dye conjugates for use in such methods, for example, strategies that minimize or avoid photodegradation and improve the activity of the conjugate when used for PIT. Provided are methods and conjugates that meet such needs.

Summary

[0005] Provided in some examples is a method of manufacturing a phthalocyanine dye-conjugate. In some examples, the method includes contacting a targeting molecule, such as a macromolecule, with a phthalocyanine dye, which in some cases contains a reactive chemical group, under conditions to produce a conjugate containing the phthalocyanine dye linked to, such as covalently bound to, the targeting molecule. In some examples, the method includes formulating the conjugate in a pharmaceutically acceptable buffer. In some examples, before, during, and/or after preparation of the conjugate, the only light to which the dye and conjugate are exposed has a wavelength within a range from about 400 nm to about 650 nm. In some examples, before, during, and/or after preparation of the conjugate, such as during the contacting and/or formulating steps, the only light to which the dye and conjugate are exposed has an intensity of less than 500 lux.

[0005a] In one aspect, the present disclosure refers to a method of manufacturing a phthalocyanine dye-targeting molecule conjugate, comprising:

a) contacting a targeting molecule with a phthalocyanine dye under conditions to produce a conjugate comprising the phthalocyanine dye linked to the targeting molecule; and

b) formulating the conjugate in a pharmaceutically acceptable buffer,

wherein in each of steps a) – b):

the only light to which the dye and conjugate are exposed has a wavelength within a range from 400 nm to 650 nm, or

the only light to which the dye and conjugate are exposed has an intensity of less than 500 lux.

[0006] In some examples, the method includes contacting a targeting molecule with a phthalocyanine dye at a molar ratio of dye to targeting molecule of from or from about 1:1 to 1000:1. In some examples, the dye includes a reactive chemical group, under conditions to produce a conjugate containing the phthalocyanine dye covalently linked to an attachment group of the targeting molecule. In some examples, the method includes formulating the conjugate in a pharmaceutically acceptable buffer to a concentration from or from about 0.01 mg/mL to 1000.0 mg/mL. In

(NTN2), netrin-4 (NTN4), netrin-G1 (NTNG1) and netrin-G2 (NTNG2), ephrin A1 (EFNA1), ephrin A2 (EFNA2), ephrin A3 (EFNA3), ephrin A4 (EFNA4), ephrin A5 (EFNA5), semaphorin 3A (SEMA3A), semaphorin 3B (SEMA3B), semaphorin 3C (SEMA3C), semaphorin 3D (SEMA3D), semaphorin 3F (SEMA3F), semaphorin 3G (SEMA3G), semaphorin 4A (SEMA4A), semaphorin 4B (SEMA4B), semaphorin 4C (SEMA4C), semaphorin 4D (SEMA4D), semaphorin 4F (SEMA4F), semaphorin 4G (SEMA4G), semaphorin 5A (SEMA5A), semaphorin 5B (SEMA5B), semaphorin 6A (SEMA6A), semaphorin 6B (SEMA6B), semaphorin 6D (SEMA6D), semaphorin 7A (SEMA7A), SLIT1, SLIT2, SLIT3, SLIT and NTRK-like family, member 1 (SLITRK1), SLIT and NTRK-like family, member 2 (SLITRK2), SLIT and NTRK-like family, member 3 (SLITRK3), SLIT and NTRK-like family, member 4 (SLITRK4), SLIT and NTRK-like family, member 5 (SLITRK5), SLIT and NTRK-like family, member 6 (SLITRK6), prostaglandin E2 (PGE2), RANTES, Somatostatin-14, Somatostatin-28, stem cell factor (SCF), stromal cell derived factor 1 (SDF-1), substance P, thyroid stimulating hormone (TSH), transforming growth factor alpha (TGF-α), transforming growth factor beta (TGF-b), tumor necrosis factor alpha (TNF-α), thrombin, vasoactive intestinal peptide (VIP), Wntl, Wnt2, Wnt2b/13, Wnt3, Wnt3a, Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7a, Wnt7b, Wnt7c, Wnt8, Wnt8a, Wnt8b, Wnt8c, Wntl0a, Wntl0b, Wnt11, Wnt14, Wnt15, or Wnt16, Sonic hedgehog, Desert hedgehog, and Indian hedgehog.

[0035] In some examples, the targeting molecule is an antibody or an antibody fragment.

[0036] In some examples, the antibody is cetuximab, panitumumab, zalutumumab, nimotuzumab, trastuzumab, Ado-trastuzumab emtansine, Tositumomab (Bexxar ®), Rituximab (Rituxan, Mabthera), Ibritumomab tiuxetan (Zevalin), Daclizumab (Zenapax), Gemtuzumab (Mylotarg), Alemtuzumab, CEA-scan Fab fragment, OC125 monoclonal antibody, ab75705, B72.3, Bevacizumab (Avastin ®), Afatinib, Axitinib, Bosutinib, Cabozantinib, Ceritinib, Crizotinib, Dabrafenib, Dasatinib, Erlotinib, Everolimus, Ibrutinib, Imatinib, Lapatinib, Lenvatinib, Nilotinib, Olaparib, Palbociclib, Pazopanib, Pertuzumab, Ramucirumab, Regorafenib, Ruxolitinib, Sorafenib, Sunitinib, Temsirolimus, Trametinib, Vandetanib, Vemurafenib, Vismodegib, Basiliximab, Ipilimumab, Nivolumab, pembrolizumab, MPDL3280A, Pidilizumab (CT-011), AMP-224, MSB0010718C, or MEDI4736, or is an antigen-binding fragment thereof. In some examples, the antibody binds to a cell surface target molecule, such as HER1/EGFR, HER2, PD-L1, CD25, EpCAM, EphA2, CD206, CD20, CD44, CD133, Mesothelin, Glypican-3, or carcinoembryonic antigen

greater than about 5 grams or greater than or greater than about 10 grams. In some examples, the conjugate is produced using good manufacturing practice (GMP).

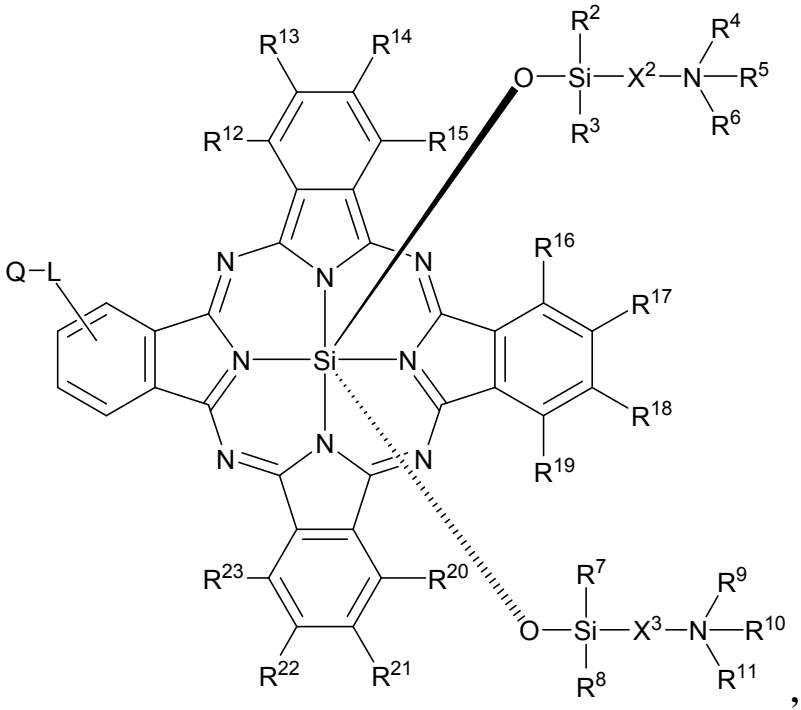
[0045] Provided in some examplesis a conjugate produced, formulated or packaged by the method described herein. In some examples, the conjugate is stable for greater than three months, such as with greater than 90% of the conjugate present as a main monomer component.

[0046] Provided in some examplesis a stable conjugate containing a phthalocyanine dye linked to a targeting molecule. In some examples, the stable conjugate is stable for greater than three months, such as with greater than 90% of the conjugate present as a main monomer component.

[0046a] In another aspect, the present disclosure refers to a stable conjugate, comprising the phthalocyanine dye-targeting molecule conjugate manufactured by the method described herein, wherein the conjugate is stable for greater than three months or greater than 6 months or greater than 12 months.

[0047] In some examples, the conjugate is stable if it retains greater than or greater than about 30%, 40%, 50%, 60%, 70%, 80%, 90% or more of its potency, activity or purity for greater than 3 months compared to the conjugate prior to the storage for the time. In some examples, the conjugate is stable if greater than 90% of the conjugate or stable conjugate is present as a main monomer component. In some examples, the conjugate is stable if greater than 95% of the conjugate or stable conjugate is present as a main monomer component. In some examples, the conjugate or stable conjugate is stable for greater than 6 months or greater than 12 months. In some examples, the conjugate or stable conjugate is stable at a temperature of less than 30 ℃.

[0048] In some examples, the phthalocyanine dye contained in the stable conjugate includes the formula:



, wherein:

L is a linker;

Q is a reactive group for attachment of the dye to the targeting molecule;

R^2, R^3, R^7, and R^8 are each independently selected from among optionally substituted alkyl and optionally substituted aryl;

R^4, R^5, R^6, R^9, R^{10}, and R^{11} are each independently selected from among hydrogen, optionally substituted alkyl, optionally substituted alkanoyl, optionally substituted alkoxycarbonyl, optionally substituted alkylcarbamoyl, and a chelating ligand, wherein at least one of R^4, R^5, R^6, R^9, R^{10}, and R^{11} comprises a water soluble group;

R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{20}, R^{21}, R^{22} and R^{23} are each independently selected from among hydrogen, halogen, optionally substituted alkylthio, optionally substituted alkylamino and optionally substituted alkoxy; and

X^2 and X^3 are each independently C_1–C_{10} alkylene, optionally interrupted by a heteroatom.

[0048a] In another aspect, the present disclosure refers to a stable conjugate, comprising the phthalocyanine dye-targeting molecule conjugate manufactured by a method comprising:

a) contacting a targeting molecule with a phthalocyanine dye under conditions to produce a conjugate comprising the phthalocyanine dye linked to the targeting molecule; and

b) formulating the conjugate in a pharmaceutically acceptable buffer,

wherein in each of steps a) – b):

Ramucirumab, Regorafenib, Ruxolitinib, Sorafenib, Sunitinib, Temsirolimus, Trametinib, Vandetanib, Vemurafenib, Vismodegib, Basiliximab, Ipilimumab, Nivolumab, pembrolizumab, lambrolizumab, MPDL3280A, Pidilizumab (CT-011), MSB0010718C, BMS-935559 or MEDI4736, AMP-224, or is an antigen-binding fragment thereof. In some examples, the antibody binds to a cell surface target molecule, such as HER1/EGFR, HER2, PD-L1, CD25, EpCAM, EphA2, CD206, CD20, CD44, CD133, Mesothelin, Glypican-3, or carcinoembryonic antigen (CEA). In some examples, the antibody is cetuximab, Panitumumab, Trastuzumab, BMS-935559, MEDI4736, MPDL3280A or MSB0010718C or is an antigen-binding fragment thereof.

[0063] In some examples, the stable dye-targeting molecule conjugate is cetuximab-IR700, Panitumumab-IR700, Trastuzumab-IR700, BMS-935559-IR700, MEDI4736-IR700, MPDL3280A-IR700 or MSB0010718C-IR700.

[0064] In some examples, the stable conjugate includes from or from about 1 to about 1000 phthalocyanine dye molecules per targeting molecule, from or from about 1 to about 10 or from or from about 2 to about 5 phthalocyanine dye molecules per targeting molecule.

[0065] Provided in some examplesis a composition containing the conjugate or stable conjugate.

[0066] Provided in some examplesis a pharmaceutical composition containing the conjugate or stable conjugate and a pharmaceutically acceptable excipient.

[0066a] In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising the stable conjugate described herein and a pharmaceutically acceptable excipient.

[0067] In some examples, the composition is formulated in phosphate buffered saline. In some examples, composition has a pH of greater than 6.0.

[0068] Provided in some examplesis a pharmaceutical composition containing a phthalocyanine dye linked to a targeting molecule and a pharmaceutically acceptable excipient. In some such examples, the composition has a pH of greater than 6.0 and the conjugate in the composition is stable for greater than three months, such as with greater than 90% of the conjugate present as a main monomer component. In some such examples, the conjugate in the composition is stable if it retains greater than or greater than about 30%, 40%, 50%, 60%, 70%, 80%, 90% or more of its potency, activity or purity for greater than 3 months compared to the conjugate prior to the storage for the time. In some such examples, the conjugate in the composition is stable if greater than 90% of the conjugate is present as a main monomer component. In some examples, the pH of the composition is greater than 6.0 or is from or from about pH 6.0 to about 8.0, inclusive.

[0069] In some examples, the concentration of the conjugate in the composition is from or from about 0.01 mg/mL to about 200 mg/mL. In some examples, the concentration of the conjugate in the composition is from or from about 0.5 mg/mL to about 10 mg/mL. In some examples, the concentration of the conjugate in the composition is from or from about 1.0 to about 5.0 mg/mL. In some examples, the concentration of the conjugate in the composition is from or from about 1.8 to about 2.1 mg/mL.

[0070] In some examples, the volume of the composition is from or from about 0.5 mL to about 100 mL, from or from about 1 mL to about 50 mL or from or from about 1 mL to about 10 mL.

[0071] Provided in some examplesis a container comprising the conjugate or stable conjugate. In some examples, the container protects from transmission of light having a wavelength from or from about 500 nm to 725 nm or 650 nm to 725 nm. In some examples, the container protects from transmission of light such that the percentage of light transmission by the container is less than 50%, less than 40%, less than 30%, less than 20%, less than 10% or less than 5%. In some examples, the container is green, blue, amber, translucent, opaque, or is covered by a material with light transmission of less than 50%, less than 40%, less than 30%, less than 20%, less than 10% or less than 5%. In some examples, the container is wrapped in an opaque foil.

[0072] Provided in some examplesis a packaging system for protecting a phthalocyanine dye-targeting molecule conjugate from light. In some examples, the packaging system includes an internal packaging material comprising the container described herein. In some examples, the internal packaging material has a light transmittance of no more than 5%. In some examples, an external packaging material contains the internal packaging material. In some examples, the external packaging material has a light transmittance of no more than 5%. In some examples, the internal packaging material includes an opaque foil.

[0072a] In yet another aspect, the present disclosure refers to a packaging system comprising the stable conjugate described herein, and further comprising a container, wherein the container protects from transmission of light such that the percentage of transmission of light is less than 50%.

[0073] Provided in some examplesis a packaging system for protecting a phthalocyanine dye-targeting molecule conjugate from light that includes a first container,

the conjugate, the conjugate is not exposed to an intensity of environmental light greater than 500 lux.

[0094] In some examples, the method is performed *in vitro* or *ex vivo*. In some examples, the *ex vivo* irradiation is method is performed using an extracorporeal device. Provided in some examplesis a method of treating hyperplasia or a tumor in a subject. In some examples, the method includes administering to the subject a therapeutically effective amount of the conjugate or stable conjugate or composition, where prior to and during the administration step the conjugate is not exposed to an intensity of environmental light greater than 500 lux. In some examples, the method further includes irradiating the hyperplasia or the tumor at a wavelength of 660 to 740 nm at a dose of at least 1 J cm^{-2} or 1 J/cm of fiber length, thereby treating the tumor in the subject.

[0094a] In yet another aspect, the present disclosure refers to use of a phthalocyanine dye-targeting molecule conjugate manufactured according to the method described herein for the preparation of a medicament for use in a photoimmunotherapyfor treating a hyperplasia or a tumor in a subject, wherein:

a) a therapeutically effective amount of the phthalocyanine dye-targeting molecule conjugate is to be administered to the subject, wherein prior to and during the administration the conjugate is not exposed to an intensity of environmental light greater than 500 lux; and
b) the photoimmunotherapy comprises irradiation of the hyperplasia or the tumor at a wavelength of 660 to 740 nm at a dose of at least 1 J cm^{-2} or 1 J/cm of fiber length to thereby treat the tumor in the subject

[0095] Provided in some examplesis a method of treating hyperplasia or a tumor in a subject. In some such examples, the method includes administering to the subject a therapeutically effective amount of a conjugate containing IRDye 700DX (IR700) linked to a targeting molecule. In some examples, the conjugate is targeted to the hyperplasia or the tumor and prior to and during the administration step the conjugate is not exposed to an intensity of environmental light greater than 500 lux. In some examples, the method further includes irradiating the tumor at a wavelength of 600 to 800 nm at a dose of at least 1 J cm^{-2} or 1 J/cm of fiber length thereby treating the tumor in the subject.

[0095a] In yet another aspect, the present disclosure refers to use of the stable conjugate described herein for the preparation of a medicament for use in a photoimmunotherapy for treating a hyperplasia or a tumor in a subject, wherein:

a) a therapeutically effective amount of the stable conjugate is to be administered to the subject, wherein prior to and during the administration the conjugate is not exposed to an intensity of environmental light greater than 500 lux; and

b) the photoimmunotherapy comprises irradiation of the hyperplasia or the tumor at a wavelength of 660 to 740 nm at a dose of at least 1 J cm^{-2} or 1 J/cm of fiber length to thereby treat the tumor in the subject.

[0096] Provided in some examplesis a method of treating hyperplasia or a tumor in a sample. In some examples, the method includes administering to the sample a therapeutically effective amount of the conjugate or stable conjugate or composition, where prior to and during the administration step the conjugate is not exposed to an intensity of environmental light greater than 500 lux. In some examples, the method further includes irradiating the hyperplasia or the tumor at a wavelength of 660 to 740 nm at a dose of at least 1 J cm^{-2} or 1 J/cm of fiber length, thereby treating the tumor in the sample.

[0096a] In yet another aspect, the present disclosure refers to a pharmaceutical composition described herein for use in a photoimmunotherapy for treating a hyperplasia or a tumor in a subject, the photoimmunotherapy comprising:

a) a therapeutically effective amount of the phthalocyanine dye-targeting molecule conjugate that is to be administered to the subject, wherein prior to and during the administration the conjugate is not exposed to an intensity of environmental light greater than 500 lux; and

b) the hyperplasia or the tumor is to be irradiated at a wavelength of 660 to 740 nm at a dose of at least 1 J cm^{-2} or 1 J/cm of fiber length to thereby treat the tumor in the subject.

[0097] Provided in some examplesis a method of treating hyperplasia or a tumor in a sample. In some such examples, the method includes administering to the sample a therapeutically effective amount of a conjugate containing IRDye 700DX (IR700) linked to a targeting molecule. In some examples, the conjugate is targeted to the hyperplasia or the tumor and prior to and during the administration step the conjugate is not exposed to an intensity of environmental light greater than 500 lux. In some examples, the method further includes irradiating the tumor at a wavelength of 600 to 800 nm at a dose of at least 1 J cm^{-2} or 1 J/cm of fiber length thereby treating the tumor in the sample.

[0097a] In yet another aspect, the present disclosure refers to use of the pharmaceutical composition described herein for the preparation of a medicament for use in a photoimmunotherapy for treating a hyperplasia or a tumor in a subject, wherein:

a) a therapeutically effective amount of the pharmaceutical composition is to be administered to the subject, wherein prior to and during the administration the conjugate in the pharmaceutical composition is not exposed to an intensity of environmental light greater than 500 lux; and

b) the photoimmunotherapy comprises irradiation of the hyperplasia or the tumor at a wavelength of 660 to 740 nm at a dose of at least 1 J cm^{-2} or 1 J/cm of fiber length to thereby treat the tumor in the subject.

[0097b] In yet another aspect, the present disclosure refers to a method of removing unwanted cells or pathogens in a sample comprising:

a) administering to a sample, the sample comprising unwanted cells or pathogens, a therapeutically effective amount of the conjugate manufactured according to the method described herein, wherein prior to and during the administration step the conjugate is not exposed to an intensity of environmental light greater than 500 lux; and

b) irradiating the unwanted cells or pathogens at a wavelength of 660 to 740 nm at a dose of at least 1 J cm^{-2} or 1 J/cm of fiber length thereby removing the unwanted cells or pathogens in the sample, optionally wherein the method is performed *in vitro* or *ex vivo*.

[0097c] In yet another aspect, the present disclosure refers to use of a phthalocyanine dye-targeting molecule conjugate manufactured according to the method described herein for the preparation of a medicament for use in a photoimmunotherapy for removing unwanted cells or pathogens in a subject, wherein the unwanted cells or pathogens are associated with a disease or condition in the subject, wherein the disease or condition is tumor or cancer, an infection, an inflammatory disease or condition, or a neuronal disease or condition, and wherein:

a) a therapeutically effective amount of the conjugate is to be administered to the subject, wherein prior to and during the administration the conjugate is not exposed to an intensity of environmental light greater than 500 lux; and

b) the photoimmunotherapy comprises irradiation of the unwanted cells or pathogens at a wavelength of 660 to 740 nm at a dose of at least 1 J cm^{-2} or 1 J/cm of fiber length to thereby remove the unwanted cells or pathogens in the subject and treat the disease or condition in the subject.

[0097d] In yet another aspect, the present disclosure refers to a pharmaceutical composition described herein for use in a photoimmunotherapy for removing unwanted cells or pathogens in a subject, wherein the unwanted cells or pathogens are associated with a disease or condition in the subject and the photoimmunotherapy comprises:

[0112] In some examplesof the methods provided herein, prior to administration of the conjugate the targeting molecule is administered, *e.g.*, administered to the subject or the sample. In some examples, the targeting molecule is administered at a dose within a range from or from about 10 mg/m^2 to about 500 mg/m^2.

[0113] In some examples, the targeting molecule is an antibody or antigen binding fragment. In some examples, the antibody is cetuximab.

[0114] Provided in some examplesis a conjugate containing a phthalocyanine dye and a targeting molecule. In some such examples, the targeting molecule is a tissue-specific homing peptide, an RGD polypeptide, an iRGD polypeptide, a Lyp-1 polypeptide, a cripto-1 binding polypeptide, a somatostatin receptor binding polypeptide, a prohibitin binding polypeptide, a NGR polypeptide, an iNGR polypeptide, an activatable cell penetrating peptide (ACPP) comprised of a polycationic cell penetrating peptide (CPP) connected via a cleavable linker to a neutralizing polyanion, or an antibody, such as Ado-trastuzumab emtansine, Afatinib, Axitinib, Bosutinib, Cabozantinib, Ceritinib, Crizotinib, Dabrafenib, Dasatinib, Everolimus, Ibrutinib, Imatinib, Lenvatinib, Nilotinib, Olaparib, Palbociclib, Pazopanib, Ramucirumab, Regorafenib, Ruxolitinib, Sorafenib, Sunitinib, Temsirolimus, Trametinib, Vandetanib, Vemurafenib, Vismodegib, Ipilimumab, Nivolumab, pembrolizumab, MPDL3280A, Pidilizumab (CT-011), AMP-224, MSB0010718C, or MEDI4736, or an antigen-binding fragment thereof.

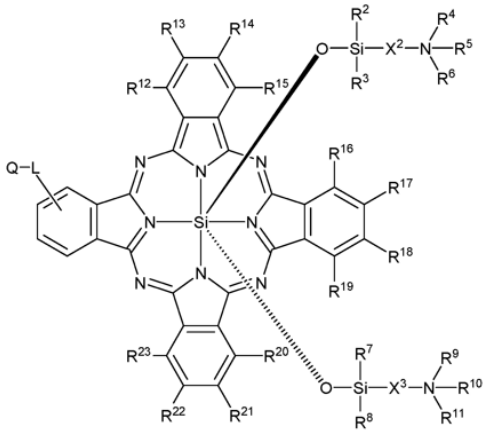
[0114a] In yet another example, the present disclosure provides a conjugate, comprising a phthalocyanine dye and a targeting molecule, wherein the targeting molecule is selected from among a tissue-specific homing peptide, an RGD polypeptide, an iRGD polypeptide, a Lyp-1 polypeptide, a cripto-1 binding polypeptide, a somatostatin receptor binding polypeptide, a prohibitin binding polypeptide, a NGR polypeptide, an iNGR polypeptide, an activatable cell penetrating peptide (ACPP) comprised of a polycationic cell penetrating peptide (CPP) connected via a cleavable linker to a neutralizing polyanion, and an antibody selected from Ado-trastuzumab emtansine, Afatinib, Axitinib, Bosutinib, Cabozantinib, Ceritinib, Crizotinib, Dabrafenib, Dasatinib, Everolimus, Ibrutinib, Imatinib, Lenvatinib, Nilotinib, Olaparib, Palbociclib, Pazopanib, Ramucirumab, Regorafenib, Ruxolitinib, Sorafenib, Sunitinib, Temsirolimus, Trametinib, Vandetanib, Vemurafenib, Vismodegib, Ipilimumab, Nivolumab, pembrolizumab, pidilizumab, lambrolizumab, BMS-935559, MPDL3280A, Pidilizumab (CT-011), AMP-224, MSB0010718C, or MEDI4736, or an antigen-binding fragment thereof.

[0115] In some examples, the ACPP comprises the structure: A-X1-B-, wherein B is a peptide portion of about 5 to about 20 basic amino acid residues, which is suitable for cellular uptake; A is a peptide portion of about 2 to about 20 acidic amino acid residues, which when linked with portion B is effective to inhibit or prevent cellular uptake of portion B; X1 is a cleavable linker of about 2 to about 100 atoms; and one or more of L-Y is linked to the C-terminus of peptide portion B.

[0116] In some examples, the homing peptide has a sequence as set forth in any of SEQ ID NOs: 1-52. In some examples, the dye is IR700.

[0116a] In another aspect, the present disclosure refers to a pharmaceutical composition comprising a conjugate of a phthalocyanine dye and a targeting molecule; wherein:

the phthalocyanine dye comprises the formula:



, wherein:

L is a linker;

Q is a reactive group for attachment of the dye to the targeting molecule;

R^2, R^3, R^7, and R^8 are each independently selected from among optionally substituted alkyl and optionally substituted aryl;

R^4, R^5, R^6, R^9, R^{10}, and R^{11} are each independently selected from among hydrogen, optionally substituted alkyl, optionally substituted alkanoyl, optionally substituted alkoxycarbonyl, optionally substituted alkylcarbamoyl, and a chelating ligand, wherein at least one of R^4, R^5, R^6, R^9, R^{10}, and R^{11} comprises a water soluble group;

R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{20}, R^{21}, R^{22} and R^{23} are each independently selected from among hydrogen, halogen, optionally substituted alkylthio, optionally substituted alkylamino and optionally substituted alkoxy; and

X^2 and X^3 are each independently C_1-C_{10} alkylene, optionally interrupted by a heteroatom; and

the pharmaceutical composition comprises the conjugate of the phthalocyanine dye and the having the following features: (a) less than or equal to 3% free dye, (b) high molecular weight species less than or equal to 5%, (c) low molecular weight species less than or equal to 5%, and (d) greater than or equal to 92% monomer.

Brief Description of the Drawings

[0117] **FIG.1A** displays the relative amounts of high molecular weight species (aggregates) and monomer form of the Cetuximab-IRDye 700DX as assessed by High Pressure Liquid Chromatography using a Size Exclusion column (HPLC-SEC) of a sample in a clear vial before 24 hours of light exposure.

58. The method of any of embodiments 1-53, wherein the targeting molecule is an antibody or an antibody fragment.

59. The method of embodiment 58, wherein the antibody is selected from among cetuximab, panitumumab, zalutumumab, nimotuzumab, trastuzumab, Ado-trastuzumab emtansine, Tositumomab (Bexxar ®), Rituximab (Rituxan, Mabthera), Ibritumomab tiuxetan (Zevalin), Daclizumab (Zenapax), Gemtuzumab (Mylotarg), Alemtuzumab, CEA-scan Fab fragment, OC125 monoclonal antibody, ab75705, B72.3, Bevacizumab (Avastin ®), Afatinib, Axitinib, Bosutinib, Cabozantinib, Ceritinib, Crizotinib, Dabrafenib, Dasatinib, Erlotinib, Everolimus, Ibrutinib, Imatinib, Lapatinib, Lenvatinib, Nilotinib, Olaparib, Palbociclib, Pazopanib, Pertuzumab, Ramucirumab, Regorafenib, Ruxolitinib, Sorafenib, Sunitinib, Temsirolimus, Trametinib, Vandetanib, Vemurafenib, Vismodegib, Basiliximab, Ipilimumab, Nivolumab, pembrolizumab, BMS-935559, MPDL3280A, Pidilizumab (CT-011), AMP-224, MSB0010718C, and MEDI4736, or is an antigen-binding fragment thereof.

60. The method of embodiment 58, wherein the antibody binds to a cell surface target molecule selected from among HER1/EGFR, HER2, PD-L1, and carcinoembryonic antigen (CEA).

61. The method of any of embodiments 58-60, wherein the antibody is selected from among cetuximab, Panitumumab, Trastuzumab, BMS-935559, MEDI4736, MPDL3280A and MSB0010718C, or is an antigen-binding fragment thereof.

62. The method of any of embodiments 1-61, wherein the dye-targeting molecule conjugate is selected from among cetuximab-IR700, Panitumumab-IR700, Trastuzumab-IR700, BMS-935559-IR700, MEDI4736-IR700, MPDL3280A-IR700 and MSB0010718C-IR700.

63. The method of any of embodiments 1-62, wherein step b) of contacting a targeting molecule with the phthalocyanine dye is at a molar ratio of dye to targeting molecule from 1:1 to 100:1 or 1:1 to 10:1.

64. The method of any of embodiments 1-63, wherein the molar ratio of dye to targeting molecule is at least or at least about 4:1 or is at least or at least about 10:1.

65. The method of any of embodiments 1-64, wherein the produced conjugate comprises from or from about 1 to about 1000 phthalocyanine dye molecules per targeting molecule, from or from about 1 to about 10 phthalocyanine dye molecules per targeting molecule or from or from about 2 to about 5 phthalocyanine dye molecules per targeting molecule.

Bevacizumab (Avastin ®), Afatinib, Axitinib, Bosutinib, Cabozantinib, Ceritinib, Crizotinib, Dabrafenib, Dasatinib, Erlotinib, Everolimus, Ibrutinib, Imatinib, Lapatinib, Lenvatinib, Nilotinib, Olaparib, Palbociclib, Pazopanib, Pertuzumab, Ramucirumab, Regorafenib, Ruxolitinib, Sorafenib, Sunitinib, Temsirolimus, Trametinib, Vandetanib, Vemurafenib, Vismodegib, Basiliximab, Ipilimumab, Nivolumab, pembrolizumab, BMS-935559, MPDL3280A, Pidilizumab (CT-011), AMP-224, MSB0010718C, and MEDI4736, or is an antigen-binding fragment thereof.

120. The conjugate or stable conjugate of embodiment 118 or embodiment 119, wherein the antibody binds to a cell surface target molecule selected from among HER1/EGFR, HER2 PD-L1, and carcinoembryonic antigen (CEA).

121. The conjugate or stable conjugate of any of embodiments 118-120, wherein the antibody is selected from among cetuximab, Panitumumab, Trastuzumab, BMS-935559, MEDI4736, MPDL3280A and MSB0010718C, or is an antigen-binding fragment thereof.

122. The conjugate or stable conjugate of any of embodiments 118-121, wherein the dye-targeting molecule conjugate is selected from among cetuximab-IR700, Panitumumab-IR700, Trastuzumab-IR700, BMS-935559-IR700, MEDI4736-IR700, MPDL3280A-IR700 and MSB0010718C-IR700.

123. The conjugate or stable conjugate of any of embodiments 90-122, wherein the conjugate comprises from or from about 1 to about 1000 phthalocyanine dye molecules per targeting molecule, from or from about 1 to about 10 or from or from about 2 to about 5 phthalocyanine dye molecules per targeting molecule.

124. A composition comprising the conjugate or stable conjugate of any of embodiments 90-123 and 234-236.

125. A pharmaceutical composition comprising the conjugate or stable conjugate of any of embodiments 90-123 and 234-236 and a pharmaceutically acceptable excipient.

126. The composition of embodiment 124 or embodiment 125, that is formulated in phosphate buffered saline.

127. The composition of any of embodiments 124-126 that has a pH of greater than 6.0.

128. A pharmaceutical composition, comprising a phthalocyanine dye linked to a targeting molecule and a pharmaceutically acceptable excipient, wherein:

the composition has a pH of greater than 6.0; and

the conjugate in the composition is stable for greater than three months.

the conjugate is administered in an amount that is at least or about at least or is or is about 20 mg/m^2, 40 mg/m^2, 160 mg/m^2, 320 mg/m^2, 640 mg/m^2, 1280 mg/m^2 or 2560 mg/m^2.

229. The method of any of embodiments 166-228, wherein prior to administration of the conjugate the targeting molecule is administered.

230. The method of any of embodiments 166-229, wherein the targeting molecule is administered up to 96 hours prior to administration of the conjugate.

231. The method of embodiment 229 or embodiment 230, wherein the targeting molecule is administered at a dose within a range from or from about 10 mg/m^2 to about 500 mg/m^2.

232. The method of any of embodiments 166-231, wherein the targeting molecule is an antibody or antigen binding fragment.

233. The method of embodiment 232, wherein the antibody is cetuximab.

234. A conjugate, comprising a phthalocyanine dye and a targeting molecule, wherein the targeting molecule is selected from among a tissue-specific homing peptide, an RGD polypeptide, an iRGD polypeptide, a Lyp-1 polypeptide, a cripto-1 binding polypeptide, a somatostatin receptor binding polypeptide, a prohibitin binding polypeptide, a NGR polypeptide, an iNGR polypeptide, an activatable cell penetrating peptide (ACPP) comprised of a polycationic cell penetrating peptide (CPP) connected via a cleavable linker to a neutralizing polyanion, and an antibody selected from Ado-trastuzumab emtansine, Afatinib, Axitinib, Bosutinib, Cabozantinib, Ceritinib, Crizotinib, Dabrafenib, Dasatinib, Everolimus, Ibrutinib, Imatinib, Lenvatinib, Nilotinib, Olaparib, Palbociclib, Pazopanib, Ramucirumab, Regorafenib, Ruxolitinib, Sorafenib, Sunitinib, Temsirolimus, Trametinib, Vandetanib, Vemurafenib, Vismodegib, Ipilimumab, Nivolumab, pembrolizumab, pidilizumab, lambrolizumab, BMS-935559, MPDL3280A, Pidilizumab (CT-011), AMP-224, MSB0010718C, or MEDI4736, or an antigen-binding fragment thereof.

235. The conjugate of embodiment 234, wherein the homing peptide has a sequence as set forth in any of SEQ ID NOs: 1-52.

236. The conjugate of embodiment 234 or embodiment 235, wherein the dye is IR700.

V. EXAMPLES

[0434] The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

CLAIMS

1. A method of manufacturing a phthalocyanine dye-targeting molecule conjugate, comprising:

a) contacting a targeting molecule with a phthalocyanine dye under conditions to produce a conjugate comprising the phthalocyanine dye linked to the targeting molecule; and

b) formulating the conjugate in a pharmaceutically acceptable buffer,

wherein in each of steps a) – b):

the only light to which the dye and conjugate are exposed has a wavelength within a range from 400 nm to 650 nm, or

the only light to which the dye and conjugate are exposed has an intensity of less than 500 lux.

2. The method of claim 1, further comprising a quenching step subsequent to step (a) and before step (b), wherein, during the quenching step:

the only light to which the conjugate is exposed has a wavelength within a range from about 400 nm to about 650 nm, or

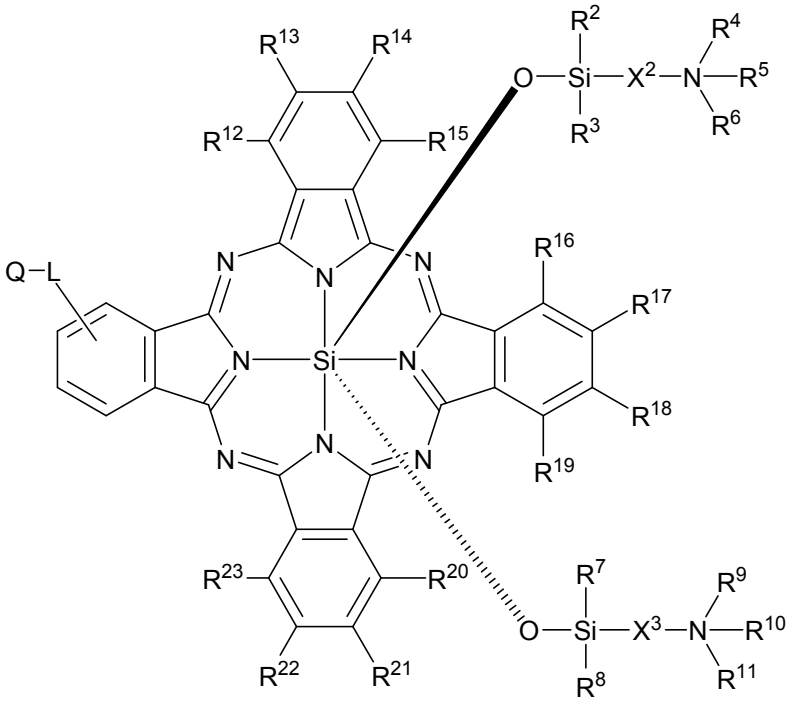
the only light to which the conjugate are exposed has an intensity of less than 500 lux.

3. The method of claim 2, wherein the quenching step comprises contacting the conjugate with glycine.

4. The method of any one of claims 1-3, wherein the contacting step a) further comprises contacting the targeting molecule with the phthalocyanine dye at a molar ratio of dye to targeting molecule of from 1:1 to 100:1, and wherein the conjugate is formulated to a concentration from 0.1 mg/mL to 200.0 mg/mL.

5. The method of any one of claims 1-4, wherein the conjugate is formulated to a concentration from 0.5 mg/mL to 10.0 mg/mL.

6. The method of any one of claims 1-5, wherein the phthalocyanine dye comprises the formula:



, wherein:

L is a linker;

Q is a reactive group for attachment of the dye to the targeting molecule;

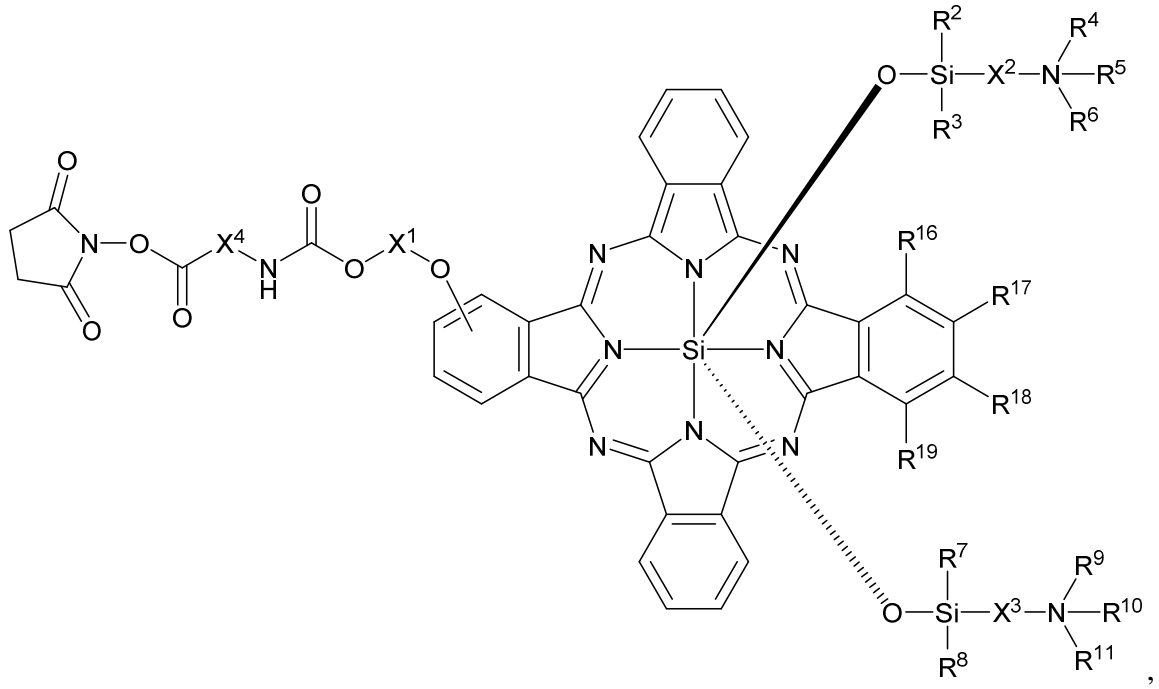
R^2, R^3, R^7, and R^8 are each independently selected from among optionally substituted alkyl and optionally substituted aryl;

R^4, R^5, R^6, R^9, R^{10}, and R^{11} are each independently selected from among hydrogen, optionally substituted alkyl, optionally substituted alkanoyl, optionally substituted alkoxycarbonyl, optionally substituted alkylcarbamoyl, and a chelating ligand, wherein at least one of R^4, R^5, R^6, R^9, R^{10}, and R^{11} comprises a water soluble group;

R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{20}, R^{21}, R^{22} and R^{23} are each independently selected from among hydrogen, halogen, optionally substituted alkylthio, optionally substituted alkylamino and optionally substituted alkoxy; and

X^2 and X^3 are each independently C_1-C_{10} alkylene, optionally interrupted by a heteroatom.

7. The method of any one of claims 1-6, wherein the phthalocyanine dye comprises the formula:



wherein:

X^1 and X^4 are each independently a C_1-C_{10} alkylene optionally interrupted by a heteroatom;

R^2, R^3, R^7, and R^8 are each independently selected from optionally substituted alkyl and optionally substituted aryl;

R^4, R^5, R^6, R^9, R^{10}, and R^{11} are each independently selected from among hydrogen, optionally substituted alkyl, optionally substituted alkanoyl, optionally substituted alkoxycarbonyl, optionally substituted alkylcarbamoyl, and a chelating ligand, wherein at least one of R^4, R^5, R^6, R^9, R^{10}, and R^{11} comprises a water soluble group; and

R^{16}, R^{17}, R^{18} and R^{19} are each independently selected from among hydrogen, halogen, optionally substituted alkylthio, optionally substituted alkylamino and optionally substituted alkoxy.

8. The method of any one of claims 1-7, wherein the phthalocyanine dye comprises IRDye 700DX-NHS (IR700-NHS).

9. The method of any one of claims 1-8, wherein the targeting molecule binds to an antigen, protein or cell surface target molecule.

10. The method of claim 9, wherein the cell surface target molecule is selected from among cell membrane phospholipids, prokaryotic peptidoglycans, bacterial cell envelop

proteins, viral capsid proteins, ACTHR, endothelial cell Anxa-1, aminopeptidase N, IL-6R, alpha-4-integrin, alpha-5-beta-3 integrin, alpha-5-beta-5 integrin, alpha-fetoprotein (AFP), ANPA, ANPB, APA, APN, APP, 1AR, 2AR, AT1, B1, B2, BAGE1, BAGE2, B-cell receptor BB 1, BB2, BB4, calcitonin receptor, cancer antigen 125 (CA 125), CCK1, CCK2, CD5, CD10, CD11a, CD13, CD14, CD19, CD20, CD22, CD25, CD30, CD33, CD38, CD45, CD52, CD56, CD68, CD90, CD133, CD7, CD15, CD34, CD44, CD206, CD271, CEA (Carcinoembryonic Antigen), CGRP, chemokine receptors, cell-surface annexin-1, cell-surface plectin-1, Cripto-1, CRLR, CXCR2, CXCR4, DCC, DLL3, E2 glycoprotein, EGFR, EGFRvIII, EMR1, Endosialin, EP2, EP4, EpCAM, EphA2, ET receptors, Fibronectin, Fibronectin ED-B, FGFR, frizzled receptors, GAGE1, GAGE2, GAGE3, GAGE4, GAGE5, GAGE6, GLP-1 receptor, G-protein coupled receptors of the Family A (Rhodopsin-like), G-protein coupled receptors of the Family B (Secretin receptor-like), G-protein coupled receptors of the Family C (Metabotropic Glutamate Receptor-like), GD2, GP100, GP120, Glypican-3, hemagglutinin, Heparin sulfates, HER1, HER2, HER3, HER4, HMFG, HPV 16/18 and E6/E7 antigens, hTERT, IL11-R, IL-13R, IL-2 receptor, CTLA-4, PD-L1, ITGAM, Kalikrien-9, Lewis Y, LH receptor, LHRH-R, LPA1, MAC-1, MAGE 1, MAGE 2, MAGE 3, MAGE 4, MART1, MC 1R, Mesothelin, MUC 1, MUC 16, Neu (cell-surface Nucleolin), Neprilysin, Neuropilin-1, Neuropilin-2, NG2, NK1, NK2, NK3, NMB-R, Notch-1, NY-ESO-1, OT-R, mutant p53, p97 melanoma antigen, NTR2, NTR3, p32 (p32/gC1q-R/HABP1), p75, PAC1, PAR1, Patched (PTCH), PDGFR, PDFG receptors, PDT, Protease-cleaved collagen IV, proteinase 3, prohibitin, protein tyrosine kinase 7, PSA, PSMA, purinergic P2X family, P2X1-5, mutant Ras, RAMP1, RAMP2, RAMP3 patched, RET receptor, plexins, smoothened, sst1, sst2A, sst2B, sst3, sst4, sst5, substance P, TEMs, T-cell CD3 Receptor, TAG72, TGFBR1, TGFBR2, Tie-1, Tie-2, Trk-A, Trk-B, Trk-C, TR1, TRPA, TRPC, TRPV, TRPM, TRPML, TRPP, TRPV1-6, TRPA1, TRPC1-7, TRPM1-8, TRPP1-5, TRPML1-3, TSH receptor, VEGF receptors, VEGFR1 or Flt-1, VEGFR2 or FLK-1/KDR, and VEGF-3 or FLT-4, voltage-gated ion channels, VPAC1, VPAC2, Wilms tumor 1, Y1, Y2, Y4, and Y5.

11. The method of any one of claims 1-10, wherein the targeting molecule is an antibody or an antibody fragment.

12. The method of any one of claims 1-11, wherein the targeting molecule is cetuximab.

13. A stable conjugate, comprising the phthalocyanine dye-targeting molecule conjugate manufactured by the method of any one of claims 1-12, wherein the conjugate is stable for greater than three months or greater than 6 months or greater than 12 months.

14. The stable conjugate of claim 13, wherein the conjugate is stable if it retains greater than 30%, 40%, 50%, 60%, 70%, 80%, 90% or more of its potency, activity or purity for greater than 3 months of storage compared to the conjugate's potency, activity or purity prior to storage.

15. A stable conjugate, comprising the phthalocyanine dye-targeting molecule conjugate manufactured by a method comprising:

a) contacting a targeting molecule with a phthalocyanine dye under conditions to produce a conjugate comprising the phthalocyanine dye linked to the targeting molecule; and

b) formulating the conjugate in a pharmaceutically acceptable buffer,

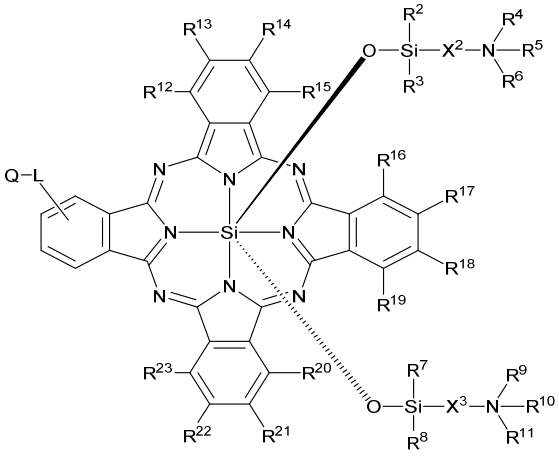
wherein in each of steps a) – b):

the only light to which the dye and conjugate are exposed has a wavelength within a range from 400 nm to 650 nm, or

the only light to which the dye and conjugate are exposed has an intensity of less than 500 lux; and

wherein the conjugate is stable for greater than three months or greater than 6 months or greater than 12 months; and

wherein the phthalocyanine dye comprises the formula:



, wherein:

L is a linker;

Q is a reactive group for attachment of the dye to the targeting molecule;

R^2, R^3, R^7, and R^8 are each independently selected from among optionally substituted alkyl and optionally substituted aryl;

R^4, R^5, R^6, R^9, R^{10}, and R^{11} are each independently selected from among hydrogen, optionally substituted alkyl, optionally substituted alkanoyl, optionally substituted alkoxycarbonyl, optionally substituted alkylcarbamoyl, and a chelating ligand, wherein at least one of R^4, R^5, R^6, R^9, R^{10}, and R^{11} comprises a water soluble group;

R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{20}, R^{21}, R^{22} and R^{23} are each independently selected from among hydrogen, halogen, optionally substituted alkylthio, optionally substituted alkylamino and optionally substituted alkoxy; and

X^2 and X^3 are each independently C_1-C_{10} alkylene, optionally interrupted by a heteroatom.

16. The stable conjugate of claim 15, wherein the phthalocyanine dye comprises IRDye 700DX-NHS (IR700-NHS).

17. The stable conjugate of claim 15 or claim 16, wherein the targeting molecule is cetuximab.

18. A pharmaceutical composition comprising the stable conjugate of any one of claims 13-17 and a pharmaceutically acceptable excipient.

19. The pharmaceutical composition of claim 18, wherein greater than 90% of the conjugate is present as a main monomer component.

20. A packaging system comprising the stable conjugate of any one of claims 13-17, and further comprising a container, wherein the container protects from transmission of light such that the percentage of transmission of light is less than 50%.

21. The packaging system of claim 20, wherein the percentage of transmission of light is less than 10%.

22. The packaging system of claim 20 or claim 21, wherein the container protects from transmission of light having a wavelength from 250 nm to 800 nm.

23. Use of a phthalocyanine dye-targeting molecule conjugate manufactured according to the method of any one of claims 1-12 for the preparation of a medicament for use in a photoimmunotherapy for treating a hyperplasia or a tumor in a subject, wherein:

a) a therapeutically effective amount of the phthalocyanine dye-targeting molecule conjugate is to be administered to the subject, wherein prior to and during the administration the conjugate is not exposed to an intensity of environmental light greater than 500 lux; and

b) the photoimmunotherapy comprises irradiation of the hyperplasia or the tumor at a wavelength of 660 to 740 nm at a dose of at least 1 J cm^{-2} or 1 J/cm of fiber length to thereby treat the tumor in the subject.

24. Use of the stable conjugate of any one of claims 13-17 for the preparation of a medicament for use in a photoimmunotherapy-for treating a hyperplasia or a tumor in a subject, wherein:

a) a therapeutically effective amount of the stable conjugate is to be administered to the subject, wherein prior to and during the administration the conjugate is not exposed to an intensity of environmental light greater than 500 lux; and

b) the photoimmunotherapy comprises irradiation of the hyperplasia or the tumor at a wavelength of 660 to 740 nm at a dose of at least 1 J cm^{-2} or 1 J/cm of fiber length to thereby treat the tumor in the subject.

25. A pharmaceutical composition of claim 18 or claim 19 for use in a photoimmunotherapy for treating a hyperplasia or a tumor in a subject, the photoimmunotherapy comprising:

a) a therapeutically effective amount of the phthalocyanine dye-targeting molecule conjugate that is to be administered to the subject, wherein prior to and during the administration the conjugate is not exposed to an intensity of environmental light greater than 500 lux; and

b) the hyperplasia or the tumor is to be irradiated at a wavelength of 660 to 740 nm at a dose of at least 1 J cm^{-2} or 1 J/cm of fiber length to thereby treat the tumor in the subject.

26. Use of the pharmaceutical composition of claim 18 or claim 19 for the preparation of a medicament for use in a photoimmunotherapy for treating a hyperplasia or a tumor in a subject, wherein:

a) a therapeutically effective amount of the pharmaceutical composition is to be administered to the subject, wherein prior to and during the administration the conjugate in the pharmaceutical composition is not exposed to an intensity of environmental light greater than 500 lux; and

b) the photoimmunotherapy comprises irradiation of the hyperplasia or the tumor at a wavelength of 660 to 740 nm at a dose of at least 1 J cm^{-2} or 1 J/cm of fiber length to thereby treat the tumor in the subject.

27. A method of removing unwanted cells or pathogens in a sample comprising:

a) administering to a sample, the sample comprising unwanted cells or pathogens, a therapeutically effective amount of the conjugate manufactured according to the method of any one of claims 1-12, wherein prior to and during the administration step the conjugate is not exposed to an intensity of environmental light greater than 500 lux; and

b) irradiating the unwanted cells or pathogens at a wavelength of 660 to 740 nm at a dose of at least 1 J cm^{-2} or 1 J/cm of fiber length thereby removing the unwanted cells or pathogens in the sample, optionally wherein the method is performed *in vitro* or *ex vivo*.

28. Use of a phthalocyanine dye-targeting molecule conjugate manufactured according to the method of any one of claims 1-12 for the preparation of a medicament for use in a photoimmunotherapy for removing unwanted cells or pathogens in a subject, wherein the unwanted cells or pathogens are associated with a disease or condition in the subject, wherein the disease or condition is tumor or cancer, an infection, an inflammatory disease or condition, or a neuronal disease or condition, and wherein:

a) a therapeutically effective amount of the conjugate is to be administered to the subject, wherein prior to and during the administration the conjugate is not exposed to an intensity of environmental light greater than 500 lux; and

b) the photoimmunotherapy comprises irradiation of the unwanted cells or pathogens at a wavelength of 660 to 740 nm at a dose of at least 1 J cm^{-2} or 1 J/cm of fiber length to thereby remove the unwanted cells or pathogens in the subject and treat the disease or condition in the subject.

29. A pharmaceutical composition of claim 18 or claim 19 for use in a photoimmunotherapy for removing unwanted cells or pathogens in a subject, wherein the

unwanted cells or pathogens are associated with a disease or condition in the subject and the photoimmunotherapy comprises:

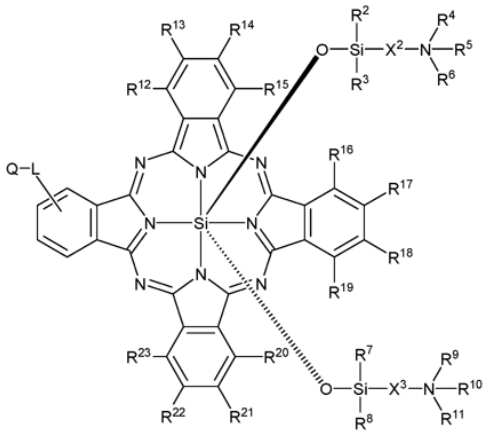
a) a therapeutically effective amount of the conjugate that is to be administered to the subject, wherein prior to and during the administration the conjugate is not exposed to an intensity of environmental light greater than 500 lux; and

b) the unwanted cells or pathogens are to be irradiated at a wavelength of 660 to 740 nm at a dose of at least 1 J cm^{-2} or 1 J/cm of fiber length to thereby remove the unwanted cells or pathogens in the subject and treat the disease or condition in the subject.

30. The pharmaceutical composition for use of claim 29, wherein disease or condition is a hyperplasia or tumor, an infection, an inflammatory disease or condition, or a neuronal disease or condition.

31. A pharmaceutical composition comprising a conjugate of a phthalocyanine dye and a targeting molecule; wherein:

the phthalocyanine dye comprises the formula:



, wherein:

L is a linker;

Q is a reactive group for attachment of the dye to the targeting molecule;

R^2, R^3, R^7, and R^8 are each independently selected from among optionally substituted alkyl and optionally substituted aryl;

R^4, R^5, R^6, R^9, R^{10}, and R^{11} are each independently selected from among hydrogen, optionally substituted alkyl, optionally substituted alkanoyl, optionally substituted alkoxycarbonyl, optionally substituted alkylcarbamoyl, and a chelating ligand, wherein at least one of R^4, R^5, R^6, R^9, R^{10}, and R^{11} comprises a water soluble group;

R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{20}, R^{21}, R^{22} and R^{23} are each independently selected from among hydrogen, halogen, optionally substituted alkylthio, optionally substituted alkylamino and optionally substituted alkoxy; and

X^2 and X^3 are each independently C_1-C_{10} alkylene, optionally interrupted by a heteroatom; and

the pharmaceutical composition comprises the conjugate of the phthalocyanine dye and the having the following features: (a) less than or equal to 3% free dye, (b) high molecular weight species less than or equal to 5%, (c) low molecular weight species less than or equal to 5%, and (d) greater than or equal to 92% monomer.

32. The pharmaceutical composition of claim 31, wherein the phthalocyanine dye comprises IRDye 700DX-NHS (IR700-NHS).

33. The pharmaceutical composition of claim 31 or claim 32, wherein the targeting molecule is selected from among a tissue-specific homing peptide, an RGD polypeptide, an iRGD polypeptide, a Lyp-1 polypeptide, a cripto-1 binding polypeptide, a somatostatin receptor binding polypeptide, a prohibitin binding polypeptide, a NGR polypeptide, an iNGR polypeptide, an activatable cell penetrating peptide (ACPP) comprised of a polycationic cell penetrating peptide (CPP) connected via a cleavable linker to a neutralizing polyanion, and an antibody selected from Ado-trastuzumab emtansine, Ipilimumab, Nivolumab, pembrolizumab, pidilizumab, lambrolizumab, BMS-935559, MPDL3280A, Pidilizumab (CT-011), AMP-224, MSB0010718C, or MEDI4736, or an antigen-binding fragment thereof.

34. The pharmaceutical composition of claim 31 or claim 32, wherein the targeting molecule is an antibody or an antigen-binding fragment.

35. The pharmaceutical composition of any one of claims 31, 32 and 34, wherein the targeting molecule is cetuximab.