodiment, the diagnostic conjugate is a gamma-, beta- or a positron-emitting isotope. A marker moiety refers to a molecule that will generate a signal under predetermined conditions. Examples of marker moieties include radioisotopes, enzymes, fluorescent labels, chemiluminescent labels, bioluminescent labels and paramagnetic labels. The binding of marker moieties to bioactive assemblies can be accomplished using standard techniques known to the art. Typical methodology in this regard is described by Kennedy et al., Clin. Chim. Acta 70: 1 (1976), Schurs et al., Clin. Chim. Acta 81: 1 (1977), Shih et al., Infl J. Cancer 46: 1101 (1990).
Methods of diagnostic imaging with labeled peptides or MAbs are well-known. For example, in the technique of immunoscintigraphy, ligands or antibodies are labeled with a gamma-emitting radioisotope and introduced into a patient. A gamma camera is used to detect the location and distribution of gamma-emitting radioisotopes. See, for example, Srivastava (ed.), RADIOLABELED MONOCLONAL ANTIBODIES FOR IMAGING AND THERAPY (Plenum Press 1988), Chase, "Medical Applications of Radioisotopes," in REMINGTON'S PHARMACEUTICAL SCIENCES, 18th Edition, Gennaro et al. (eds.), pp. 624-652 (Mack Publishing Co., 1990), and Brown, "Clinical Use of Monoclonal Antibodies," in BIOTECHNOLOGY AND PHARMACY 227-49, Pezzuto et al. (eds.) (Chapman & Hall 1993). Also preferred is the use of positron-emitting radionuclides (PET isotopes), such as with an energy of 511 keV, such as $^{18}$F, $^{68}$Ga, $^{64}$Cu, and $^{124}$I. Such imaging can be conducted by direct labeling of the bioactive assembly, or by a pretargeted imaging method, as described in Goldenberg et al, "Antibody Pre-targeted Advances Cancer Radioimmunodetection and Radioimmunotherapy," (J Clin Oncol 2006;24:823-834), see also U.S. Patent Publication Nos. 20050002945, 20040018557, 20030148409 and 20050014207, each incorporated herein by reference.

The radiation dose delivered to the patient is maintained at as low a level as possible through the choice of isotope for the best combination of minimum half-life, minimum retention in the body, and minimum quantity of isotope which will permit detection and accurate measurement. Examples of radioisotopes that are appropriate for diagnostic imaging include $^{99m}$Tc and $^{111}$In.

The bioactive assemblies, or haptens or carriers that bind to them, also can be labeled with paramagnetic ions and a variety of radiological contrast agents for purposes of in vivo diagnosis. Contrast agents that are particularly useful for magnetic resonance imaging comprise gadolinium, manganese, dysprosium, lanthanum, or iron ions. Additional agents include chromium, copper, cobalt, nickel, rhenium, europium, terbium, holmium, or neodymium. ligands, antibodies and fragments thereof can also be conjugated to ultrasound contrast/enhancing agents. For example, one ultrasound contrast agent is a liposome that comprises a humanized IgG or fragment thereof. Also preferred, the ultrasound contrast agent is a liposome that is gas filled.
Imaging agents and radioisotopes

[0104] Many appropriate imaging agents are known in the art, as are methods for their attachment to proteins or peptides (see, e.g., U.S. patents 5,021,236 and 4,472,509, both incorporated herein by reference). Certain attachment methods involve the use of a metal chelate complex employing, for example, an organic chelating agent such as DTPA attached to the protein or peptide (U.S. Patent 4,472,509). Proteins or peptides also may be reacted with an enzyme in the presence of a coupling agent such as glutaraldehyde or periodate. Conjugates with fluorescein markers are prepared in the presence of these coupling agents or by reaction with an isothiocyanate.

[0105] Non-limiting examples of paramagnetic ions of potential use as imaging agents include chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III) and erbium (III), with gadolinium being particularly preferred. Ions useful in other contexts, such as X-ray imaging, include but are not limited to lanthanum (III), gold (III), lead (II), and especially bismuth (III).

[0106] Radioisotopes of potential use as imaging or therapeutic agents include astatine$^{211}$, carbon$^{14}$, chromium$^{51}$, chlorine$^{36}$, cobalt$^{57}$, cobalt$^{58}$, copper$^{62}$, copper$^{64}$, copper$^{67}$, Eu$^{152}$, fluorine$^{18}$, gallium$^{67}$, gallium$^{68}$, hydrogen$^{3}$, iodine$^{123}$, iodine$^{124}$, iodine$^{125}$, iodine$^{131}$, indium$^{111}$, iron$^{52}$, iron$^{59}$, lutetium$^{177}$, phosphorus$^{32}$, phosphorus$^{33}$, rhenium$^{186}$, rhenium$^{188}$, Sc$^{47}$, selenium$^{74}$, silver$^{111}$, sulphur$^{35}$, technetium$^{99m}$, technetium$^{99}$, yttrium$^{86}$ and yttrium$^{90}$, and zirconium$^{89}$. I$^{125}$ is often being preferred for use in certain embodiments, and technetium$^{99m}$ and indium$^{111}$ are also often preferred due to their low energy and suitability for long-range detection.

[0107] Radioactively labeled proteins or peptides may be produced according to well-known methods in the art. For instance, they can be iodinated by contact with sodium or potassium iodide and a chemical oxidizing agent such as sodium hypochlorite, or an enzymatic oxidizing agent, such as lactoperoxidase. Proteins or peptides may be labeled with technetium$^{99m}$ by ligand exchange process, for example, by reducing pertechnate with stannous solution, chelating the reduced technetium onto a Sephadex column and applying the peptide to this column or by direct labeling techniques, e.g., by incubating pertechnate, a reducing agent such as SNCI$_2$, a buffer solution such as sodium-potassium phthalate solution,
and the peptide. Intermediary functional groups which are often used to bind radioisotopes which exist as metallic ions to peptides include diethyleneetriaminepentaacetic acid (DTPA), DOTA, NOTA, porphyrin chelators and ethylene diaminetetraacetic acid (EDTA). Also contemplated for use are fluorescent labels, including rhodamine, fluorescein isothiocyanate and renographin.

[0108] In certain embodiments, the proteins or peptides may be linked to a secondary binding ligand or to an enzyme (an enzyme tag) that will generate a colored product upon contact with a chromogenic substrate. Examples of suitable enzymes include urease, alkaline phosphatase, (horseradish) hydrogen peroxidase and glucose oxidase. Preferred secondary binding ligands are biotin and avidin or streptavidin compounds. The use of such labels is well known to those of skill in the art in light and is described, for example, in U.S. Patents 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149 and 4,366,241; each incorporated herein by reference. These fluorescent labels are preferred for in vitro uses, but may also be of utility in in vivo applications, particularly endoscopic or intravascular detection procedures.

[0109] In alternative embodiments, ligands, antibodies, or other proteins or peptides may be tagged with a fluorescent marker. Non-limiting examples of photodetectable labels include Alexa 350, Alexa 430, AMCA, aminoacridine, BODIPY 630/650, BODIPY 650/665, BODIPY-FL, BODIPY-R6G, BODIPY-TMR, BODIPY-TRX, 5-carboxy-4',5'-dichloro-2,7L dimethoxy fluorescein, 5-carboxy-2',4',5',7'-tetrachlorofluorescein, 5-carboxyfluorescein, 5-carboxyrhodamine, 6-carboxyrhodamine, 6-carboxytetramethyl amino, Cascade Blue, Cy2, Cy3, Cy5,6-FAM, dansyl chloride, Fluorescein, HEX, 6-JOE, NBD (7-nitrobenz-2-oxa-1,3-diazole), Oregon Green 488, Oregon Green 500, Oregon Green 514, Pacific Blue, phthalic acid, terephthalic acid, isophthalic acid, cresyl fast violet, cresyl blue violet, brilliant cresyl blue, para-aminobenzoic acid, erythrosine, phthalocyanines, azomethines, cyanines, xanthines, succinylfluoresceins, rare earth metal cryptates, europium trisbipyridine diamine, a europium cryptate or chelate, diame, dicyanins, La Jolla blue dye, allopycocyanin, allococyanin B, phycocyanin C, phycocyanin R, thiamine, phycoerythrocyanin, phycoerythrin R, REG, Rhodamine Green, rhodamine isothiocyanate, Rhodamine Red, ROX, TAMRA, TET, TRIT (tetramethyl rhodamine isothiol), Tetramethylrhodamine, Edans and Texas Red. These and other luminescent labels may be obtained from commercial sources such as Molecular Probes (Eugene, OR), and EMD Biosciences (San Diego, CA).
[0110] Chemiluminescent labeling compounds of use may include luminol, isoluminol, an aromatic acridinium ester, an imidazole, an acridinium salt and an oxalate ester, or a bioluminescent compound such as luciferin, luciferase and aequorin. Diagnostic conjugates may be used, for example, in intraoperative, endoscopic, or intravascular tumor or disease diagnosis.

[0111] In various embodiments, labels of use may comprise metal nanoparticles. Methods of preparing nanoparticles are known. (See e.g., U.S. Patent Nos. 6,054,495; 6,127,120; 6,149,868; Lee and Meisel, J. Phys. Chem. 86:3391-3395, 1982.) Nanoparticles may also be obtained from commercial sources (e.g., Nanoprobes Inc., Yaphank, NY; Polysciences, Inc., Warrington, PA). Modified nanoparticles are available commercially, such as Nanogold® nanoparticles from Nanoprobes, Inc. (Yaphank, NY). Functionalized nanoparticles of use for conjugation to proteins or peptides may be commercially obtained.

**Therapeutic agents**

*Pharmaceutical Compositions*

[0112] In some embodiments, a bioactive assembly and/or one or more other therapeutic agents may be administered to a subject, such as a subject with cancer. Such agents may be administered in the form of pharmaceutical compositions. Generally, this will entail preparing compositions that are essentially free of impurities that could be harmful to humans or animals. One skilled in the art would know that a pharmaceutical composition can be administered to a subject by various routes including, for example, orally or parenterally, such as intravenously.

[0113] In certain embodiments, an effective amount of a therapeutic agent must be administered to the subject. An "effective amount" is the amount of the agent that produces a desired effect. An effective amount will depend, for example, on the efficacy of the agent and on the intended effect. For example, a lesser amount of an antiangiogenic agent may be required for treatment of a hyperplastic condition, such as macular degeneration or endometriosis, compared to the amount required for cancer therapy in order to reduce or eliminate a solid tumor, or to prevent or reduce its metastasizing. An effective amount of a particular agent for a specific purpose can be determined using methods well known to those in the art.
Chemotherapeutic Agents

[0114] In certain embodiments, chemotherapeutic agents may be administered. Anti-cancer chemotherapeutic agents of use include, but are not limited to, 5-fluorouracil, bleomycin, busulfan, camptothecins, carboplatin, chlorambucil, cisplatin (CDDP), cyclophosphamide, dactinomycin, daunorubicin, doxorubicin, estrogen receptor binding agents, etoposide (VP 16), farnesyl-protein transferase inhibitors, gemcitabine, ifosfamide, mechlorethamine, melphalan, methotrexate, mitomycin, navelbine, nitrosurea, plicomycin, procarbazine, raloxifene, tamoxifen, taxol, temazolomide (an aqueous form of DTIC), transplatinum, vinblastine and methotrexate, vincristine, or any analog or derivative variant of the foregoing. Chemotherapeutic agents of use against infectious organisms include, but are not limited to, acyclovir, albendazole, amantadine, amikacin, amoxicillin, amphotericin B, ampicillin, aztreonam, azithromycin, bacitracin, bactrim, Batrafen®, bifonazole, carbenicillin, caspofungin, cefaclor, cefazolin, cephalosporins, cefepime, ceftriaxone, cefotaxime, chloramphenicol, cidofovir, Cipro®, clarithromycin, clavulanic acid, clotrimazole, cloxacillin, doxycycline, econazole, erythrocycline, erythromycin, flagyl, fluconazole, flucytosine, foscarvet, furazolidone, ganciclovir, gentamycin, imipenem, isoniazid, itraconazole, kanamycin, ketoconazole, linecomycin, linezolid, meropenem, miconazole, minocycline, naftifme, nalidixic acid, neomycin, netilmicin, nitrofurantoin, nystatin, oseltamivir, oxacillin, paromomycin, penicillin, pentamidine, pipercillin-tazobactam, rifabutin, rifampin, rimantadine, streptomycin, sulfamethoxazole, sulfasalazine, tetracycline, tioconazole, tobramycin, tolciclate, tolnaftate, trimethoprim sulfamethoxazole, valacyclovir, vancomycin, zanamir, and zithromycin.

[0115] Chemotherapeutic agents and methods of administration, dosages, etc., are well known to those of skill in the art (see for example, the "Physicians Desk Reference", Goodman & Gilman's "The Pharmacological Basis of Therapeutics" and in "Remington's Pharmaceutical Sciences", incorporated herein by reference in relevant parts). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.
Hormones

Corticosteroid hormones can increase the effectiveness of other chemotherapy agents, and consequently, they are frequently used in combination treatments. Prednisone and dexamethasone are examples of corticosteroid hormones. Progestins, such as hydroxyprogesterone caproate, medroxyprogesterone acetate, and megestrol acetate, have been used in cancers of the endometrium and breast. Estrogens such as diethylstilbestrol and ethinyl estradiol have been used in cancers such as prostate cancer. Antiestrogens such as tamoxifen have been used in cancers such as breast cancer. Androgens such as testosterone propionate and fluoxymesterone have also been used in treating breast cancer.

Angiogenesis inhibitors

In certain embodiments, anti-angiogenic agents, such as angiotatin, baculostatin, canstatin, maspin, anti-VEGF antibodies, anti-PIGF peptides and antibodies, anti-vascular growth factor antibodies, anti-Flk-1 antibodies, anti-Fit-1 antibodies and peptides, laminin peptides, fibronectin peptides, plasminogen activator inhibitors, tissue metalloproteinase inhibitors, interferons, interleukin-12, IP-10, Gro-β, thrombospondin, 2-methoxyoestradiol, proliferin-related protein, carboxiamidotriazole, CMIOI, Marimastat, pentosan polysulphate, angiopoietin-2, interferon-alpha, herbimycin A, PNU145156E, 16K prolactin fragment, Linomide, thalidomide, pentoxifylline, genistein, TNP-470, endostatin, paclitaxel, accutin, angiotatin, cidofovir, vincristine, bleomycin, AGM-1470, platelet factor 4 or minocycline maybe of use.

Immunomodulators

As used herein, the term "immunomodulator" includes cytokines, stem cell growth factors, lymphotoxins and hematopoietic factors, such as interleukins, colony-stimulating factors, interferons (e.g., interferons-α, -β and -γ) and the stem cell growth factor designated "SI factor." Examples of suitable immunomodulator moieties include IL-2, IL-6, IL-10, IL-12, IL-18, IL-21, interferon-gamma, TNF-alpha, and the like.

The term "cytokine" is a generic term for proteins or peptides released by one cell population which act on another cell as intercellular mediators. As used broadly herein, examples of cytokines include lymphokines, monokines, growth factors and traditional polypeptide hormones. Included among the cytokines are growth hormones such as human
growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; prostaglandin, fibroblast growth factor; prolactin; placental lactogen, OB protein; tumor necrosis factor-α and -β; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-B; platelet-growth factor; transforming growth factors (TGFs) such as TGF-α and TGF-β; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-α, -β, and -γ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-21, LIF, G-CSF, GM-CSF, M-CSF, EPO, kit-ligand or FLT-3, angiostatin, thrombospondin, endostatin, tumor necrosis factor and LT. As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

[0120] Chemokines generally act as chemoattractants to recruit immune effector cells to the site of chemokine expression. Chemokines include, but are not limited to, RANTES, MCAF, MIP-α, MIP-β, and IP-10. The skilled artisan will recognize that certain cytokines are also known to have chemoattractant effects and could also be classified under the term chemokines. Similarly, the terms immunomodulator and cytokine overlap in their respective members.

**Radioisotope therapy and Radioimmunotherapy**

[0121] In some embodiments, the peptides and/or proteins may be of use in radionuclide therapy or radioimmunotherapy methods (see, e.g., Govindan et al., 2005, *Technology in Cancer Research & Treatment*, 4:375-91; Sharkey and Goldenberg, 2005, *J. Nucl. Med.* 46:1 15S-127S; Goldenberg et al. (J Clin Oncol 2006; 24:823-834), "Antibody Pre-targeting Advances Cancer Radioimmunodetection and Radioimmunotherapy," each incorporated herein by reference.) In specific embodiments, bioactive assemblies may be directly tagged with a radioisotope of use and administered to a subject. In alternative embodiments, radioisotope(s) may be administered in a pre-targeting method as discussed above, using a
haptenic peptide or Hg and that is radiolabeled and injected after administration of a bispecific bioactive assembly that localizes at the site of elevated expression in the diseased tissue.

Radioactive isotopes useful for treating diseased tissue include, but are not limited to: $^{111}$In, $^{177}$Lu, $^{212}$Bi, $^{213}$Bi, $^{211}$At, $^{62}$Cu, $^{67}$Cu, $^{90}$Y, $^{125}$I, $^{131}$I, $^{32}$P, $^{33}$P, $^{47}$Sc, $^{111}$Ag, $^{67}$Ga, $^{142}$Pr, $^{153}$Sm, $^{161}$Tb, $^{166}$Ho, $^{168}$Re, $^{188}$Re, $^{189}$Re, $^{212}$Pb, $^{223}$Ra, $^{225}$Ac, $^{59}$Fe, $^{75}$Se, $^{77}$As, $^{89}$Sr, $^{99}$Mo, $^{105}$Rh, $^{109}$Pd, $^{143}$Pr, $^{149}$Pm, $^{169}$Er, $^{194}$Ir, $^{198}$Au, $^{199}$Au, and $^{211}$Pb. The therapeutic radionuclide preferably has a decay energy in the range of 20 to 6,000 keV, preferably in the ranges 60 to 200 keV for an Auger emitter, 100-2,500 keV for a beta emitter, and 4,000-6,000 keV for an alpha emitter. Maximum decay energies of useful beta-particle-emitting nuclides are preferably 20-5,000 keV, more preferably 100-4,000 keV, and most preferably 500-2,500 keV. Also preferred are radionuclides that substantially decay with Auger-emitting particles. For example, Co-58, Ga-67, Br-80m, Tc-99m, Rh-103m, Pt-109, In-111, Sb-119, 1-125, Ho-161, Os-189m and Ir-192. Decay energies of useful beta-particle-emitting nuclides are preferably <1,000 keV, more preferably <100 keV, and most preferably <70 keV. Also preferred are radionuclides that substantially decay with generation of alpha-particles. Such radionuclides include, but are not limited to: Dy-152, At-211, Bi-212, Ra-223, Rn-219, Po-215, Bi-211, Ac-225, Fr-221, At-217, Bi-213 and Fm-255. Decay energies of useful alpha-particle-emitting radionuclides are preferably 2,000-10,000 keV, more preferably 3,000-8,000 keV, and most preferably 4,000-7,000 keV.

For example, $^{67}$Cu, considered one of the more promising radioisotopes for radioimmunotherapy due to its 61.5 hour half-life and abundant supply of beta particles and gamma rays, can be conjugated to a protein or peptide using the chelating agent p-bromoacetamido-benzyl-tetraethylaminetetraacetic acid (TETA). Alternatively, $^{90}$Y, which emits an energetic beta particle, can be coupled to a peptide, antibody, fusion protein, or fragment thereof, using diethylentriaminepentaacetic acid (DTPA).

Additional potential radioisotopes include $^{11}$C, $^{13}$N, $^{15}$O, $^{75}$Br, $^{198}$Au, $^{224}$Ac, $^{126}$I, $^{131}$I, $^{77}$Br, $^{111}$In, $^{95}$Ru, $^{97}$Ru, $^{103}$Ru, $^{105}$Ru, $^{107}$Hg, $^{203}$Hg, $^{122}$Te, $^{125}$Te, $^{165}$Tm, $^{167}$Tm, $^{168}$Tm, $^{197}$Pt, $^{109}$Pd, $^{105}$Rh, $^{142}$Pr, $^{143}$Pr, $^{161}$Tb, $^{166}$Ho, $^{199}$Au, $^{57}$Co, $^{58}$Co, $^{51}$Cr, $^{59}$Fe, $^{75}$Se, $^{201}$Tl, $^{225}$Ac, $^{76}$Br, $^{169}$Y and the like.
In another embodiment, a radiosensitizer can be used. The addition of the radiosensitizer can result in enhanced efficacy. Radiosensitizers are described in D. M. Goldenberg (ed.), CANCER THERAPY WITH RADIOLABELED ANTIBODIES, CRC Press (1995), which is incorporated herein by reference in its entirety.

The peptide, antibody, antibody fragment, or fusion protein that has a boron addend-loaded carrier for thermal neutron activation therapy will normally be effected in similar ways. However, it will be advantageous to wait until non-targeted immunoconjugate clears before neutron irradiation is performed. Clearance can be accelerated using an antibody that binds to the ligand. See U.S. Pat. No. 4,624,846 for a description of this general principle. For example, boron addends such as carboranes, can be attached to antibodies. Carboranes can be prepared with carboxyl functions on pendant side chains, as is well-known in the art. Attachment of carboranes to a carrier, such as aminodextran, can be achieved by activation of the carboxyl groups of the carboranes and condensation with amines on the carrier. The intermediate conjugate is then conjugated to the antibody. After administration of the conjugate, a boron addend is activated by thermal neutron irradiation and converted to radioactive atoms which decay by alpha-emission to produce highly toxic, short-range effects.

Kits

Various embodiments may concern kits containing components suitable for treating or diagnosing diseased tissue in a patient. Exemplary kits may contain at least one bioactive assembly. If the composition containing components for administration is not formulated for delivery via the alimentary canal, such as by oral delivery, a device capable of delivering the kit components through some other route may be included. One type of device, for applications such as parenteral delivery, is a syringe that is used to inject the composition into the body of a subject. Inhalation devices may also be used.

The kit components may be packaged together or separated into two or more separate containers. In some embodiments, the containers may be vials that contain sterile, lyophilized formulations of a composition that are suitable for reconstitution. A kit may also contain one or more buffers suitable for reconstitution and/or dilution of other reagents. Other containers that may be used include, but are not limited to, a pouch, tray, box, tube, or the
like. Kit components may be packaged and maintained steriley within the containers. Another component that can be included is instructions to a person using a kit for its use.

EXAMPLES

[0129] The following examples are provided to illustrate, but not to limit the claims of the present invention.

Example 1. Generation of a fusion protein comprising a heat shock protein, AD2 and AD3 (type-a adaptor module) and use

[0130] A fusion protein in which AD2 and AD3 are linked, respectively, to the amino and carboxyl termini of a heat shock protein such as HSP70 or gp96 can be further docked and locked with two peripheral modules, one consisting of a DDD2-lmked homodimer (X₂) and the other consisting of a DDD3C-linked homodimer (Y₂) to form a complex composed of X₂ (Ma)Y₂. One choice of the two peripheral modules is the Ig-like domains 1 and 2 of human CD22 and the extracellular region of human CD20, which upon conjugation to the HSP-based adaptor module is of use as a therapeutic vaccine for B cell lymphomas. Another choice of the two peripheral modules is the N-Al and the A3-B3 domains of CEACAM5, which upon conjugation to the HSP-based adaptor module is of use as a therapeutic vaccine for CEA-expressing cancers. The two peripheral modules can also be hLL1 Fab and the extracellular region of HER2, which upon conjugation to the HSP-based adaptor module is of use as a therapeutic vaccine for cancers over-expressing HER2.

[0131] A vaccine comprising AD2-HSP70-AD3 stably linked to the N-Al and the A3-B3 domains of CEACAM5 produced as described above is formulated in saline or other physiologically compatible solution and administered to patients following surgical removal of colorectal cancer. The therapeutic vaccine is given once weekly for a minimal of four weeks at a dosage in the range of 100 and 5000 µg, with the preferred dosage being about 500 µg. The route of injection is subcutaneous but the site of injection can be varied each time with the same site of injection repeated after a gap of one or more injections. For example, the first injection is given on the left thigh, the second injection on the right thigh, the third injection on the left arm, the fourth injection on the right arm, the fifth injection on the left thigh, the sixth injection on the right arm, etc. After the first cycle of four weekly injections, two more injections are given biweekly, followed by a regimen of monthly
injections. The effect of the vaccine on eliciting anti-cancer immune response is evaluated by measuring (1) delayed hypersensitivity as an assessment of cellular immunity; (2) activity of cytolytic T cells in vitro; (3) levels of circulating CEA; (4) changes in tumor size using various imaging techniques such as CT scan; and (5) other biomarkers associated with CEA-expressing cancers.

Example 2. Treating colorectal cancer with a vaccine comprising AD2-HSP70-AD3 stably linked to the N-Al and the A3-B3 domains of CEACAM5

[0132] Patient DN is a 62-year-old male with a resected left colon carcinoma of 4 cm in diameter, diagnosed with T2N1M0 disease refused post-operative chemotherapy and is given an experimental vaccine treatment. The patient receives four weekly injections of the vaccine comprising AD2-HSP70-AD3 stably linked to the N-Al and the A3-B3 domains of CEACAM5 in saline at a dosage of 500 μg, followed by two bi-weekly injections of the same dosage and thereafter, one monthly injection of the same dosage. The first injection is given on the left arm, the second on the right arm, the third on the right thigh, and the fourth on the left thigh. The site of the injection is then repeated. The patient is premedicated with Tylenol and antihistamines in order to mitigate any side effects.

[0133] During the injections, only grade 1 or 2 local erythema or itching at the injection sites is noted, and shortness of breadth after the fourth injection, all resolving within 4 hours. At the follow-up of 3, 6, and 12 months, which includes diagnostic imaging tests (CT and one FDG-PET study at 12 months) and serum CEA assays, no abnormalities are detected. At the 2-year follow-up, the patient is considered to be free of disease, and avoided the side effects of aggressive chemotherapy by having this experimental vaccine.

Example 3. Generation of a polypeptide comprising DDD2-hPl-DDD3C (type-b adaptor module) and use

[0134] A polypeptide in which DDD2 and DDD3C are fused, respectively, to the amino and carboxyl termini of hPl will self-associate into a structure composed of a homodimer of hPl linked via disulfide bonds, which upon reduction with a thiol-containing agent is further docked and locked with two peripheral modules, one consisting of an entity derivatized with AD2 (X) and the other consisting of an entity derivatized with AD3 (Y) to form a complex composed of X(hPl)₂Y. Suitable choices for X and Y include receptor-targeting ligands, antibody fragments, and immunostimulatory molecules. For example, a construct of X(hPl)₂Y in which one of the two peripheral modules is based on anti-hTfR (human
transferin receptor) Fab and the other anti-hIR (human insulin receptor) Fab can be used to carry therapeutic siRNAs or genes across the blood brain barrier (BBB) and further into glioma cells to treat brain cancers. (Zhang et al., Clin Cancer Res, 2004, 10: 3667-3677).

**Example 4. Generation of a polypeptide comprising DDD3-hPl-AD2 (type-c adaptor module) and use**

[0135] A polypeptide in which DDD3 and AD2 are fused, respectively, to the amino and carboxyl termini of hPl will self-associate into a homodimer of hPl, which can be docked and locked with two identical DDD2-linked peripheral modules (X2) to form a complex composed of X2(hPl)2X2. One area of particular promise for a construct of X2(hPl)2X2 is to deliver nonviral vectors across the blood-brain barrier for gene therapy of brain disorders. For example, a construct of X2(iPl)2X2 in which the peripheral module (X) is based on anti-hTfR Fab can be used to carry a DNA vector encoding the gene of tyrosine hydroxylase across the BBB for treating Parkinson's disease (Pardridge, NeuroRx®, 2005, 2: 129-138).

**Example 5. Generation of a polypeptide comprising DDD2-CH2-CH3-DDD3C (type-b adaptor module) and use**

[0136] A polypeptide in which DDD2 and DDD3C are fused, respectively, to the amino and carboxyl termini of the CH2 and CH3 domains of human IgGl, will self-associate into a structure composed of two Fc subunits linked via disulfide bonds, which can be converted into a single Fc subunit upon reduction with a thiol-containing agent and further docked and locked with two peripheral modules, one consisting of an entity derivatized with AD2 (X) and the other consisting of an entity derivatized with AD3 (Y) to form a complex composed ofX(Fc)Y. When the two AD-containing entities are each derived from a Fab of different specificity, the resulting assembly is an IgG-like bispecific antibody with an intact Fc.

**Example 6. Generation of a polypeptide comprising DDD3-CH2-CH3-AD2 (type-c adaptor module) and use**

[0137] A polypeptide in which DDD3 and AD2 are fused, respectively, to the amino and carboxyl termini of the CH2 and CH3 domains of human IgGl, will self-associate into an Fc-containing structure, which can be docked and locked with two identical DDD2-linked peripheral modules (X2) to form a complex composed ofX2(Fc)X2.
Example 7. Generation of a polypeptide comprising DDD3C-CH2-CH3-AD2 (type-d adaptor module) and use

A polypeptide in which DDD3C and AD2 are fused, respectively, to the amino and carboxyl termini of the CH2 and CH3 domains of human IgG1, will self-associate into a structure composed of two Fc subunits linked via disulfide bonds, which can be converted into a single Fc subunit upon reduction with a thiol-containing agent and docked and locked with three peripheral modules, two of which are identical DDD2-linked homodimers (X2) and the third consists of an AD3-linked entity (Y), to form a complex composed of X2(Fc)YX2.

Example 8. Molecular engineering of DDD3-CH2-CH3-AD2 and DDD3C-CH2-CH3-AD2

Two PCR reactions were performed to generate the DDD3 and DDD3C sequences using a human RIa cDNA clone (Invitrogen IMAGE clone #5531 156) as a template. Both reactions used the oligonucleotide RIBgIIIright as the 3' PCR primer. The oligonucleotides RIBspHIleft and RICBspHIleft were used as 5' primers for DDD3 and DDD3C, respectively.

BiBgIIRight

BI-BspH1left

Bi-CBspHIleft

FcBgIUleft

The Fc (CH2 and CH3 domains) was amplified using the pdHL2 vector as a template and the oligonucleotides FcBgIIIleft and Fc Bam-EcoRIright as primers.

FcBgIUleft

BlBgIIIright

BIBspH1left

Bi-CBspHIleft

[0140] 5'-AGATCTGCTTTCCTCCTCCTCCTCTC-S' (SEQ ID NO:11)

[0141] 5'-TCATGAGCTTCGAGAATGTGAGCTC-S' (SEQ ID NO:12)

[0142] 5'-TCATGAGTTGTGGCGGAAGCCTTCGAGAATGTGAGC-S' (SEQ ID NO:13)

[0143] 5'-AGATCTGGCGCACCTGAACTCCTG-S' (SEQ ID NO:14)
Each of the amplimers was cloned in the pGemT PCR cloning vector. The Fc insert fragment was excised from pGemT with BgIII and EcoRI restriction enzymes and cloned into those same sites in the SV3 shuttle vector to generate the intermediate clone Fc-SV3.

The DDD3 and DDD3C inserts were then excised from the pGemT vectors with BspHI and BgIII and ligated with Fc-SV3 vector that was digested with Ncol (BspHI compatible ends) and BgIII to generate the shuttle vectors DDD3-Fc-SV3 and DDD3C-Fc-SV3, respectively. Finally, the expression cassettes were excised from the SV3 shuttle vectors with Xbal and BamHI and ligated with AD2-pdHL2 vector that was prepared by digestion of h679-AD2-pdHL2 with Xbal and BamHI. The final expression constructs are DDD3-Fc-AD2-pDHL2 and DDD3C-Fc-AD2-pdHL2.

The amino acid sequence of DDD3C-Fc-AD2 is shown in Figure 6. The amino acid sequence of DDD3-Fc-AD2 is the same except that the 5 amino-terminal residues of DDD3C-Fc-AD2, namely MSCGG, are replaced with MS.

Both expression vectors are transfected into Sp/EEE cells. Positive clones are screened by ELISA using Protein-A coated plates for capture and HRP-conjugated antibody for detection. Purification is accomplished using protein-A affinity chromatography.

Example 9. Treating focal, transient brain ischemia with an agent composed of DDD3C-CH2-CH3-AD2 linked to brain-derived neurotrophic factor (BDNF) and a monoclonal antibody to human transferrin receptor (hTfR)

Within one hour of onset of the symptoms of a stroke, patient TF is given an i.v. injection of 10 mg of the complex comprising four BDNF-DDD2 modules and one anti-hTfR Fab-AD3 module stably linked to the DDD3C-Fc-AD2 module in saline. The timely intervention reduces the total hemisphere infarct volume as shown by MRI, with the presenting signs and symptoms of partial limb paralysis, speech difficulty, confusion improving markedly within 48 hours while the patient also receives other supportive and anti-coagulant therapy.
Table 1. Compositions of selected $X_2(Ma)Y_2$ assemblies

<table>
<thead>
<tr>
<th>Ma</th>
<th>X</th>
<th>Y</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSP70</td>
<td>Anti-CD74 Fab</td>
<td>N-A1-B1</td>
<td>CEA cancer vaccine</td>
</tr>
<tr>
<td></td>
<td>Anti-CD74 Fab</td>
<td>A3-B3</td>
<td>CEA cancer vaccine</td>
</tr>
<tr>
<td></td>
<td>Anti-CD205 fab</td>
<td>N-A1-B1</td>
<td>CEA cancer vaccine</td>
</tr>
<tr>
<td></td>
<td>Anti-CD205 Fab</td>
<td>A3-B3</td>
<td>CEA cancer vaccine</td>
</tr>
<tr>
<td></td>
<td>Anti-CD209 Fab</td>
<td>N-A1-B1</td>
<td>CEA cancer vaccine</td>
</tr>
<tr>
<td></td>
<td>Anti-CD209 Fab</td>
<td>A3-B3</td>
<td>CEA cancer vaccine</td>
</tr>
<tr>
<td>$\alpha_2$macroglobulin</td>
<td>Anti-CD74 Fab</td>
<td>N-A1-B1</td>
<td>CEA cancer vaccine</td>
</tr>
<tr>
<td></td>
<td>Anti-CD74 Fab</td>
<td>A3-B3</td>
<td>CEA cancer vaccine</td>
</tr>
<tr>
<td></td>
<td>Anti-CD205 fab</td>
<td>N-A1-B1</td>
<td>CEA cancer vaccine</td>
</tr>
<tr>
<td></td>
<td>Anti-CD205 Fab</td>
<td>A3-B3</td>
<td>CEA cancer vaccine</td>
</tr>
<tr>
<td></td>
<td>Anti-CD209 Fab</td>
<td>N-A1-B1</td>
<td>CEA cancer vaccine</td>
</tr>
<tr>
<td></td>
<td>Anti-CD209 Fab</td>
<td>A3-B3</td>
<td>CEA cancer vaccine</td>
</tr>
<tr>
<td>HSA</td>
<td>Anti-hTfR Fab</td>
<td>BDNF</td>
<td>Neuroprotection following strokes</td>
</tr>
<tr>
<td></td>
<td>Anti-hTfR Fab</td>
<td>Neuropeptides</td>
<td>Treating CNS disorders</td>
</tr>
<tr>
<td>Human transferrin (hTf)</td>
<td>G-CSF</td>
<td>Anti-EGFR Fab</td>
<td>Treating solid tumors</td>
</tr>
<tr>
<td></td>
<td>Anti-VEGF Fab</td>
<td>Anti-EGFR Fab</td>
<td>Treating solid tumors</td>
</tr>
</tbody>
</table>

Table 2. Compositions of selected $X(Mb)_2Y$ assemblies

<table>
<thead>
<tr>
<th>(Mb)$_2$</th>
<th>X</th>
<th>Y</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fc</td>
<td>Anti-CD20 Fab</td>
<td>Anti-CD22 Fab</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td></td>
<td>Anti-CD20 Fab</td>
<td>Anti-CD19 Fab</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td></td>
<td>Anti-HER2 Fab</td>
<td>Anti-EGFR Fab</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td></td>
<td>Anti-IGF-1R Fab</td>
<td>Anti-EGFR Fab</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td></td>
<td>Anti-VEGF R1 Fab</td>
<td>Anti-VEGF R2 Fab</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td></td>
<td>Anti-VEGF R3 Fab</td>
<td>Anti-VEGF R2 Fab</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td></td>
<td>Anti-CD19 Fab</td>
<td>Anti-CD3 Fab</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td></td>
<td>Anti-CD19 Fab</td>
<td>Anti-CD64 Fab</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td></td>
<td>Anti-HER2 Fab</td>
<td>Anti-CD89 Fab</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td></td>
<td>Anti-HER2 Fab</td>
<td>Anti-CD16 Fab</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td></td>
<td>Anti-HER2 Fab</td>
<td>Anti-CD64 Fab</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td></td>
<td>Anti-HER2 Fab</td>
<td>Anti-CD3 Fab</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td></td>
<td>Anti-HER2 Fab</td>
<td>Anti-HER3 Fab</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td></td>
<td>Anti-EGFR Fab</td>
<td>Anti-CD2 Fab</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td></td>
<td>Anti-EGFR Fab</td>
<td>Anti-CD16 Fab</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td></td>
<td>Anti-EGFR Fab</td>
<td>Anti-CD64 Fab</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td></td>
<td>Anti-EGFR Fab</td>
<td>Anti-CD89 Fab</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td></td>
<td>Anti-MUC1 Fab</td>
<td>Anti-CD64 Fab</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td></td>
<td>Anti-CD19 Fab</td>
<td>Anti-CD22 Fab</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td></td>
<td>Anti-hTfR Fab</td>
<td>BDNF</td>
<td>Neuroprotection</td>
</tr>
</tbody>
</table>
Table 3. Compositions of selected $X_2(Mc)_2X_2$ assemblies

<table>
<thead>
<tr>
<th>(Mc)$_2$</th>
<th>X</th>
<th>Y</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fc</td>
<td>Protamine/DNA-based vaccines</td>
<td>DNA vaccines</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Protamine/siRNAs</td>
<td>Therapy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-VEGF Fab</td>
<td>Cancer therapy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-CD20 Fab</td>
<td>Cancer therapy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-CD22 Fab</td>
<td>Cancer therapy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Soluble Tumor Necrosis Factor receptor (sTNFR)</td>
<td>As indicated for Enbrel®</td>
<td></td>
</tr>
<tr>
<td>(hP1)$_2$</td>
<td>Anti-hTfR Fab</td>
<td>Gene therapy for brain disorders</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Compositions of selected $X_2(Md)_2YX_2$ assemblies

<table>
<thead>
<tr>
<th>(Md)$_2$</th>
<th>X</th>
<th>Y</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fc</td>
<td>Aβ12-28P</td>
<td>Anti-hTfR Fab</td>
<td>Treating Alzheimer’s disease</td>
</tr>
<tr>
<td>Fc</td>
<td>BDNF</td>
<td>Anti-hTfR Fab</td>
<td>Neuroprotection following stroke</td>
</tr>
<tr>
<td>(hP1)$_2$</td>
<td>Anti-hTfR Fab</td>
<td>Anti-IGF-1R Fab</td>
<td>Gene therapy for brain cancers</td>
</tr>
</tbody>
</table>
WHAT IS CLAIMED IS:

1. A bioactive assembly comprising:
   a) one or more adaptor modules;
   b) one or more anchoring domains (AD) and/or dimerization docking domains (DDD) attached to the one or more adaptor modules, wherein each anchoring domain binds to two dimerization and docking domains.

2. The assembly of claim 1, wherein the adaptor modules are type-a (Ma), type-b (Mb), type-c (Mc) or type-d (Md) adaptor modules.

3. The assembly of claim 2, further comprising one or more effector moieties attached to the assembly.

4. The assembly of claim 1, wherein the anchoring domains are selected from the group consisting of AD2 (SEQ ID NO:2) or AD3 (SEQ ID NO:5).

5. The assembly of claim 1, wherein the dimerization and docking domains are selected from the group consisting of DDD2 (SEQ ID NO:1), DDD3 (SEQ ID NO:3) and DDD3C (SEQ ID NO:4).

6. The assembly of claim 3, wherein the bioactive assembly has a composition of $X_2$-AD2-(Ma)-AD3-$Y_2$, wherein X represents one or more effector moieties attached to DDD2 and Y represents one or more effector moieties attached to DDD3C.

7. The assembly of claim 3, wherein the bioactive assembly has a composition of $X$-(DDD2-Mb-DDD3C)$_2$-$Y$, wherein X represents one or more effector moieties attached to AD2 and Y represents one or more effector moieties attached to AD3.

8. The assembly of claim 3, wherein the bioactive assembly has a composition of $X_2$-(AD2-Mc-DDD3)$_2$-$X_2$, wherein X represents one or more effector moieties attached to DDD2.

9. The assembly of claim 3, wherein the bioactive assembly has a composition of $X_4$-(AD2-Md-DDD3C)-$Y$, wherein X represents one or more effector moieties attached to DDD2 and Y represents one or more effector moieties attached to AD3.

10. The assembly of claim 1, wherein the bioactive assembly comprises DDD3C-CH$_2$-CH$_3$-AD2, wherein CH2 has an amino acid sequence of SEQ ID NO:7 and CH3 has an amino acid sequence of SEQ ID NO:8.
11. The assembly of claim 2, wherein the adaptor modules are selected from the group consisting of HSP70, α2.macroglubin, HSA, Fc and hPl.

12. The assembly of claim 2, wherein the adaptor module is a heat shock protein, a human protamine or an Fc fragment of a human antibody.

13. The assembly of claim 3, wherein the effector moieties are selected from the group consisting of aptamers, avimers, antibodies, antibody fragments, monoclonal antibodies, monoclonal antibody fragments, chimeric antibodies, chimeric antibody fragments, humanized antibodies, humanized antibody fragments, Fd fragments, Fab fragments, F(ab)_2 fragments, Fab' fragments, F(ab')_2 fragments, Fv, scFv, dsFv, sFv, diabody and triabody.

14. The assembly of claim 1, wherein the assembly components are covalently or non-covalently attached.

15. The assembly of claim 14, wherein at least two components of the assembly are covalently attached by disulfide bonds.

16. The assembly of claim 3, wherein the effector comprises a protein, peptide, peptide mimetic, polynucleotide, RNA/, oligosaccharide, natural or synthetic polymeric substance, nanoparticle, quantum dot, organic compound or inorganic compound.

17. The assembly of claim 13, wherein the Fab fragment is selected from the group consisting of the Fab fragments of hMN-14, L19, hA20, hLL2, L243, hCC49, 7E3, hLL1, hPAM4, hRS7, rHI, L49, anti-CD14, anti-CD1 11, Humira®, REMICADE®, Xolair®, Synagis® and hMN-15.

18. The assembly of claim 16, wherein the effector comprises a protein selected from the group consisting of a bacterial toxin, a plant toxin, ricin, abrin, a ribonuclease (RNase), DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtherin toxin, Pseudomonas exotoxin, Pseudomonas endotoxin, Ranpirnase (Rap), Rap (N69Q), PE38, dgA, DT390, PLC, tPA, a cytokine, a growth factor, a soluble receptor component, surfactant protein D, IL-4, sIL-4R, sIL-13R, VEGF_{121}, TPO, EPO, a clot-dissolving agent, an enzyme, a fluorescent protein, sTNFα-R, and a nanobody.

19. The assembly of claim 13, wherein the effector has at least one binding site for carbonic anhydrase IX, alpha-fetoprotein, A3, antigen specific for A33 antibody, Ba 733, BrE3-antigen, CA125, CD1, CD1a, CD3, CD5, CD15, CD16, CD19, CD20.
CD21, CD22, CD23, CD25, CD30, CD33, CD38, CD45, CD74, CD79a, CD80, CD138, colon-specific antigen-p (CSAp), CEA (CEACAM5), CEACAM6, CSAp, EGFR, EGP-I, EGP-2, Ep-CAM, Flt-I, Flt-3, folate receptor, HLA-DR, human chorionic gonadotropin (HCG) and its subunits, HER2/neu, hypoxia inducible factor (HIF-I), Ia, IL-2, IL-6, IL-8, insulin growth factor-1 (IGF-I), KC4-antigen, KS-I-antigen, KS1-4, Le-Y, macrophage inhibition factor (MIF), MAGE, MUC1, MUC2, MUC3, MUC4, MUC16, NCA66, NCA95, NCA90, antigen specific for PAM-4 antibody, placental growth factor, p53, prostatic acid phosphatase, PSA, PSMA, RS5, SiOO, TAC, TAG-72, tenascin, TRAIL receptors, Tn antigen, Thomson-Friedenreich antigens, tumor necrosis antigens, VEGF, ED-B fibronectin, 17-IA-antigen, an angiogenesis marker, an oncogene marker or an oncogene product.

20. The assembly of claim 3, wherein the effector is a diagnostic agent, a therapeutic agent, a chemotherapeutic agent, a radioisotope, an imaging agent, an anti-angiogenic agent, a cytokine, a chemokine, a growth factor, a drug, a prodrug, an enzyme, a binding molecule, an aptamers, a ligand for a cell surface receptor, a chelator, an immunomodulator, an oligonucleotide, an interference RNA, a hormone, a photodetectable label, a dye, a peptide, a toxin, a contrast agent, a paramagnetic label, an ultrasound label, a pro-apoptotic agent, a liposome, a nanoparticle or a combination thereof.

21. The assembly of claim 20, wherein the anti-angiogenic agent is angiotatin, baculostatin, canstatin, maspin, anti-VEGF antibodies or peptides, anti-placental growth factor antibodies or peptides, anti-Flk-1 antibodies, anti-Flt-1 antibodies or peptides, laminin peptides, fibronectin peptides, plasminogen activator inhibitors, tissue metalloproteinase inhibitors, interferons, interleukin 12, IP-10, Gro-β, thrombospondin, 2-methoxyoestradiol, proliferin-related protein, carboxiamidotriazole, CMIOI, Marimastat, pentosan polysulphate, angiopoietin 2, interferon-alpha, herbimycin A, PNU145156E, 16K prolactin fragment, Linomide, thalidomide, pentoxifylline, genistein, TNP-470, endostatin, paclitaxel, accutin, angiotatin, cidofovir, vincristine, bleomycin, AGM-1470, platelet factor 4 or minocycline.

22. The assembly of claim 20, wherein the therapeutic agent is abrin, amantadine, amoxicillin, amphotericin B, ampicillin, aplidin, azaridine, anastrozole, azacytidine, aztreonam, azithromycin, bacitracin, bactrim, Batrafen®, bifonazole, bleomycin, bortezomib, bryostatin-1, busulfan, calicheamycin, camptothecin, 10-
hydroxycamptothecin, carbenicillin, caspofungin, carmustine, cefaclor, cefazolin, cefalosporins, cefepime, cefotaxime, celebrex, chlorambucil, chloramphenicol, Cipro®, cisplatin, irinotecan (CPT-II), SN-38, carboplatin, cladribine, cyclophosphamide, cytarabine, dacomabazine, docetaxel, dactinomycin, daunomycin glucuronide, daunorubicin, dexamethasone, diethylstilbestrol, diphtheria toxin, DNase I, doxorubicin, 2-pyrroloidoxorubicine (2P-DOX), doxycycline, cyano-morpholino doxorubicin, doxorubicin glucuronide, epirubicin glucuronide, ethinyl estradiol, estramustine, estrogen receptor binding agents, etoposide, etoposide glucuronide, etoposide phosphate, erythrocyctose, erythromycin, flagyl, farnesyl-protein transferase inhibitors, floxuridine (FUDR), 3',5'-O-dioleoyl-FudR (FUDR-dO), fludarabine, flutamide, fluorouracil, fluoxymesterone, ganciclovir, gentamycin, gelonin, gemcitabine, hydroxyprogesterone caproate, hydroxyurea, idarubicin, ifosfamide, isoniazid, iraconazole, kanamycin, ketoconazole, L-asparaginase, leucovorin, lomustine, mechloretamine, medroprogesterone acetate, megestrol acetate, melphalan, mercaptopurine, 6-mercaptopurine, methotrexate, mitoxantrone, mithramycin, mitomycin, mitotane, minocycline, naftifine, nalidixic acid, neomycin, navelbine, nitrosurea, nystatin, onconase, oxacillin, paromomycin, pentamidine, pipericillin-tazobactam, phenyl butyrate, prednisone, procarbazine, paclitaxel, pentostatin, pokeweed antiviral protein, PSI-341, Pseudomonas exotoxin, Pseudomonas endotoxin, raloxifene, rapLRI, ribonuclease, ricin, semustine, rifabutin, rifampin, rimantadine, streptomycin, sulfamethoxazole, sulfosalazone, Staphylococcal enterotoxin-A, streptozocin, tamoxifen, taxanes, taxol, testosterone propionate, tetracycline, thalidomide, thioguanine, thiopeta, teniposide, topotecan, transplatinum, trimethoprim sulfamethoxazole, uracil mustard, valacyclovir, vancomycin, velcade, vinblastine, vinorelbine, vincristine, zanamir, zithromycin, an antisense oligonucleotide, an interference RNA, or a combination thereof.

23. The assembly of claim 20, wherein the diagnostic or therapeutic agent is selected from the group consisting of $^{225}$Ac, $^{211}$At, $^{212}$Bi, $^{213}$Bi, $^{14}$C, $^{51}$Cr, $^{36}$Cl, $^{45}$Ti, $^{57}$Co, $^{58}$Co, $^{62}$Cu, $^{64}$Cu, $^{67}$Cu, $^{166}$Dy, $^{152}$Eu, $^{16}$F, $^{67}$Ga, $^{68}$Ga, $^{195m}$Hg, $^{166}$Ho$^{3+}$H, $^{111}$In, $^{123}$I, $^{124}$I, $^{125}$I, $^{131}$I, $^{52}$Fe, $^{59}$Fe, $^{177}$Lu, $^{191}$Os, $^{212}$Pb, $^{32}$P, $^{33}$P, $^{142}$Pr, $^{195m}$Pt, $^{223}$Ra, $^{186}$Re, $^{188}$Re, $^{189}$Re, $^{47}$Sc, $^{75}$Se $^{111}$Ag, $^{153}$Sm, $^{89}$Sr, $^{35}$S, $^{161}$Tb, $^{94m}$Tc, $^{99m}$Tc, $^{86}$Y, $^{90}$Y, and $^{89}$Zr.

24. The assembly of claim 20, wherein the imaging agent is selected from the group consisting of chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel
(II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III) erbium (III), lanthanum (III), gold (III), lead (II) and bismuth (III).

25. The assembly of claim 20, wherein the photodetectable label is selected from the group consisting of Alexa 350, Alexa 430, AMCA, aminoacridine, BODIPY 630/650, BODIPY 650/665, BODIPY-FL, BODIPY-R6G, BODIPY-TMR, BODIPY-TRX, 5-carboxy-4', 5'-dichloro-2',7'-dimethoxy fluorescein, 5-carboxy-2',4',5',7'-tetrachlorofluorescein, 5-carboxyfluorescein, 5-carboxyrhodamine, 6-carboxyrhodamine, 6-carboxytetramethyl amino, Cascade Blue, Cy2, Cy3, Cy5,6-FAM, dansyl chloride, Fluorescein, HEX, 6-JOE, NBD (7-nitrobenz-2-oxa-1,3-diazole), Oregon Green 488, Oregon Green 500, Oregon Green 514, Pacific Blue, phthalic acid, terephthalic acid, isophthalic acid, cresyl fast violet, cresyl blue violet, brilliant cresyl blue, para-aminobenzoic acid, erythrosine, phthalocyanines, azomethines, cyanines, xanthines, succinylfluoresceins, rare earth metal cryptates, europium trisbipyridine diamine, a europium cryptate or chelate, diameine, dicyanins, La Jolla blue dye, allopyocyanin, allocyocyanin B, phycocyanin C, phycocyanin R, thiamine, phycoerythrocyanin, phycoerythrin R, REG, Rhodamine Green, rhodamine isothiocyanate, Rhodamine Red, ROX, TAMRA, TET, TRIT (tetramethyl rhodamine isothiol), Tetramethylrhodamine, and Texas Red.

26. The assembly of claim 13, wherein at least one effector moiety has a binding site for a carrier or hapten.

27. The assembly of claim 26, wherein the carrier or hapten comprises at least one diagnostic or therapeutic agent.

28. The assembly of claim 3, wherein the one or more effector moieties are selected from the group consisting of N-Al-B1, A3-B3, anti-CD2 Fab, anti-CD3 Fab, anti-CD16 Fab, anti-CD19 Fab, anti-CD20 Fab, anti-CD22 Fab, anti-CD64 Fab, anti-CD74 Fab, anti-CD89 Fab, anti-CD205 Fab, anti-CD209 Fab, anti-HER2 Fab, anti-HER3 Fab, anti-EGFR Fab, anti-IGF-IR Fab, anti-VEGF Fab, anti-VEGFR1 Fab, anti-VEGFR2 Fab, anti-VEGFR3 Fab, anti-PIGF Fab, anti-MUC1 Fab, anti-HER Fab, BDNF, a neuropeptide, a protamine/DNA-based vaccine, a protamine/siRNA, soluble Tumor Necrosis Factor receptor (sTNFR) and Aβ1-28P.

29. The assembly of claim 3, wherein a first effector has an affinity for any cell surface antigen associated with a disease or a medical condition and a second effector
is selected from the group consisting of a cytokine, a growth factor, carboxypeptidase G2, penicilliamidase, β-lactamase, cytosine deaminase, nitroreductase, β-galactosidase, green fluorescence protein (GFP) or its various engineered analogs, alkaline phosphatase, horseradish peroxidase, streptavidin, a ribonuclease, a bacterial toxin, a plant toxin and surfactant protein D (Sp-D).

30. A method comprising:
   a) obtaining a bioactive assembly according to claim 3; and
   b) administering the bioactive assembly to a subject with a condition;

31. The method of claim 30, wherein the condition is cancer, hyperplasia, amyloidosis, Alzheimer's disease, an autoimmune disease, diabetic retinopathy, juvenile diabetes, late-onset diabetes, macular degeneration, inflammatory bowel disease, Crohn's disease, ulcerative colitis, rheumatoid arthritis, sarcoidosis, asthma, edema, pulmonary hypertension, psoriasis, corneal graft rejection, neovascular glaucoma, Osier-Webber Syndrome, myocardial angiogenesis, plaque neovascularization, restenosis, neointima formation after vascular trauma, telangiectasia, hemophiliac joints, angiofibroma, fibrosis associated with chronic inflammation, lung fibrosis, organ transplant rejection, deep venous thrombosis or wound granulation.

32. The method of claim 31, wherein the condition is cancer and at least one effector has a binding affinity for a tumor-associated antigen selected from the group consisting of carbonic anhydrase IX, alpha-fetoprotein, A3, antigen specific for A33 antibody, BA 733, BrE3-antigen, CA125, CD1, CDIa, CD3, CD5, CD15, CD16, CD19, CD20, CD21, CD22, CD23, CD25, CD30, CD33, CD38, CD45, CD74, CD79a, CD80, CD138, colon-specific antigen-p (CSAp), CEA (CEACAM5), CEACAM6, EGFR, EGP-1, EGP-2, Ep-CAM, Fit-1, Fllt-3, folate receptor, G250 antigen, HLA-DR, human chorionic gonadotropin (HCG) and its subunits, HER2/neu, hypoxia inducible factor (HIF-I), Ia, IL-2, IL-6, IL-8, insulin growth factor-1 (IGF-I), KC4-antigen, KS-1-antigen, KS1-4, Le-Y, macrophage inhibition factor (MIF), MAGE, MUC1, MUC2, MUC3, MUC4, MUC16, NCA66, NCA95, NCA90, antigen specific for PAM-4 antibody, placental growth factor, p53, prostatic acid phosphatase, PSA, PSMA, RS5, S100, TAC, TAG-72, tenascin, TRAIL receptors, Tn antigen, Thomson-Friedenreich antigens, tumor necrosis antigens, VEGF, ED-B fibronectin, 17-1A-antigen, an angiogenesis marker, an oncogene marker and an oncogene product.

34. The method of claim 33, further comprising administering one or more anti-cancer therapies in combination with the assembly.

35. The method of claim 34, wherein the therapy comprises administering a chemotherapeutic agent, a cytokine, radiation therapy, immunotherapy, radioimmunotherapy, localized hyperthermia, laser irradiation, an anti-angiogenic agent or surgical excision.

36. The method of claim 30, wherein the bioactive assembly comprises a first effector with a binding affinity for an antigen associated with a disease or other condition and a second effector with a binding affinity for a carrier or hapten.

37. The method of claim 36, further comprising:
   c) optionally, administering a clearing agent to remove circulating bioactive assemblies; and
   d) administering the carrier or hapten to the subject, wherein the carrier or hapten is conjugated to one or more therapeutic agents and/or diagnostic agents.

38. The method of claim 37, wherein the carrier or hapten is attached to an agent selected from the group consisting of an anti-angiogenic agent, a chemotherapeutic agent, a cytokine, a drug, a prodrug, a toxin, an interference RNA, an aptamer, an enzyme, an oligonucleotide, a radioisotope, an immunomodulator, an antibiotic, an anti-viral agent, an anti-fungal agent, anti-parasite agent, a hormone, a binding molecule, a lipid, a polymer, a micelle, a liposome, a nanoparticle or a combination thereof.

39. The method of claim 30, wherein the condition is caused by a fungus.

40. The method of claim 39, wherein the fungus is Microsporum, Trichophyton, Epidermophyton, Sporothrix schenckii, Cryptococcus neoformans, Coccidioides immitis, Histoplasma capsulatum, Blastomyces dermatitidis or Candida albicans.
41. The method of claim 30, wherein the condition is caused by a virus.

42. The method of claim 41, wherein the virus is human immunodeficiency virus (HIV), herpes virus, cytomegalovirus, rabies virus, influenza virus, human papilloma virus, hepatitis B virus, hepatitis C virus, Sendai virus, feline leukemia virus, Reo virus, polio virus, human serum parvo-like virus, simian virus 40, respiratory syncytial virus, mouse mammary tumor virus, Varicella-Zoster virus, Dengue virus, rubella virus, measles virus, adenovirus, human T-cell leukemia viruses, Epstein-Barr virus, murine leukemia virus, mumps virus, vesicular stomatitis virus, Sindbis virus, lymphocytic choriomeningitis virus or blue tongue virus.

43. The method of claim 30, wherein the condition is caused by a bacterium.

44. The method of claim 43, wherein the bacterium is Bacillus anthracis, Streptococcus agalactiae, Legionella pneumophila, Streptococcus pyogenes, Escherichia coli, Neisseria gonorrhoeae, Neisseria meningitidis, Pneumococcus spp., Hemophilus influenzae B, Treponema pallidum, Lyme disease spirochetes, Pseudomonas aeruginosa, Mycobacterium leprae, Brucella abortus, Mycobacterium tuberculosis or a Mycoplasma.

45. The method of claim 30, wherein the condition is caused by a unicellular parasite.


47. The method of claim 30, wherein the condition is an autoimmune disease.

48. The method of claim 47, wherein the autoimmune disease is acute idiopathic thrombocytopenic purpura, chronic idiopathic thrombocytopenic purpura, dermatomyositis, Sydenham's chorea, myasthenia gravis, systemic lupus erythematosus, lupus nephritis, rheumatic fever, polyglandular syndromes, bullous pemphigoid, juvenile diabetes mellitus, Henoch-Schonlein purpura, post-streptococcal nephritis, erythema nodosum, Takayasu's arteritis, Addison's disease, rheumatoid arthritis, multiple sclerosis, sarcoidosis, ulcerative colitis, erythema multiforme, IgA nephropathy, polyarteritis nodosa, ankylosing spondylitis, Goodpasture's syndrome, thromboangitis obliterans, Sjogren's syndrome, primary biliary cirrhosis, Hashimoto's thyroiditis, thyrotoxicosis
(Graves' disease), scleroderma, chronic active hepatitis, polymyositis/dermatomyositis, polychondritis, pemphigus vulgaris, Wegener's granulomatosis, membranous nephropathy, amyotrophic lateral sclerosis, tabes dorsalis, giant cell arteritis/polymyalgia, pernicious anemia, rapidly progressive glomerulonephritis, psoriasis or fibrosing alveolitis.

49. The method of claim 30, wherein the condition is myocardial infarction, ischemic heart disease, atherosclerotic plaques, graft rejection, Alzheimer's disease, atopic tissue or inflammation caused by accretion of activated granulocytes, monocytes, lymphoid cells or macrophages.

50. A method of diagnosing a condition comprising:
   a) obtaining a bioactive assembly according to claim 3, wherein a first effector moiety binds to a target molecule, composite, aggregate, cell, antigen or tissue associated with the condition and a second effector moiety binds to a diagnostic hapten;
   b) administering the assembly to a subject suspected of having a condition;
   c) administering to the same subject a diagnostic hapten that binds to the second effector; and
   d) detecting the presence of the hapten bound to the second effector;

wherein localization of the hapten to a tissue associated with the condition is diagnostic of the presence of the condition in the subject.

51. The method of claim 50, wherein the hapten is conjugated to a magnetic resonance imaging (MRI) contrast agent and the localized hapten is detected by MRI.

52. The method of claim 51, wherein the MRI contrast agent is chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysporium (III), holmium (III) or erbium (III).

53. The method of claim 50, wherein the hapten is conjugated to an ultrasound imaging enhancing agent and the localized hapten is detected by ultrasound imaging.

54. The method of claim 30 or claim 50, further comprising performing an endoscopic procedure for said therapy or said detection.

55. The method of claim 30, wherein the bioactive assembly comprises a combination of antibodies or antibody fragments selected from the group consisting of anti-CD74 X anti-CD20, anti-CD74 X anti-CD22, anti-CD22 X anti-CD20, anti-CD20 X
anti-HLA-DR, anti-CD19 X anti-CD20, anti-CD20 X anti-CD80, anti-CD2 X anti-CD25, anti-CD8 X anti-CD25, and anti-CD2 X anti-CD147.

56. A method of making a bioactive assembly comprising:
   a) obtaining an adaptor module conjugated to at least two AD and/or DDD sequences;
   b) obtaining at least one effector conjugated to DDD and/or AD sequences that are complementary to the AD and/or DDD sequences that are conjugated to the adaptor module; and
   c) exposing the complementary AD and DDD sequences under conditions allowing formation of the bioactive assembly.

57. The method of claim 56, wherein the complementary DDD/AD sequences are DDD2/AD2 or DDD3C/AD3.

58. The assembly of claim 16, wherein the effector is a peptide that binds to a tumor-associated antigen, an oncogene protein, a growth factor or a cell surface receptor.

59. The assembly of claim 58, wherein the peptide binds to EGFR, VEGFR, PIGF or Flt-I.

60. The assembly of claim 59, wherein the peptide is BPl SHRYRLAIQLHASDSSSCV (SEQ ID NO: 16), BP2 QDDHLTTGR (SEQ ID NO: 17) or BP4 RMPYSEHSAPLG (SEQ ID NO: 18).

61. The assembly of claim 20, wherein the cytokine is selected from the group consisting of human growth hormone, N-methionyl human growth hormone, bovine growth hormone, parathyroid hormone, thyroxin, insulin, proinsulin, relaxin, prorelaxin, follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), luteinizing hormone (LH), hepatic growth factor, prostaglandin, fibroblast growth factor, prolactin, placental lactogen, OB protein, tumor necrosis factor-α, tumor necrosis factor-β, mullerian-inhibiting substance, mouse gonadotropin-associated peptide, inhibin, activin, vascular endothelial growth factor, integrin, thrombopoietin (TPO), a nerve growth factor (NGF), NGF-β, platelet-growth factor, a transforming growth factors (TGF), TGF-α, TGF-β, insulin-like growth factor-I insulin-like growth factor-II, erythropoietin (EPO), an osteoinductive factor, an interferon, interferon-α, interferon-β, and interferon-γ, a colony stimulating factors (CSF), macrophage-CSF (M-CSF), granulocyte-macrophage-CSF (GM-CSF), granulocyte-CSF (G-CSF), an interleukins (IL), IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17,
IL-1β, IL-21, LIF, kit-ligand, FLT-3, angiostatin, thrombospondin, endostatin, tumor necrosis factor and LT.

62. The assembly of claim 20, wherein the chemokine is selected from the group consisting of RANTES, MCAF, MIP1-alpha, MIP1-Beta, and IP-10.

63. The assembly of claim 20, wherein the effector moiety is selected from the group consisting of adrenocorticotropic hormone, ebiratide, angiotensin, angiotensin II, asparaginase, atrial natriuretic peptides, atrial sodium diuretic peptides, bacitracin, beta-endorphins, blood coagulation factors VII, VIII and IX, blood thymic factor, bone morphogenetic factor, bone morphogenetic protein, bradykinin, caerulein, calcitonin gene related polypeptide, calcitonins, CCK-8, cell growth factors, EGF, acidic FGF, basic FGF, chemokines, cholecystokinin, cholecystokinin-8, cholecystokinin-pancreozymin, colistin, colony-stimulating factors, corticotropin-releasing factor, desmopressin, dipeptide, dismutase, dynorphin, eldeoisin, endorphins, endotoxin, endothelin-antagonistic peptides, endotherins, enkephalins, epidermal growth factor, erythropoietin, follicle-stimulating hormone, gallanin, gastrin-inhibitory polypeptide, gastrin-releasing polypeptide, gastrins, glucagon, glutathione peroxidase, glutathio-peroxidase, gonadotropin, gramicidin, gramicidines, growth factor, growth hormone-releasing factor, growth hormones, h-ANP, hormone releasing hormone, human chorionic gonadotrophin, human chorionic gonadotrophin .beta.-chain, human placental lactogen, insulin, insulin-like growth factors, IGF-I, IGF-II, interferons, interleukins, intestinal polypeptide, kallikrein, kyotorphin, luliberin, luteinizing hormone, luteinizing hormone-releasing hormone, lysozyme chloride, melanocyte-stimulating hormone, melanophore stimulating hormone, mellitin, motilin, muramyl, muramylidipeptide, nerve growth factor, nerve nutrition factors, NT-3, NT-4, CNTF, GDNF, BDNF, neuropeptide Y, neurotensin, oxytocin, pancreastatin, pancreatic polypeptide, pancreozymin, parathyroid hormone, pentagastrin, polypeptide YY, pituitary adenyl cyclase-activating polypeptides, platelet derived growth factor, polymixin B, prolactin, protein synthesis stimulating polypeptide, PTH-related protein, relaxin, renin, secretin, serum thymic factor, somatomedins, somatostatins, substance P, superoxide, superoxide dismutase, taftsin, tetragastrin, thymic humoral factor, thymosin, thymostimulin, thyroid hormone releasing hormone, thyroid-stimulating hormone, thyrotropin releasing hormone TRH, trypsin, tuftsin, tumor growth factor, tumor necrosis factor, tyrocidin, urogastrone, urokinase, vasoactive intestinal polypeptide, vasopressins, and functional equivalents.
Figure 1

A. **DDD2:**
   CGHIQIPPGLTELLQGYTVEVLRQQ
   PPDLVEFAVEYFTRLREARA (SEQ ID NO:1)

B. **AD2:**
   CGQIEYLAKQIVDNAIQQAAGC (SEQ ID NO:2)

C. **DDD3:**
   SLRECELYVQKHNIQALLKDSIVQLCTARPERPMAFLREYFERLEKEEEAK (SEQ ID NO:3)

D. **DDD3C**
   MSCGGSLRECELYVQKHNIQALLKDSIVQLCTARPERPMAFLREYFERLEKEEAK (SEQ ID NO:4)

E. **AD3**
   CGFEELAWKIAKMIWSDVFQQGC (SEQ ID NO:5)
Figure 2

Type-a adaptor module

$X_2(Ma)Y_2$
$X(Mb)_2 Y$

Type-b adaptor module

Figure 3
Figure 4

Type-c adaptor module

$X_2(Mc)_2X_2$
Figure 5

Type-d adaptor module

$X_2(Md)_2YX_2$
**Complete amino acid sequence of DDD3C-CH2-CH3-AD2**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Sequence</th>
<th>Seq ID No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DD3C</strong></td>
<td>MS C GGSLRECELYVQKHNIQALLKDSIVQLCTARP ERPMAFLREYF ERLEKEEAK</td>
<td>4</td>
</tr>
<tr>
<td><strong>Link-1</strong></td>
<td>GRSG</td>
<td>6</td>
</tr>
<tr>
<td><strong>CH2</strong></td>
<td>APELLGGPSVF LPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK</td>
<td>7</td>
</tr>
<tr>
<td><strong>CH3</strong></td>
<td>GQPREPQVYTLPSSREEMTKNQVSLTCLVKGFYPSDIAWEVESNGQPPENNYKT TPPVLDSDGSSFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGK</td>
<td>8</td>
</tr>
<tr>
<td><strong>Link-2</strong></td>
<td>GSGGGGGSGG</td>
<td>9</td>
</tr>
<tr>
<td><strong>AD2</strong></td>
<td>C CGQIEYLAKQIVDNAIIQQAG C</td>
<td>2</td>
</tr>
</tbody>
</table>
SEQUENCE LISTING

<110> Chang, Chi e n Hsing
       Goldenberg, David M .
       McBride, William J .
       Rossi , Edmund A .

<120> METHODS AND COMPOSITIONS FOR GENERATING BIOACTIVE ASSEMBLIES OF INCREASED COMPLEXITY AND USES

<130> 337630

<150> PCT/US2006/010762
<151> 2006-03-24

<150> 60/751,196
<151> 2005-12-16

<150> 60/782,332
<151> 2006-03-14

<150> 60/728,292
<151> 2005-10-19

<150> PCT/US2006/012084
<151> 2006-03-29

<150> 11/389,358
<151> 2006-03-24

<150> 11/391,584
<151> 2006-03-24

<160> 18

<170> PatentIn version 3.3

<210> 1
<211> 45
<212> PRT
<213> Artificial

<220> synthetic peptide

<400> 1

Cys Gly His lie Gin lie Pro Pro Gly Leu Thr Gin Leu Gin Gly
1 5 10 15

Tyr Thr Val Glu Val Leu Arg Gin Gin Pro Pro Asp Leu Val Glu Phe
20 25 30

Ala Val Glu Tyr Phe Thr Arg Leu Arg Gin Ala Arg Ala
35 40 45

<210> 2
<211> 21
<212> PRT
<213> Artificial

<220> synthetic peptide

<400> 2
cys Gly Gin lie Glu Tyr Leu Ala Lys Gin lie Val Asp Asn Ala lie
    1 5 10 15

Gin Gin Ala Gly cys
    20

<210> 3
<211> 50
<212> PRT
<213> Artificial

<220> synthetic peptide
<223> synthetic peptide

<400> 3
Ser Leu Arg Gln cys Gln Leu Tyr Val Gin Lys His Asn lie Gin Ala
    1 5 10 15

Leu Leu Lys Asp Ser lie Val Gin Leu Cys Thr Ala Arg Pro Glu Arg
    20 25 30

Pro Met Ala Phe Leu Arg Glu Tyr Phe Glu Arg Leu Glu Lys Glu Glu
    35 40 45

Ala Lys
    50

<210> 4
<211> 55
<212> PRT
<213> Artificial

<220> synthetic peptide
<223> synthetic peptide

<400> 4
Met Ser Cys Gly Gly Ser Leu Arg Glu Cys Glu Leu Tyr Val Gin Lys
    1 5 10 15

His Asn lie Gin Ala Leu Leu Lys Asp Ser lie Val Gin Leu Cys Thr
    20 25 30

Ala Arg Pro Glu Arg Pro Met Ala Phe Leu Arg Glu Tyr Phe Glu Arg
    35 40 45

Leu Glu Lys Glu Glu Ala Lys
    50 55

<210> 5
<211> 23
<212> PRT
<213> Artificial

<220> synthetic peptide
<400> 5
Cys Gly Phe Glu Glu Leu Ala Trp Lys lie Ala Lys Met lie Trp ser
1  5  10  15
Asp val Phe Gin Gin Gly cys
20

<210> 6
<211> 4
<212> PRT
<213> Artificial

<220> synthetic peptide

<400> 6
Gly Arg Ser Gly
1

<210> 7
<211> 110
<212> PRT
<213> Artificial

<220> synthetic peptide

<400> 7
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
1  5  10  15
Pro Lys Asp Thr Leu Met H e Ser Arg Thr Pro Glu Val Thr Cys Val
20 25 30
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
35 40 45
val Asp Gly val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
50 55 60
Gin Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
65 70 75 80
Gin Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys val ser Asn Lys
85 90 95
Ala Leu Pro Ala Pro lie Glu Lys Thr lie Ser Lys Ala Lys
100 105 110

<210> 8
<211> 107
<212> PRT
<213> Artificial
<220>   
<223>   synthetic peptide  
<400> 8  
Gly Gin Pro Arg Glu Pro Gin Val Tyr Thr Leu Pro Pro Ser Arg Glu  
1 5 10 15  
Glu Met Thr Lys Asn Gin Val Ser Leu Thr Cys Leu Val Lys Gly Phe  
20 25 30  
Tyr Pro Ser Asp lie Ala Val Glu Trp Glu Ser Asn Gly Gin Pro Glu  
35 40 45  
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe  
50 55 60  
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gin Gin Gly  
65 70 75 80  
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr  
85 90 95  
Thr Gin Lys Ser Leu Ser Leu Ser Pro Gly Lys  
100 105  

<210> 9  
<211> 9  
<212> PRT  
<213> Artificial  

<220>   
<223>   synthetic peptide  
<400> 9  
Gly Ser Gly Gly Gly Gly Ser Gly Gly  
1 5  

<210> 10  
<211> 14  
<212> PRT  
<213> Artificial  

<220>   
<223>   synthetic peptide  
<400> '10  
Arg Arg Arg Arg Arg Arg Gly Gly Arg Gly Arg Arg Arg Arg  
1 5 10  

<210> 11  
<211> 27  
<212> DNA  
<213> Artificial  

<220>   
<223>   synthetic oligonucleotide
<400>  11
  agatctgcct  tttgcctcct  ccttctc
  .  27

<210>  12
<211>  26
<212> DNA
<213> Artificial

<220>
<223> synthetic oligonucleotide

<400>  12
  tcatgacgc  tgcagaatgt  gagctc
   26

<210>  13
<211>  36
<212> DNA
<213> Artificial

<220>
<223> synthetic oligonucleotide

<400>  13
  tcatgacttg  tggcggagcc  cttcgaagat  gtgacg
   36

<210>  14
<211>  24
<212> DNA
<213> Artificial

<220>
<223> synthetic oligonucleotide

<400>  14
  agatctgacg  cacctgaact  cctg
   24

<210>  15
<211>  33
<212> DNA
<213> Artificial

<220>
<223> synthetic oligonucleotide

<400>  15
  gaattcggat  cctttacccg  gagacagggg  gag
   33

<210>  16
<211>  19
<212> PRT
<213> Artificial

<220>
<223> synthetic peptide

<400>  16

  ser  His  Arg  Tyr  Arg  Leu  Ala  lie  Gin  Leu  His  Ala  Ser  Asp  ser  Ser
  1    5    10    15

  Ser  Cys  Val
Artificial synthetic peptide

Gln Asp Asp His Leu Thr Thr Gly Arg
1 5

Arg Met Pro Tyr Ser Gln His Ser Ala Pro Leu Gly
1 5 10