

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization

International Bureau



(10) International Publication Number

WO 2017/024238 A1

(43) International Publication Date
9 February 2017 (09.02.2017)

WIPO | PCT

(51) International Patent Classification:
A61K 38/00 (2006.01) *A61K 47/00* (2006.01)
A61K 38/14 (2006.01) *A61K 47/48* (2006.01)
A61K 39/00 (2006.01)

(74) Agents: **HETZER-EGGER, Claudia, H.** et al.; Mintz Levin Cohn Ferris Glovsky and Popeo, P.C., 3580 Carmel Mountain Road, Suite 300, San Diego, CA 92130-6768 (US).

(21) International Application Number:
PCT/US2016/045817

(22) International Filing Date:
5 August 2016 (05.08.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/202,006 6 August 2015 (06.08.2015) US

(71) Applicant: **CITY OF HOPE** [US/US]; 1500 East Duarte Road, Duarte, CA 91010-3000 (US).

(72) Inventors: **HERRMANN, Andreas**; 500 S. El Molino, Pasadena, CA 91101 (US). **YU, Hua**; 123 S. Oakhart Drive, Glendora, CA 91741 (US).

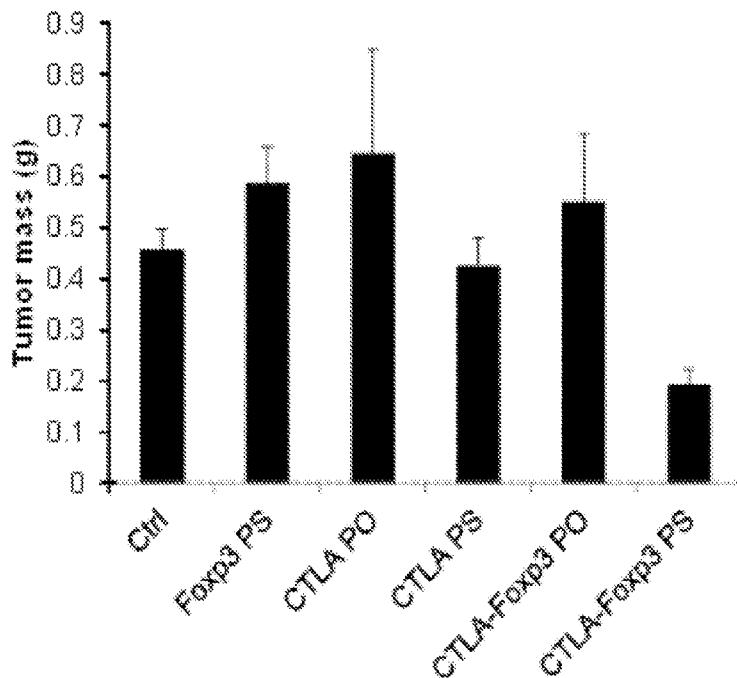
(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,

[Continued on next page]

(54) Title: CELL PENETRATING PROTEIN-ANTIBODY CONJUGATES AND METHODS OF USE

FIG. 4B



(57) Abstract: Provided herein are cell penetrating conjugates. The conjugates include non-cell penetrating proteins connected through a phosphorothioate nucleic acid, wherein the phosphorothioate nucleic acid enhances intracellular delivery of the non-cell penetrating proteins. Also provided are methods and kits including the conjugates provided herein.



LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, **Published:**
SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, — *with international search report (Art. 21(3))*
GW, KM, ML, MR, NE, SN, TD, TG).

CELL PENETRATING PROTEIN-ANTIBODY CONJUGATES AND METHODS OF USE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/202,006, filed August 6, 2015, which is hereby incorporated by reference in its entirety and for all purposes.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0002] This invention was made using support under Grant Number R01CA122976 awarded by the National Institutes of Health and National Cancer Institute. The government has certain rights to this invention.

BACKGROUND OF THE INVENTION

[0003] Antibodies have proven to be an efficacious drug modality for their easy generation, specificity and bio-durability relative to other types of drugs such as small molecule drugs. Current antibody therapy can only target extracellular molecules. However, numerous important targets for disease treatment and disease diagnosis are intracellular. For example, a number of transcriptional factors, such as STAT3, are among the most crucial yet challenging targets for cancer therapy. Provided herein are solutions for these and other needs in the art.

BRIEF SUMMARY OF THE INVENTION

[0004] There is a need to use peptides and proteins (e.g. antibodies) to target intracellular molecules. However, the ability of peptides and proteins (e.g. antibodies) to target intracellular molecules in an effective manner has proven difficult. As described throughout and demonstrated in the examples below, provided herein, *inter alia*, are novel cell penetrating compositions (e.g., antibodies, antibody conjugates) and methods of making and using the same. The compositions and methods provided herein allow for effective targeting of a broad range of intracellular molecules (e.g., proteins (e.g., oncogenic proteins, intracellularly residing viral proteins, and others)) and are useful, for example, for systemic administration.

[0005] Provided herein, *inter alia*, are cell penetrating conjugates. In one aspect, the cell penetrating conjugate includes a phosphorothioate nucleic acid connecting a first non-cell penetrating protein to a second protein. The phosphorothioate nucleic acid enhances the intracellular delivery of the both proteins.

[0006] In another aspect, a method of forming a cell penetrating conjugate is provided. The method includes hybridizing a first phosphorothioate nucleic acid attached to a first non-cell penetrating protein to a second phosphorothioate nucleic acid attached to a second protein, thereby forming a cell penetrating conjugate.

[0007] In another aspect, a cell including the cell penetrating conjugate provided herein including embodiments thereof is provided.

[0008] In another aspect, a pharmaceutical composition including the cell penetrating conjugate provided herein including embodiments thereof and a pharmaceutically acceptable carrier is provided.

[0009] In another aspect, a kit including the cell penetrating conjugate provided herein including embodiments thereof or the pharmaceutical composition provided herein including embodiments thereof and instructions for use are provided.

[0010] In another aspect, a method of delivering a non-cell penetrating protein into a cell including contacting the cell with the cell penetrating conjugate provided herein including embodiments thereof is provided.

[0011] In another aspect, a method of treating a disease in a subject is provided. The method includes administering to a subject in need thereof an effective amount of the cell penetrating conjugate provided herein including embodiments thereof, thereby treating the disease in the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] FIG. 1: Histograms showing production of bispecific antibodies via hybridization of attached DNA oligos. Hybridization was determined via gel filtration using antiSTAT3 antibody (upper right panel; STAT3-SAv-Biot-Cy5-PS antisense oligo), NFkB(p65) antibody (upper left panel; NFkB/p65-SAv-Biot-Cy5-PS sense oligo) or a combination thereof (lower left panel; STAT3/NFkB/p65-SAv-Biot-Cy5-PS double-stranded oligo and lower right panel; STAT3/NFkB/p65-SAv-Biot-Cy5-FAM double-stranded oligo). To ensure hybridization

generated dsDNA linking the antibodies, ssDNA oligos were chosen with incorporated fluorophore Quasar/Cy5 for modification of NFkB (p65) and FAM for modification of STAT3. Successful hybridization and generation of antibody linking dsDNA is indicated by both fluorophores eluting simultaneously with bi-IgG (lower right panel). All antibodies shown were purified by gel filtration and fractions at overlaying maximal amplitudes for protein (280 nm), DNA (260 nm) and fluorophore (647 nm and 495 nm) were collected for future characterization (FIG. 2). The detection channels acquired absorption at λ [nm] as indicated; 260 nm indicates DNA absorption, 280 nm indicates protein absorption, 647 nm and 495 nm indicate absorption by a fluorophore that was incorporated into the DNA oligo.

[0013] FIG. 2: Photographic image of a SDS page gel showing simultaneous recognition of intracellular antigens, STAT3 and NFkB(p65), by the cell-penetrating bispecific antibodies. Human lymphoma Ly3 cells were assessed by incubating with indicated purified antibodies at 10 mg/ml for 2 hrs. ProteinG-agarose beads were added to whole cell lysates cleared from cell debris, and Western blot analysis was performed to detect NFkB (p65) and STAT3 proteins.

[0014] FIG. 3: Histograms showing flow cytometry studies of cell penetration by modified intracellular targeting antibodies. Human B cell lymphoma Ly3 were treated with 10 μ g/ml of indicated modified antibodies for 2 hrs (upper panel); and confocal microscopy studies of the human B cell lymphoma cells incubated with fluorescently labeled modified antibodies at 10 μ g/ml. Scale 10 μ m (lower panel). Both studies confirmed cell penetration by modified single antibodies and bispecific antibodies generated by oligo-sense/antisense hybridization.

[0015] FIG. 4A and 4B: Bi-specific antibody treatment of B16 melanoma. FIG 4A: 0.1 million B16 tumor cells were injected s.c. and indicated antibodies (1 μ g/mouse) were administrated every other day after tumor inoculation from day 8 to day 26. FIG. 4B: All mice were euthanized on day 28 and tumor mass was weighted; tumor mass in gram is shown.

DETAILED DESCRIPTION OF THE INVENTION

[0016] While various embodiments and aspects of the present invention are shown and described herein, it will be obvious to those skilled in the art that such embodiments and aspects are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention.

It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention.

[0017] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in the application including, without limitation, patents, patent applications, articles, books, manuals, and treatises are hereby expressly incorporated by reference in their entirety for any purpose.

[0018] The abbreviations used herein have their conventional meaning within the chemical and biological arts. The chemical structures and formulae set forth herein are constructed according to the standard rules of chemical valency known in the chemical arts.

[0019] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art. See, e.g., Singleton et al., DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY 2nd ed., J. Wiley & Sons (New York, NY 1994); Sambrook et al., MOLECULAR CLONING, A LABORATORY MANUAL, Cold Springs Harbor Press (Cold Springs Harbor, NY 1989). Any methods, devices and materials similar or equivalent to those described herein can be used in the practice of this invention. The following definitions are provided to facilitate understanding of certain terms used frequently herein and are not meant to limit the scope of the present disclosure.

[0020] "Nucleic acid" or "oligonucleotide" or "polynucleotide" or grammatical equivalents used herein means at least two nucleotides covalently linked together. The term "Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form, or complements thereof. The term "polynucleotide" refers to a linear sequence of nucleotides. The term "nucleotide" typically refers to a single unit of a polynucleotide, *i.e.*, a monomer. Nucleotides can be ribonucleotides, deoxyribonucleotides, or modified versions thereof. Examples of polynucleotides contemplated herein include single and double stranded DNA, single and double stranded RNA (including siRNA), and hybrid molecules having mixtures of single and double stranded DNA and RNA. The terms also encompass nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs

include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, and 2-O-methyl ribonucleotides.

[0021] The term "phosphorothioate nucleic acid" refers to a nucleic acid in which one or more internucleotide linkages are through a phosphorothioate moiety (thiophosphate). The phosphorothioate moiety may be a monothiophosphate ($-P(O)_3(S)^{3-}$) or a dithiophosphate ($-P(O)_2(S)_2^{3-}$). In embodiments, the phosphorothioate moiety is a monothiophosphate ($-P(O)_3(S)^{3-}$). In embodiments, the phosphorothioate nucleic acid is a monothiophosphate nucleic acid. In embodiments, one or more of the nucleosides of a phosphorothioate nucleic acid are linked through a phosphorothioate moiety (e.g. monothiophosphate), and the remaining nucleosides are linked through a phosphodiester moiety ($-P(O)_4^{3-}$). In embodiments, one or more of the nucleosides of a phosphorothioate nucleic acid are linked through a phosphorothioate moiety (e.g. monothiophosphate), and the remaining nucleosides are linked through a methylphosphonate linkage. In embodiments, all the nucleosides of a phosphorothioate nucleic acid are linked through a phosphorothioate moiety (e.g. a monothiophosphate).

[0022] Phosphorothioate oligonucleotides (phosphorothioate nucleic acids) are typically from about 5, 6, 7, 8, 9, 10, 12, 15, 20, 25, 30, 40, 50 or more nucleotides in length, up to about 100 nucleotides in length. Phosphorothioate nucleic acids may also be longer in lengths, e.g., 200, 300, 500, 1000, 2000, 3000, 5000, 7000, 10,000, etc. As described above, in certain embodiments, the phosphorothioate nucleic acids herein contain one or more phosphodiester bonds. In other embodiments, the phosphorothioate nucleic acids include alternate backbones (e.g., mimics or analogs of phosphodiesters as known in the art, such as, boranophosphate, methylphosphonate, phosphoramidate, or O-methylphosphoroamidite linkages (see Eckstein, Oligonucleotides and Analogues: A Practical Approach, Oxford University Press). The phosphorothioate nucleic acids may also include one or more nucleic acid analog monomers known in the art, such as, peptide nucleic acid monomer or polymer, locked nucleic acid monomer or polymer, morpholino monomer or polymer, glycol nucleic acid monomer or polymer, or threose nucleic acid monomer or polymer. Other analog nucleic acids include those with positive backbones; non-ionic backbones, and nonribose backbones, including those described in U.S. Patent Nos. 5,235,033 and 5,034,506, and Chapters 6 and 7, ASC Symposium Series 580, Carbohydrate Modifications in Antisense Research, Sanghui & Cook, eds. Nucleic acids containing one or more carbocyclic sugars are

also included within one definition of nucleic acids. Modifications of the ribose-phosphate backbone may be done for a variety of reasons, e.g., to increase the stability and half-life of such molecules in physiological environments or as probes on a biochip. Mixtures of naturally occurring nucleic acids and analogs can be made; alternatively, mixtures of different nucleic acid analogs, and mixtures of naturally occurring nucleic acids and analogs may be made. Phosphorothioate nucleic acids and phosphorothioate polymer backbones can be linear or branched. For example, the branched nucleic acids are repetitively branched to form higher ordered structures such as dendrimers and the like.

[0023] In embodiments, the phosphorothioate nucleic acid includes a phosphorothioate polymer backbone. As used herein, a "phosphorothioate polymer backbone" is a chemical polymer with at least two phosphorothioate linkages (e.g. monothiophosphate) (e.g. linking together sugar subunits, cyclic subunits or alkyl subunits). The phosphorothioate polymer backbone may be a phosphorothioate sugar polymer, which is a phosphorothioate nucleic acid in which one or more (or all) of the chain of pentose sugars lack the bases (nucleobases) normally present in a nucleic acid. The phosphorothioate polymer backbone can include two or more phosphorothioate linkages. The phosphorothioate polymer backbone can include 5, 6, 7, 8, 9, 10, 12, 15, 25, 30, 40, 50 or more linkages and can contain up to about 100 phosphorothioate linkages. Phosphorothioate polymer backbones may also contain a larger number of linkages, e.g., 200, 300, 500, 1000, 2000, 3000, 5000, 7000, 10,000, and the like.

[0024] The phosphorothioate nucleic acids and phosphorothioate polymer backbones may be partially or completely phosphorothioated. For example, 50% or more of the internucleotide linkages of a phosphorothioate nucleic acid can be phosphorothioate linkages. In embodiments, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of the internucleotide linkages of a phosphorothioate nucleic acid are phosphorothioate linkages. In embodiments, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of the internucleotide linkages of a phosphorothioate nucleic acid are phosphorothioate linkages. In embodiments, 75%, 80%, 85%, 90%, 95%, or 99% of the internucleotide linkages of a phosphorothioate nucleic acid are phosphorothioate linkages. In embodiments, 90%, 95%, or 99% of the internucleotide linkages of a phosphorothioate nucleic acid are phosphorothioate linkages. In embodiments, the remaining internucleotide linkages are phosphodiester linkages. In embodiments, the remaining internucleotide linkages are methylphosphonate linkages. In embodiments, 100%

of the internucleotide linkages of the phosphorothioate nucleic acids are phosphorothioate linkages. Similarly, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%, of the intersugar linkages in a phosphorothioate polymer backbone can be phosphorothioate linkages. In embodiments, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%, of the intersugar linkages in a phosphorothioate polymer backbone can be phosphorothioate linkages. In embodiments, 75%, 80%, 85%, 90%, 95%, or 99%, of the intersugar linkages in a phosphorothioate polymer backbone can be phosphorothioate linkages. In embodiments, 90%, 95%, or 99%, of the intersugar linkages in a phosphorothioate polymer backbone can be phosphorothioate linkages. In embodiments, the remaining internucleotide linkages are phosphodiester linkages. In embodiments, the remaining internucleotide linkages are methylphosphonate linkages. In embodiments, 100% of the intersugar linkages of the phosphorothioate polymer backbone are phosphorothioate linkages.

[0025] Nucleic acids may include nonspecific sequences. As used herein, the term "nonspecific sequence" refers to a nucleic acid sequence that contains a series of residues that are not designed to be complementary to or are only partially complementary to any other nucleic acid sequence. By way of example, a nonspecific nucleic acid sequence is a sequence of nucleic acid residues that does not function as an inhibitory nucleic acid when contacted with a cell or organism. An "inhibitory nucleic acid" is a nucleic acid (e.g. DNA, RNA, polymer of nucleotide analogs) that is capable of binding to a target nucleic acid (e.g. an mRNA translatable into a protein) and reducing transcription of the target nucleic acid (e.g. mRNA from DNA) or reducing the translation of the target nucleic acid (e.g. mRNA) or altering transcript splicing (e.g. single stranded morpholino oligo).

[0026] A "labeled nucleic acid or oligonucleotide" is one that is bound, either covalently, through a linker or a chemical bond, or noncovalently, through ionic, van der Waals, electrostatic, or hydrogen bonds to a label such that the presence of the nucleic acid may be detected by detecting the presence of the detectable label bound to the nucleic acid. Alternatively, a method using high affinity interactions may achieve the same results where one of a pair of binding partners binds to the other, e.g., biotin, streptavidin. In embodiments, the phosphorothioate nucleic acid or phosphorothioate polymer backbone includes a detectable label, as disclosed herein and generally known in the art.

[0027] The words "complementary" or "complementarity" refer to the ability of a nucleic acid in a polynucleotide to form a base pair with another nucleic acid in a second polynucleotide. For example, the sequence A-G-T is complementary to the sequence T-C-A. Complementarity may be partial, in which only some of the nucleic acids match according to base pairing, or complete, where all the nucleic acids match according to base pairing.

[0028] Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are near each other, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[0029] The term "gene" means the segment of DNA involved in producing a protein; it includes regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons). The leader, the trailer as well as the introns include regulatory elements that are necessary during the transcription and the translation of a gene. Further, a "protein gene product" is a protein expressed from a particular gene.

[0030] The word "expression" or "expressed" as used herein in reference to a gene means the transcriptional and/or translational product of that gene. The level of expression of a DNA molecule in a cell may be determined on the basis of either the amount of corresponding mRNA that is present within the cell or the amount of protein encoded by that DNA produced by the cell. The level of expression of non-coding nucleic acid molecules (e.g., siRNA) may be detected by standard PCR or Northern blot methods well known in the art. *See, Sambrook et al., 1989 Molecular Cloning: A Laboratory Manual, 18.1-18.88.*

[0031] The term "recombinant" when used with reference, e.g., to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic

acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all. Transgenic cells and plants are those that express a heterologous gene or coding sequence, typically as a result of recombinant methods.

[0032] The term "heterologous" when used with reference to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid, e.g., a promoter from one source and a coding region from another source. Similarly, a heterologous protein indicates that the protein comprises two or more subsequences that are not found in the same relationship to each other in nature (e.g., a fusion

[0033] The term "exogenous" refers to a molecule or substance (e.g., a compound, nucleic acid or protein) that originates from outside a given cell or organism. For example, an "exogenous promoter" as referred to herein is a promoter that does not originate from the cell or organism it is expressed by. Conversely, the term "endogenous" or "endogenous promoter" refers to a molecule or substance that is native to, or originates within, a given cell or organism.

[0034] The term "isolated", when applied to a nucleic acid or protein, denotes that the nucleic acid or protein is essentially free of other cellular components with which it is associated in the natural state. It can be, for example, in a homogeneous state and may be in either a dry or aqueous solution. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein that is the predominant species present in a preparation is substantially purified.

[0035] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues, wherein the polymer may in embodiments be conjugated to a moiety that does not consist of amino acids. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers. A "fusion protein" refers to a

chimeric protein encoding two or more separate protein sequences that are recombinantly expressed as a single moiety.

[0036] The term "peptidyl" and "peptidyl moiety" means a monovalent peptide.

[0037] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, γ -carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid. The terms "non-naturally occurring amino acid" and "unnatural amino acid" refer to amino acid analogs, synthetic amino acids, and amino acid mimetics which are not found in nature.

[0038] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0039] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, "conservatively modified variants" refers to those nucleic acids that encode identical or essentially identical amino acid sequences. Because of the degeneracy of the genetic code, a number of nucleic acid sequences will encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is

ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

[0040] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

[0041] The following eight groups each contain amino acids that are conservative substitutions for one another:

- 1) Alanine (A), Glycine (G);
- 2) Aspartic acid (D), Glutamic acid (E);
- 3) Asparagine (N), Glutamine (Q);
- 4) Arginine (R), Lysine (K);
- 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
- 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
- 7) Serine (S), Threonine (T); and
- 8) Cysteine (C), Methionine (M)

(*see, e.g.*, Creighton, *Proteins* (1984)).

[0042] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (i.e., about 60% identity, preferably 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region, when compared and

aligned for maximum correspondence over a comparison window or designated region) as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection (*see, e.g.*, NCBI web site <http://www.ncbi.nlm.nih.gov/BLAST/> or the like). Such sequences are then said to be "substantially identical." This definition also refers to, or may be applied to, the compliment of a test sequence. The definition also includes sequences that have deletions and/or additions, as well as those that have substitutions. As described below, the preferred algorithms can account for gaps and the like. Preferably, identity exists over a region that is at least about 25 amino acids or nucleotides in length, or more preferably over a region that is 50-100 amino acids or nucleotides in length.

[0043] For specific proteins described herein (e.g., NFkB, STAT3), the named protein includes any of the protein's naturally occurring forms, or variants or homologs that maintain the protein transcription factor activity (e.g., within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to the native protein). In some embodiments, variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring form. In other embodiments, the protein is the protein as identified by its NCBI sequence reference. In other embodiments, the protein is the protein as identified by its NCBI sequence reference or functional fragment or homolog thereof.

[0044] Thus, a "STAT 3 protein" as referred to herein includes any of the recombinant or naturally-occurring forms of the Signal transducer and activator of transcription 3 (STAT3) protein or variants or homologs thereof that maintain STAT3 protein activity (e.g. within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to STAT3). In some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring STAT3 polypeptide. In embodiments, the STAT3 protein is substantially identical to the protein identified by the UniProt reference number P40763 or a variant or homolog having substantial identity thereto.

[0045] A "NFkB protein" as referred to herein includes any of the recombinant or naturally-occurring forms of the nuclear factor kappa-light-chain-enhancer of activated B cell

(NFkB) protein or variants or homologs thereof that maintain NFkB protein activity (e.g. within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to NFkB). In some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring NFkB polypeptide. In embodiments, the NFkB protein is substantially identical to the protein identified by the UniProt reference number P19838 or a variant or homolog having substantial identity thereto. In embodiments, the NFkB protein is substantially identical to the protein identified by the UniProt reference number Q04206 or a variant or homolog having substantial identity thereto. In embodiments, the NFkB protein is substantially identical to the protein identified by the UniProt reference number Q00653 or a variant or homolog having substantial identity thereto. In embodiments, the NFkB protein is substantially identical to the protein identified by the UniProt reference number Q01201 or a variant or homolog having substantial identity thereto. In embodiments, the NFkB protein is substantially identical to the protein identified by the UniProt reference number Q048648 or a variant or homolog having substantial identity thereto.

[0046] A "p65 protein" as referred to herein includes any of the recombinant or naturally-occurring forms of the Transcription factor p65 also known as nuclear factor NF-kappa-B p65 subunit (p65) protein or variants or homologs thereof that maintain p65 protein activity (e.g. within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to p65). In some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring p65 polypeptide. In embodiments, the p65 protein is substantially identical to the protein identified by the UniProt reference number Q04206 or a variant or homolog having substantial identity thereto.

[0047] The term "CTLA-4" or "CTLA-4 protein" as provided herein includes any of the recombinant or naturally-occurring forms of the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) or variants or homologs thereof that maintain CTLA-4 protein activity (e.g. within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to CTLA-4). In some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence

(e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring CTLA-4 polypeptide. In embodiments, CTLA-4 is the protein as identified by the NCBI sequence reference GI:83700231, homolog or functional fragment thereof.

[0048] The term "FOXP3" as provided herein includes any of the recombinant or naturally-occurring forms of the forkhead box P3 (FOXP3) transcription factor or variants or homologs thereof that maintain FOXP3 protein activity (e.g. within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to FOXP3). In some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring FOXP3 polypeptide. In embodiments, FOXP3 is the protein as identified by the UniProt reference number Q9BZS1, homolog or functional fragment thereof. In embodiments, FOXP3 is the protein as identified by the UniProt reference number Q99JB6, homolog or functional fragment thereof.

[0049] "Antibody" refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. Typically, the antigen-binding region of an antibody will be most critical in specificity and affinity of binding. In some embodiments, antibodies or fragments of antibodies may be derived from different organisms, including humans, mice, rats, hamsters, camels, etc. Antibodies of the invention may include antibodies that have been modified or mutated at one or more amino acid positions to improve or modulate a desired function of the antibody (e.g. glycosylation, expression, antigen recognition, effector functions, antigen binding, specificity, etc.).

[0050] An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (VL) and variable heavy chain (VH) refer to these light and heavy chains respectively. The Fc (i.e. fragment crystallizable region)

is the "base" or "tail" of an immunoglobulin and is typically composed of two heavy chains that contribute two or three constant domains depending on the class of the antibody. By binding to specific proteins the Fc region ensures that each antibody generates an appropriate immune response for a given antigen. The Fc region also binds to various cell receptors, such as Fc receptors, and other immune molecules, such as complement proteins.

[0051] Antibodies exist, for example, as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab)'2, a dimer of Fab which itself is a light chain joined to VH-CH1 by a disulfide bond. The F(ab)'2 may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the F(ab)'2 dimer into an Fab' monomer. The Fab' monomer is essentially the antigen dinging portion with part of the hinge region (see Fundamental Immunology (Paul ed., 3d ed. 1993). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized de novo either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized de novo using recombinant DNA methodologies (e.g., single chain Fv) or those identified using phage display libraries (see, e.g., McCafferty et al., *Nature* 348:552-554 (1990)).

[0052] A single-chain variable fragment (scFv) is typically a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of immunoglobulins, connected with a short linker peptide of 10 to about 25 amino acids. The linker may usually be rich in glycine for flexibility, as well as serine or threonine for solubility. The linker can either connect the N-terminus of the VH with the C-terminus of the VL, or vice versa.

[0053] For preparation of suitable antibodies of the invention and for use according to the invention, e.g., recombinant, monoclonal, or polyclonal antibodies, many techniques known in the art can be used (see, e.g., Kohler & Milstein, *Nature* 256:495-497 (1975); Kozbor et al., *Immunology Today* 4: 72 (1983); Cole et al., pp. 77-96 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. (1985); Coligan, *Current Protocols in Immunology* (1991); Harlow & Lane, *Antibodies, A Laboratory Manual* (1988); and Goding, *Monoclonal Antibodies: Principles and Practice* (2d ed. 1986)). The genes encoding the heavy and light chains of an antibody of interest can be cloned from a cell, e.g., the genes encoding a

monoclonal antibody can be cloned from a hybridoma and used to produce a recombinant monoclonal antibody. Gene libraries encoding heavy and light chains of monoclonal antibodies can also be made from hybridoma or plasma cells. Random combinations of the heavy and light chain gene products generate a large pool of antibodies with different antigenic specificity (see, e.g., Kuby, Immunology (3rd ed. 1997)). Techniques for the production of single chain antibodies or recombinant antibodies (U.S. Patent 4,946,778, U.S. Patent No. 4,816,567) can be adapted to produce antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized or human antibodies (see, e.g., U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, Marks et al., *Bio/Technology* 10:779-783 (1992); Lonberg et al., *Nature* 368:856-859 (1994); Morrison, *Nature* 368:812-13 (1994); Fishwild et al., *Nature Biotechnology* 14:845-51 (1996); Neuberger, *Nature Biotechnology* 14:826 (1996); and Lonberg & Huszar, *Intern. Rev. Immunol.* 13:65-93 (1995)). Alternatively, phage display technology can be used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens (see, e.g., McCafferty et al., *Nature* 348:552-554 (1990); Marks et al., *Biotechnology* 10:779-783 (1992)). Antibodies can also be made bispecific, i.e., able to recognize two different antigens (see, e.g., WO 93/08829, Traunecker et al., *EMBO J.* 10:3655-3659 (1991); and Suresh et al., *Methods in Enzymology* 121:210 (1986)). Antibodies can also be heteroconjugates, e.g., two covalently joined antibodies, or immunotoxins (see, e.g., U.S. Patent No. 4,676,980, WO 91/00360; WO 92/200373; and EP 03089).

[0054] Methods for humanizing or primatizing non-human antibodies are well known in the art (e.g., U.S. Patent Nos. 4,816,567; 5,530,101; 5,859,205; 5,585,089; 5,693,761; 5,693,762; 5,777,085; 6,180,370; 6,210,671; and 6,329,511; WO 87/02671; EP Patent Application 0173494; Jones et al. (1986) *Nature* 321:522; and Verhoyen et al. (1988) *Science* 239:1534). Humanized antibodies are further described in, e.g., Winter and Milstein (1991) *Nature* 349:293. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as import residues, which are typically taken from an import variable domain. Humanization can be essentially performed following the method of Winter and co-workers (see, e.g., Morrison et al., *PNAS USA*, 81:6851-6855 (1984), Jones et al., *Nature* 321:522-525 (1986); Riechmann et al., *Nature* 332:323-327 (1988); Morrison and Oi, *Adv. Immunol.*, 44:65-92 (1988), Verhoeven et al., *Science* 239:1534-1536 (1988) and Presta,

Curr. Op. Struct. Biol. 2:593-596 (1992), Padlan, Molec. Immun., 28:489-498 (1991); Padlan, Molec. Immun., 31(3):169-217 (1994)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies. For example, polynucleotides comprising a first sequence coding for humanized immunoglobulin framework regions and a second sequence set coding for the desired immunoglobulin complementarity determining regions can be produced synthetically or by combining appropriate cDNA and genomic DNA segments. Human constant region DNA sequences can be isolated in accordance with well known procedures from a variety of human cells.

[0055] A "chimeric antibody" is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity. The preferred antibodies of, and for use according to the invention include humanized and/or chimeric monoclonal antibodies.

[0056] Techniques for conjugating therapeutic agents to antibodies are well known (see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery" in *Controlled Drug Delivery* (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review" in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera et al. (eds.), pp. 475-506 (1985); and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.*, 62:119-58 (1982)). As used herein, the term "antibody-drug conjugate" or "ADC" refers to a therapeutic agent conjugated or otherwise covalently bound to an antibody. A "therapeutic agent" as referred to herein, is a composition useful in

treating or preventing a disease such as cancer. In embodiments, the therapeutic agent is an antibody.

[0057] The phrase "specifically (or selectively) binds" to an antibody or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein, often in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and more typically more than 10 to 100 times background. Specific binding to an antibody under such conditions requires an antibody that is selected for its specificity for a particular protein. For example, polyclonal antibodies can be selected to obtain only a subset of antibodies that are specifically immunoreactive with the selected antigen and not with other proteins. This selection may be achieved by subtracting out antibodies that cross-react with other molecules. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (see, e.g., Harlow & Lane, *Using Antibodies, A Laboratory Manual* (1998) for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity).

[0058] A "ligand" refers to an agent, e.g., a polypeptide or other molecule, capable of binding to a receptor.

[0059] A "label" or a "detectable moiety" is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means. For example, useful labels include ³²P, fluorescent dyes, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin, digoxigenin, or haptens and proteins or other entities which can be made detectable, e.g., by incorporating a radiolabel into a peptide or antibody specifically reactive with a target peptide. Any appropriate method known in the art for conjugating an antibody to the label may be employed, e.g., using methods described in Hermanson, *Bioconjugate Techniques* 1996, Academic Press, Inc., San Diego.

[0060] The term "biotin" as provided herein refers to a compound characterized by an ureido (tetrahydroimidizalone) ring fused with a tetrahydrothiophene ring. Thus, "biotin" as provided herein refers to 5-[(3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]pentanoic acid, and in the customary sense, refers to CAS Registry No. 58-85-5. A

"biotin-binding domain" as used herein is a protein domain that is capable of binding biotin. Non-limiting examples of biotin-binding domains include avidin, streptavidin and neutravidin. In embodiments, the biotin-binding domain binds biotin non-covalently.

[0061] The term "avidin" or "streptavidin" as provided herein includes any of the avidin or streptavidin naturally occurring forms, homologs, variants or derivatives (e.g., neutravidin) that maintain the activity of the naturally occurring form of avidin or streptavidin (e.g., within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to the native protein). In some embodiments, variants have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring form. In embodiments, avidin is the protein as identified by the UniProt sequence reference P02701, homolog or functional fragment thereof. In embodiments, streptavidin is the protein as identified by the UniProt sequence reference P22629, homolog or functional fragment thereof.

[0062] "Contacting" is used in accordance with its plain ordinary meaning and refers to the process of allowing at least two distinct species (e.g. chemical compounds including biomolecules or cells) to become sufficiently proximal to react, interact or physically touch. It should be appreciated, however, the resulting reaction product can be produced directly from a reaction between the added reagents or from an intermediate from one or more of the added reagents which can be produced in the reaction mixture.

[0063] The term "contacting" may include allowing two species to react, interact, or physically touch, wherein the two species may be, for example, a biotin domain as described herein and a biotin-binding domain. In embodiments contacting includes, for example, allowing a biotin domain as described herein to interact with a biotin-binding domain.

[0064] A "control" sample or value refers to a sample that serves as a reference, usually a known reference, for comparison to a test sample. For example, a test sample can be taken from a test condition, *e.g.*, in the presence of a test compound, and compared to samples from known conditions, *e.g.*, in the absence of the test compound (negative control), or in the presence of a known compound (positive control). A control can also represent an average value gathered from a number of tests or results. One of skill in the art will recognize that controls can be designed for assessment of any number of parameters. For example, a control can be devised to compare therapeutic benefit based on pharmacological data (*e.g.*, half-life)

or therapeutic measures (e.g., comparison of side effects). One of skill in the art will understand which controls are valuable in a given situation and be able to analyze data based on comparisons to control values. Controls are also valuable for determining the significance of data. For example, if values for a given parameter are widely variant in controls, variation in test samples will not be considered as significant.

[0065] A "control" or "standard control" refers to a sample, measurement, or value that serves as a reference, usually a known reference, for comparison to a test sample, measurement, or value. For example, a test sample can be taken from a patient suspected of having a given disease (e.g. an autoimmune disease, inflammatory autoimmune disease, cancer, infectious disease, immune disease, or other disease) and compared to a known normal (non-diseased) individual (e.g. a standard control subject). A standard control can also represent an average measurement or value gathered from a population of similar individuals (e.g. standard control subjects) that do not have a given disease (i.e. standard control population), e.g., healthy individuals with a similar medical background, same age, weight, etc. A standard control value can also be obtained from the same individual, e.g. from an earlier-obtained sample from the patient prior to disease onset. One of skill will recognize that standard controls can be designed for assessment of any number of parameters (e.g. RNA levels, protein levels, specific cell types, specific bodily fluids, specific tissues, synoviocytes, synovial fluid, synovial tissue, fibroblast-like synoviocytes, macrophagelike synoviocytes, etc).

[0066] One of skill in the art will understand which standard controls are most appropriate in a given situation and be able to analyze data based on comparisons to standard control values. Standard controls are also valuable for determining the significance (e.g. statistical significance) of data. For example, if values for a given parameter are widely variant in standard controls, variation in test samples will not be considered as significant.

[0067] "Patient" or "subject in need thereof" refers to a living organism suffering from or prone to a disease or condition that can be treated by administration of a composition or pharmaceutical composition as provided herein. Non-limiting examples include humans, other mammals, bovines, rats, mice, dogs, monkeys, goat, sheep, cows, deer, and other non-mammalian animals. In some embodiments, a patient is human.

[0068] The terms "disease" or "condition" refer to a state of being or health status of a patient or subject capable of being treated with a compound, pharmaceutical composition, or

method provided herein. In embodiments, the disease is cancer (e.g. lung cancer, ovarian cancer, osteosarcoma, bladder cancer, cervical cancer, liver cancer, kidney cancer, skin cancer (e.g., Merkel cell carcinoma), testicular cancer, leukemia, lymphoma, head and neck cancer, colorectal cancer, prostate cancer, pancreatic cancer, melanoma, breast cancer, neuroblastoma). The disease may be an autoimmune, inflammatory, cancer, infectious, metabolic, developmental, cardiovascular, liver, intestinal, endocrine, neurological, or other disease.

[0069] As used herein, the term "cancer" refers to all types of cancer, neoplasm or malignant tumors found in mammals, including leukemias, lymphomas, melanomas, neuroendocrine tumors, carcinomas and sarcomas. Exemplary cancers that may be treated with a compound, pharmaceutical composition, or method provided herein include lymphoma, sarcoma, bladder cancer, bone cancer, brain tumor, cervical cancer, colon cancer, esophageal cancer, gastric cancer, head and neck cancer, kidney cancer, myeloma, thyroid cancer, leukemia, prostate cancer, breast cancer (e.g. triple negative, ER positive, ER negative, chemotherapy resistant, herceptin resistant, HER2 positive, doxorubicin resistant, tamoxifen resistant, ductal carcinoma, lobular carcinoma, primary, metastatic), ovarian cancer, pancreatic cancer, liver cancer (e.g. hepatocellular carcinoma), lung cancer (e.g. non-small cell lung carcinoma, squamous cell lung carcinoma, adenocarcinoma, large cell lung carcinoma, small cell lung carcinoma, carcinoid, sarcoma), glioblastoma multiforme, glioma, melanoma, prostate cancer, castration-resistant prostate cancer, breast cancer, triple negative breast cancer, glioblastoma, ovarian cancer, lung cancer, squamous cell carcinoma (e.g., head, neck, or esophagus), colorectal cancer, leukemia, acute myeloid leukemia, lymphoma, B cell lymphoma, or multiple myeloma. Additional examples include, cancer of the thyroid, endocrine system, brain, breast, cervix, colon, head & neck, esophagus, liver, kidney, lung, non-small cell lung, melanoma, mesothelioma, ovary, sarcoma, stomach, uterus or Medulloblastoma, Hodgkin's Disease, Non-Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, glioma, glioblastoma multiforme, ovarian cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, primary brain tumors, cancer, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, endometrial cancer, adrenal cortical cancer, neoplasms of the endocrine or exocrine pancreas, medullary thyroid cancer, medullary thyroid carcinoma, melanoma, colorectal cancer, papillary thyroid cancer,

hepatocellular carcinoma, Paget's Disease of the Nipple, Phyllodes Tumors, Lobular Carcinoma, Ductal Carcinoma, cancer of the pancreatic stellate cells, cancer of the hepatic stellate cells, or prostate cancer.

[0070] The term "leukemia" refers broadly to progressive, malignant diseases of the blood-forming organs and is generally characterized by a distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow. Leukemia is generally clinically classified on the basis of (1) the duration and character of the disease-acute or chronic; (2) the type of cell involved; myeloid (myelogenous), lymphoid (lymphogenous), or monocytic; and (3) the increase or non-increase in the number abnormal cells in the blood-leukemic or aleukemic (subleukemic). Exemplary leukemias that may be treated with a compound, pharmaceutical composition, or method provided herein include, for example, acute nonlymphocytic leukemia, chronic lymphocytic leukemia, acute granulocytic leukemia, chronic granulocytic leukemia, acute promyelocytic leukemia, adult T-cell leukemia, aleukemic leukemia, a leukocythemic leukemia, basophytic leukemia, blast cell leukemia, bovine leukemia, chronic myelocytic leukemia, leukemia cutis, embryonal leukemia, eosinophilic leukemia, Gross' leukemia, hairy-cell leukemia, hemoblastic leukemia, hemocytoblastic leukemia, histiocytic leukemia, stem cell leukemia, acute monocytic leukemia, leukopenic leukemia, lymphatic leukemia, lymphoblastic leukemia, lymphocytic leukemia, lymphogenous leukemia, lymphoid leukemia, lymphosarcoma cell leukemia, mast cell leukemia, megakaryocytic leukemia, micromyeloblastic leukemia, monocytic leukemia, myeloblastic leukemia, myelocytic leukemia, myeloid granulocytic leukemia, myelomonocytic leukemia, Naegeli leukemia, plasma cell leukemia, multiple myeloma, plasmacytic leukemia, promyelocytic leukemia, Rieder cell leukemia, Schilling's leukemia, stem cell leukemia, subleukemic leukemia, or undifferentiated cell leukemia.

[0071] The term "sarcoma" generally refers to a tumor which is made up of a substance like the embryonic connective tissue and is generally composed of closely packed cells embedded in a fibrillar or homogeneous substance. Sarcomas that may be treated with a compound, pharmaceutical composition, or method provided herein include a chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, Abemethy's sarcoma, adipose sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilms' tumor sarcoma, endometrial sarcoma, stromal sarcoma, Ewing's sarcoma, fascial sarcoma,

fibroblastic sarcoma, giant cell sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented hemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphoma, immunoblastic sarcoma of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, or telangiectaltic sarcoma.

[0072] The term "melanoma" is taken to mean a tumor arising from the melanocytic system of the skin and other organs. Melanomas that may be treated with a compound, pharmaceutical composition, or method provided herein include, for example, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, nodular melanoma, subungual melanoma, or superficial spreading melanoma.

[0073] The term "carcinoma" refers to a malignant new growth made up of epithelial cells tending to infiltrate the surrounding tissues and give rise to metastases. Exemplary carcinomas that may be treated with a compound, pharmaceutical composition, or method provided herein include, for example, medullary thyroid carcinoma, familial medullary thyroid carcinoma, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatous, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, ductal carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epiermoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniform carcinoma, gelatinous carcinoma, giant cell carcinoma, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypernephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular

carcinoma, carcinoma lenticulare, lipomatous carcinoma, lobular carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, nasopharyngeal carcinoma, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhous carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tubular carcinoma, tuberous carcinoma, verrucous carcinoma, or carcinoma villosum.

[0074] As used herein, the terms "metastasis," "metastatic," and "metastatic cancer" can be used interchangeably and refer to the spread of a proliferative disease or disorder, e.g., cancer, from one organ or another non-adjacent organ or body part. Cancer occurs at an originating site, e.g., breast, which site is referred to as a primary tumor, e.g., primary breast cancer. Some cancer cells in the primary tumor or originating site acquire the ability to penetrate and infiltrate surrounding normal tissue in the local area and/or the ability to penetrate the walls of the lymphatic system or vascular system circulating through the system to other sites and tissues in the body. A second clinically detectable tumor formed from cancer cells of a primary tumor is referred to as a metastatic or secondary tumor. When cancer cells metastasize, the metastatic tumor and its cells are presumed to be similar to those of the original tumor. Thus, if lung cancer metastasizes to the breast, the secondary tumor at the site of the breast consists of abnormal lung cells and not abnormal breast cells. The secondary tumor in the breast is referred to a metastatic lung cancer. Thus, the phrase metastatic cancer refers to a disease in which a subject has or had a primary tumor and has one or more secondary tumors. The phrases non-metastatic cancer or subjects with cancer that is not metastatic refers to diseases in which subjects have a primary tumor but not one or more secondary tumors. For example, metastatic lung cancer refers to a disease in a subject with or with a history of a primary lung tumor and with one or more secondary tumors at a second location or multiple locations, e.g., in the breast.

[0075] As used herein, an "autoimmune disease" refers to a disease or disorder that arises from altered immune reactions by the immune system of a subject, e.g., against substances tissues and/or cells normally present in the body of the subject. Autoimmune diseases include, but are not limited to, arthritis, rheumatoid arthritis, psoriatic arthritis, juvenile idiopathic arthritis, scleroderma, systemic scleroderma, multiple sclerosis, systemic lupus erythematosus (SLE), myasthenia gravis, juvenile onset diabetes, diabetes mellitus type 1, Guillain-Barre syndrome, Hashimoto's encephalitis, Hashimoto's thyroiditis, ankylosing spondylitis, psoriasis, Sjogren's syndrome, vasculitis, glomerulonephritis, auto-immune thyroiditis, Behcet's disease, Crohn's disease, ulcerative colitis, bullous pemphigoid, sarcoidosis, psoriasis, ichthyosis, Graves ophthalmopathy, inflammatory bowel disease, Addison's disease, Vitiligo, asthma, and allergic asthma.

[0076] As used herein, an "inflammatory disease" refers to a disease or disorder associated with abnormal or altered inflammation. Inflammation is a biological response initiated by the immune system as part of the healing process in response to a pathogen, damaged cells or tissues or irritants. Chronic inflammation can lead to a variety of diseases. Inflammatory diseases include, but are not limited to, atherosclerosis, allergies, asthma, rheumatoid arthritis, transplant rejection, celiac disease, chronic prostatitis, inflammatory bowel diseases, pelvic inflammatory diseases, and inflammatory myopathies.

[0077] As used herein, "metabolic disorders" refer to diseases or disorders involving abnormal metabolism of a variety of molecules and substances including, for example, carbohydrates, amino acids, and organic acids. Metabolic disorders include, but are not limited to, disorders of carbohydrate metabolism, e.g., glycogen storage disease, disorders of amino acid metabolism, e.g., phenylketonuria, maple syrup urine disease, glutaric aciduria type 1, urea cycle disorder or urea cycle defects, e.g., carbamoyl phosphate synthetase I deficiency, disorders of organic acid metabolism (organic acidurias), e.g., alcaptonuria, disorders of fatty acid oxidation and mitochondrial metabolism, e.g., medium-chain acyl-coenzyme A dehydrogenase deficiency, disorders of porphyrin metabolism, e.g., acute intermittent porphyria, disorders of purine or pyrimidine metabolism, e.g., Lesch-Nyhan syndrome, disorders of steroid metabolism, e.g., lipoid congenital adrenal hyperplasia, congenital adrenal hyperplasia, disorders of mitochondrial function, e.g., Kearns-Sayre syndrome, disorders of peroxisomal function, e.g., Zellweger syndrome, and lysosomal storage disorders, e.g., Gaucher's disease, and Niemann Pick disease.

[0078] As used herein, "developmental disorders" refer to diseases or disorders often originating in childhood associated with language disorders, learning disorders, motor disorders and neurodevelopmental disorders. Examples include, but are not limited to, autism spectrum disorders and attention deficit disorders.

[0079] As used herein, "cardiovascular diseases" refer to diseases associated with the heart, blood vessels or both. Cardiovascular diseases include, but are not limited to, coronary heart disease, cardiomyopathy, hypertensive heart disease, heart failure, cardiac dysrhythmias, inflammatory heart disease, peripheral arterial disease, cerebrovascular disease and inflammatory heart disease.

[0080] As used herein, "liver diseases" refer to diseases associated with the abnormalities in the liver and/or liver function. Liver diseases include, but are not limited to, hepatitis, alcoholic liver disease, fatty liver disease, cirrhosis, Budd-Chiari syndrome, Gilbert's syndrome and cancer.

[0081] As used herein, the term "intestinal disease" refers to diseases or disorders associated with abnormalities in the intestine (small or large). Intestinal diseases include, but are not limited to, gastroenteritis, colitis, ileitis, appendicitis, coeliac disease, Chron's disease, enteroviruses, irritable bowel syndrome, and diverticular disease.

[0082] As used herein, the term "endocrine disease" refers to diseases or disorders of the endocrine system including endocrine gland hyposecretion, endocrine gland hypersecretion and tumors. Endocrine diseases include, but are not limited to, Addison's disease, diabetes, Conn's syndrome, Cushing's syndrome, glucocorticoid remediable aldosteronism, hypoglycemia, hyperthyroidism, hypothyroidism, thyroiditis, hypopituitarism, hypogonadism and parathyroid gland disorders.

[0083] As used herein, the term "neurological disorder" refers to diseases or disorders of the bodies nervous system including structural, biochemical or electrical abnormalities. Neurological disorders include, but are not limited to, brain damage, brain dysfunction, spinal cord disorders, peripheral neuropathies, cranial nerve disorders, autonomic nervous system disorders, seizure disorders, movement disorders, e.g., Parkinson's disease and Multiple Sclerosis, and central neuropathies.

[0084] As used herein, the term "infectious disease" refers to diseases or disorders associate with infection, presence and/or growth of a pathogenic agent in a host subject.

Infectious pathogenic agents include, but are not limited to, viruses, bacteria, fungi, protozoa, multicellular parasites and aberrant proteins, e.g., prions. Viruses associated with infectious disease include but are not limited to, herpes simplex viruses, cytomegalovirus, Epstein-Barr virus, Varicella-zoster virus, herpesviruses, Vesicular stomatitis virus, Hepatitis viruses, Rhinovirus, Coronavirus, Influenza viruses, Measles virus, Polyomavirus, Human Papilomavirus, Respiratory syncytial virus, Adenovirus, Coxsackie virus, Dengue virus, Mumps virus, Poliovirus, Rabies virus, Rous sarcoma virus, Yellow fever virus, Ebola virus, Simian Immunodeficiency viruses, Human Immunodeficiency viruses. Bacteria associated with infectious disease include, but are not limited to, *M. tuberculosis*, *Salmonella* species, *E. coli*, *Chlamydia* species, *Staphylococcus* species, *Bacillus* species, and *Pseudomonas* species.

[0085] The term "associated" or "associated with" in the context of a substance or substance activity or function associated with a disease (e.g., diabetes, cancer (e.g. prostate cancer, renal cancer, metastatic cancer, melanoma, castration-resistant prostate cancer, breast cancer, triple negative breast cancer, glioblastoma, ovarian cancer, lung cancer, squamous cell carcinoma (e.g., head, neck, or esophagus), colorectal cancer, leukemia, acute myeloid leukemia, lymphoma, B cell lymphoma, or multiple myeloma)) means that the disease (e.g. lung cancer, ovarian cancer, osteosarcoma, bladder cancer, cervical cancer, liver cancer, kidney cancer, skin cancer (e.g., Merkel cell carcinoma), testicular cancer, leukemia, lymphoma, head and neck cancer, colorectal cancer, prostate cancer, pancreatic cancer, melanoma, breast cancer, neuroblastoma) is caused by (in whole or in part), or a symptom of the disease is caused by (in whole or in part) the substance or substance activity or function.

[0086] The term "aberrant" as used herein refers to different from normal. When used to describe enzymatic activity, aberrant refers to activity that is greater or less than a normal control or the average of normal non-diseased control samples. Aberrant activity may refer to an amount of activity that results in a disease, wherein returning the aberrant activity to a normal or non-disease-associated amount (e.g. by using a method as described herein), results in reduction of the disease or one or more disease symptoms.

[0087] As used herein, the terms "cell-penetrating" or "cell-penetration" refer to the ability of a molecule (e.g., an antibody) to pass from the extracellular environment into a cell in a significant or effective amount. Thus, a cell-penetrating conjugate is a molecule that passes from the extracellular environment, through the membrane of a cell and into a cell.

[0088] As used herein, the terms "non-cell penetrating" or "non-cell penetration" refers to the inability of a molecule (e.g., an antibody) to pass from the extracellular environment into a cell in a significant or effective amount. Thus, non-cell penetrating peptides or proteins generally are not capable of passing from the extracellular environment, through the cell membrane, and into a cell to achieve a significant biological effect on a population of cells, organ or organism. The term does not exclude the possibility that one or more of the small number of peptides or proteins may enter the cell. However, the term refers to molecules that are generally not able to enter a cell from the extracellular environment to a significant degree. Examples of non-cell penetrating molecules and substances include, but are not limited to, large molecules such as, for example, high molecular weight proteins (e.g., antibodies). Peptides or proteins can be determined to be non-cell penetrating using methods known to those of skill in the art. By way of example, a peptide or protein can be fluorescently labeled and the ability of the peptide or protein to pass from the extracellular environment into the cell can be determined *in vitro* by flow cytometric analysis or confocal microscopy. In some embodiments, a "non-cell penetrating protein" refers to a protein (e.g., an antibody) that penetrates a cell at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 10,000 or 100,000 fold less than the same protein attached to a phosphorothioate nucleic acid or phosphorothioate polymer backbone. In some embodiments, a "non-cell penetrating protein" refers to a protein that does not measurably penetrate a cell.

[0089] As used herein, "molecular weight" (M.W.) or "molecular mass" refers to the sum of the atomic weights of all the atoms in a molecule. With respect to molecules, a molecule with a high molecular weight typically has a molecular weight of 25 kDa or more. By way of example, a high molecular weight protein can have a M.W. from about 25 kDa to 1000 kDa or more.

[0090] As used herein, the term "intracellular" means inside a cell. As used herein, an "intracellular target" is a target, e.g., nucleic acid, polypeptide or other molecule (e.g., carbohydrate) that is located inside of a cell and is a target to which the non-cell penetrating proteins provided herein bind. Binding can be direct or indirect. In embodiments, the non-cell penetrating protein selectively binds the intracellular target. By selectively binds, selectively binding, or specifically binding refers to the agent (e.g., a non-cell penetrating protein) binding one agent (e.g., intracellular target) to the partial or complete exclusion of other

agents. By binding is meant a detectable binding at least about 1.5 times the background of the assay method. For selective or specific binding such a detectable binding can be detected for a given agent but not a control agent. Alternatively, or additionally, the detection of binding can be determined by assaying the presence of down-stream molecules or events.

[0091] As used herein, the term "conjugate" refers to the association between atoms or molecules. The association can be direct or indirect. For example, a conjugate between a nucleic acid (e.g., a phosphorothioate nucleic acid) and a protein (e.g., an antibody provided herein) can be direct, e.g., by covalent bond, or indirect, e.g., by non-covalent bond (e.g. electrostatic interactions (e.g. ionic bond, hydrogen bond, halogen bond), van der Waals interactions (e.g. dipole-dipole, dipole-induced dipole, London dispersion), ring stacking (pi effects), hydrophobic interactions and the like). Where the conjugate is a non-covalent conjugate, the non-cell penetrating protein and the phosphorothioate nucleic acid are connected through a non-covalent linker. In embodiments, the non-covalent linker includes a biotin-binding domain and a biotin domain. In embodiments, conjugates are formed using conjugate chemistry including and are not limited to nucleophilic substitutions (e.g., reactions of amines and alcohols with acyl halides, active esters), electrophilic substitutions (e.g., enamine reactions) and additions to carbon-carbon and carbon-heteroatom multiple bonds (e.g., Michael reaction, Diels-Alder addition). These and other useful reactions are discussed in, for example, March, ADVANCED ORGANIC CHEMISTRY, 3rd Ed., John Wiley & Sons, New York, 1985; Hermanson, BIOCONJUGATE TECHNIQUES, Academic Press, San Diego, 1996; and Feeney et al., MODIFICATION OF PROTEINS; Advances in Chemistry Series, Vol. 198, American Chemical Society, Washington, D.C., 1982. In embodiments, the phosphorothioate nucleic acid, phosphorothioate backbone polymer or non-cell penetrating protein are non-covalently attached to the biotin-binding domain or biotin domain through a non-covalent chemical reaction between a component of the phosphorothioate nucleic acid, phosphorothioate backbone polymer (e.g. a monothiophosphate) or non-cell penetrating protein and a component of the biotin-binding domain or biotin domain (e.g. an amino acid). In other embodiments, the phosphorothioate nucleic acid, phosphorothioate backbone polymer or non-cell penetrating protein include one or more reactive moieties, e.g., a covalent reactive moiety, as described herein (e.g., an amino acid reactive moiety such as a vinyl sulfone moiety (-S(O)₂CH=CH₂)). The one or more reactive moieties may be reacted with a second reactive moiety of the biotin-binding domain or biotin domain, thereby forming a covalent bond. Without limitation, the reactive moiety

of the biotin domain or the biotin-binding domain may react with a primary amine, a sulfhydryl moiety, a carboxyl moiety, a carbohydrate moiety, a tyrosine side chain or a histidine side chain of the non-cell penetrating protein or a guanidine or cytosine base of the phosphorothioate nucleic acid or phosphorothioate backbone polymer. The reaction chemistry of biotinylation (binding with a biotin domain) or avidinylation (binding with a biotin-binding domain) and other useful reactions are discussed in, for example, Nelson WM, Wojnar WA. The use of photobiotinylated PCR primers for magnetic bead-based solid phase sequencing. Human Genome III October 21-23, 1991 San Diego, CA. Poster no. T41; and Thermo Scientific Avidin-Biotin Technical Handbook published March, 2009, as 1601675_AvBi_HB_INTL.pdf.

[0092] Useful reactive moieties including covalent reactive moieties or functional groups used for conjugate chemistries herein include, for example:

- (a) carboxyl groups and various derivatives thereof including, but not limited to, N-hydroxysuccinimide esters, N-hydroxybenztriazole esters, acid halides, acyl imidazoles, thioesters, p-nitrophenyl esters, alkyl, alkenyl, alkynyl and aromatic esters;
- (b) hydroxyl groups which can be converted to esters, ethers, aldehydes, etc.
- (c) haloalkyl groups wherein the halide can be later displaced with a nucleophilic group such as, for example, an amine, a carboxylate anion, thiol anion, carbanion, or an alkoxide ion, thereby resulting in the covalent attachment of a new group at the site of the halogen atom;
- (d) dienophile groups which are capable of participating in Diels-Alder reactions such as, for example, maleimido groups;
- (e) aldehyde or ketone groups such that subsequent derivatization is possible via formation of carbonyl derivatives such as, for example, imines, hydrazones, semicarbazones or oximes, or via such mechanisms as Grignard addition or alkylolithium addition;
- (f) sulfonyl halide groups for subsequent reaction with amines, for example, to form sulfonamides;
- (g) thiol groups, which can be converted to disulfides, reacted with acyl halides, or bonded to metals such as gold;

- (h) amine or sulphydryl groups, which can be, for example, acylated, alkylated or oxidized;
- (i) alkenes, which can undergo, for example, cycloadditions, acylation, Michael addition, etc;
- (j) epoxides, which can react with, for example, amines and hydroxyl compounds;
- (k) phosphoramidites and other standard functional groups useful in nucleic acid synthesis;
- (l) metal silicon oxide bonding;
- (m) metal bonding to reactive phosphorus groups (e.g. phosphines) to form, for example, phosphate diester bonds; and
- (n) sulfones, for example, vinyl sulfone.

[0093] The reactive functional groups can be chosen such that they do not participate in, or interfere with, the chemical stability of the proteins described herein. By way of example, the nucleic acids can include a vinyl sulfone or other reactive moiety. For example, a nucleic acid with a vinyl sulfone reactive moiety may be formed from a nucleic acid with an S-S-R moiety, wherein R is $-(CH_2)_6-OH$. A nucleic acid with a vinyl sulfone may further be formed from a nucleic acid with a terminal phosphate (PS).

[0094] A "control" or "standard control" refers to a sample, measurement, or value that serves as a reference, usually a known reference, for comparison to a test sample, measurement, or value. For example, a test sample can be taken from a patient suspected of having a given disease (e.g. an autoimmune disease, inflammatory autoimmune disease, cancer, infectious disease, immune disease, or other disease) and compared to a known normal (non-diseased) individual (e.g. a standard control subject). A standard control can also represent an average measurement or value gathered from a population of similar individuals (e.g. standard control subjects) that do not have a given disease (i.e. standard control population), e.g., healthy individuals with a similar medical background, same age, weight, etc. A standard control value can also be obtained from the same individual, e.g. from an earlier-obtained sample from the patient prior to disease onset. One of skill will recognize that standard controls can be designed for assessment of any number of parameters (e.g. RNA levels, protein levels, specific cell types, specific bodily fluids, specific tissues,

synoviocytes, synovial fluid, synovial tissue, fibroblast-like synoviocytes, macrophagelike synoviocytes, etc).

[0095] One of skill in the art will understand which standard controls are most appropriate in a given situation and be able to analyze data based on comparisons to standard control values. Standard controls are also valuable for determining the significance (e.g. statistical significance) of data. For example, if values for a given parameter are widely variant in standard controls, variation in test samples will not be considered as significant.

[0096] The term "diagnosis" refers to a relative probability that a disease (e.g. an autoimmune, inflammatory autoimmune, cancer, infectious, immune, or other disease) is present in the subject. Similarly, the term "prognosis" refers to a relative probability that a certain future outcome may occur in the subject with respect to a disease state. For example, in the context of the present invention, prognosis can refer to the likelihood that an individual will develop a disease (e.g. an autoimmune, inflammatory autoimmune, cancer, infectious, immune, or other disease), or the likely severity of the disease (e.g., duration of disease). The terms are not intended to be absolute, as will be appreciated by any one of skill in the field of medical diagnostics.

[0097] "Biological sample" or "sample" refer to materials obtained from or derived from a subject or patient. A biological sample includes sections of tissues such as biopsy and autopsy samples, and frozen sections taken for histological purposes. Such samples include bodily fluids such as blood and blood fractions or products (e.g., serum, plasma, platelets, red blood cells, and the like), sputum, tissue, cultured cells (e.g., primary cultures, explants, and transformed cells) stool, urine, synovial fluid, joint tissue, synovial tissue, synoviocytes, fibroblast-like synoviocytes, macrophage-like synoviocytes, immune cells, hematopoietic cells, fibroblasts, macrophages, T cells, etc. A biological sample is typically obtained from a eukaryotic organism, such as a mammal such as a primate e.g., chimpanzee or human; cow; dog; cat; a rodent, e.g., guinea pig, rat, mouse; rabbit; or a bird; reptile; or fish.

[0098] A "cell" as used herein, refers to a cell carrying out metabolic or other functions sufficient to preserve or replicate its genomic DNA. A cell can be identified by well-known methods in the art including, for example, presence of an intact membrane, staining by a particular dye, ability to produce progeny or, in the case of a gamete, ability to combine with a second gamete to produce a viable offspring. Cells may include prokaryotic and eukaryotic cells. Prokaryotic cells include but are not limited to bacteria. Eukaryotic cells include but

are not limited to yeast cells and cells derived from plants and animals, for example mammalian, insect (e.g., spodoptera) and human cells. Cells may be useful when they are naturally nonadherent or have been treated not to adhere to surfaces, for example by trypsinization.

CELL-PENETRATING CONJUGATES

[0099] Provide herein are, *inter alia*, are cell penetrating conjugates including a first non-cell penetrating protein and a second protein and phosphorothioate nucleic acid molecules. The second protein provided herein may be a non-cell penetrating protein. In embodiments, the second protein is an antibody. The conjugate as provided herein may include a phosphorothioate nucleic acid connecting a first non-cell penetrating protein (e.g., first antibody) to a second non-cell penetrating protein (e.g., second antibody), wherein the phosphorothioate nucleic acid enhances intracellular delivery of the first non-cell penetrating protein and the second non-cell penetrating protein. The first and second non-cell penetrating protein may bind to the same target (e.g., intracellular protein or surface-expressed protein) or to different targets. Where the first and second non-cell penetrating protein bind to the same target, the first non-cell penetrating protein may bind a different epitope compared the second non-cell penetrating protein. Covalent or non-covalent attachment (e.g., through a non-covalent linker) of the phosphorothioate nucleic acid to the first and second non-cell penetrating protein forms the non-cell penetrating protein provided herein capable to enter a cell through penetration. The conjugates provided herein are useful, *inter alia*, for the intracellular delivery of antibodies and targeting of intracellular targets (e.g., signaling proteins) for therapeutic and diagnostic purposes.

[0100] In one aspect, a cell penetrating conjugate including a phosphorothioate nucleic acid connecting a first non-cell penetrating protein to a second non-cell penetrating protein is provided. The phosphorothioate nucleic acid enhances intracellular delivery of the first non-cell penetrating protein and the second non-cell penetrating protein. In another aspect, a cell penetrating conjugate including a phosphorothioate polymer backbone connecting a first non-cell penetrating protein to a second non-cell penetrating protein is provided. The phosphorothioate polymer backbone enhances intracellular delivery of the first non-cell penetrating protein and the second non-cell penetrating protein. As discussed above, polymer backbones contain the same structure (i.e., contains a chain of two or more sugar residues linked together) as a nucleic acid sequence with the exception that the polymer backbone

lacks the bases normally present in a nucleic acid sequence. Therefore, for all embodiments and aspects provided herein phosphorothioate polymer backbones may be used on behalf or in combination with the phosphorothioate nucleic acid described herein.

[0101] The first non-cell penetrating protein and the second non-cell penetrating protein may be covalently or non-covalently attached to the phosphorothioate nucleic acid. In embodiments, the phosphorothioate nucleic acid is covalently attached to the first non-cell penetrating protein and the second non-cell penetrating protein. In embodiments, the phosphorothioate nucleic acid is non-covalently attached to the first non-cell penetrating protein and the second non-cell penetrating protein. In embodiments, the phosphorothioate nucleic acid is covalently attached to the first non-cell penetrating protein and non-covalently attached to the second non-cell penetrating protein. In embodiments, the phosphorothioate nucleic acid is non-covalently attached to the first non-cell penetrating protein and covalently attached to the second non-cell penetrating protein. In embodiments, the phosphorothioate nucleic acid is independently attached to a lysine, arginine, cysteine, or histidine of the first non-cell penetrating protein. In embodiments, the phosphorothioate nucleic acid is independently attached to a lysine, arginine, cysteine, or histidine of the second non-cell penetrating protein. In embodiments, the phosphorothioate nucleic acid is attached to a cysteine of the first non-cell penetrating protein. In embodiments, the phosphorothioate nucleic acid is attached to a cysteine of the second non-cell penetrating protein.

[0102] In embodiments, the phosphorothioate nucleic acid is attached to the first non-cell penetrating protein through a first linker and to the second non-cell penetrating protein through a second linker. The first and second linker provided herein may be a chemical linker. In embodiments, the chemical linker is a covalent linker or a non-covalent linker. In embodiments, the covalent linker is a bond, a substituted or unsubstituted alkylene, substituted or unsubstituted heteroalkylene, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkylene, substituted or unsubstituted arylene or substituted or unsubstituted heteroarylene or any combination thereof. Thus, a chemical linker as provided herein may include a plurality of chemical moieties, wherein each of the plurality of moieties is chemically different.

[0103] The phosphorothioate nucleic acid may be attached to the first non-cell penetrating protein and the second non-cell penetrating protein through a non-covalent linker. A non-covalent linker as provided herein may include a first member of a biotin binding pair and a

second member of a biotin binding pair. The first member of the biotin binding pair may be a biotin-binding domain (e.g., avidin, streptavidin) or a biotin domain (e.g., biotin). The second member of the biotin binding pair may be a biotin-binding domain (e.g., avidin, streptavidin) or a biotin domain (e.g., biotin). The non-covalent linker is formed through non-covalent binding between the first member of the biotin binding pair and the second member of the biotin binding pair. In embodiments, the first member of the biotin binding pair is a biotin-binding domain (e.g., avidin, streptavidin) and the second member of the biotin binding pair is a biotin domain (e.g., biotin). In embodiments, the first member of the biotin binding pair is a biotin domain (e.g., biotin) and the second member of the biotin binding pair is a biotin-binding domain (e.g., avidin, streptavidin).

[0104] In embodiments, the first linker is a non-covalent linker. In embodiments, the first linker is a covalent linker. In embodiments, the second linker is a non-covalent linker. In embodiments, the second linker is a covalent linker. In embodiments, the first linker is a non-covalent linker and the second linker is a covalent linker. In embodiments, the first linker is a covalent linker and the second linker is a non-covalent linker. In embodiments, the first linker or the second linker is a bond. In embodiments, the first linker is a bond. In embodiments, the second linker is a bond.

[0105] In embodiments, the first linker is a non-covalent linker. A non-covalent linker as provided herein is a linker including a first binding domain (e.g., a biotin domain or biotin-binding domain) non-covalently attached to a second binding domain (e.g., a biotin-binding domain or biotin domain). Thus, a non-covalent linker is formed through non-covalent binding between a first binding domain and a second binding domain.

[0106] In embodiments, the first linker includes a first binding domain and a second binding domain. In embodiments, the second linker includes a third binding domain and a forth binding domain. The first binding domain, the second binding domain, the third binding domain and the forth binding domain may be the same or independently different. In embodiments, the first binding domain is a first biotin-binding domain (e.g., avidin, streptavidin). In embodiments, the second binding domain is a first biotin domain (e.g., biotin). In embodiments, the third binding domain is a second biotin-binding domain (e.g., avidin, streptavidin). In embodiments, the forth binding domain is a second biotin domain (e.g., biotin). In embodiments, the first binding domain is a first biotin domain (e.g., biotin). In embodiments, the second binding domain is a first biotin-binding domain (e.g., avidin,

streptavidin). In embodiments, the third binding domain is a second biotin domain (e.g., biotin). In embodiments, the forth binding domain is a second biotin-binding domain (e.g., avidin, streptavidin). In embodiments, the first linker includes a first biotin-binding domain non-covalently attached to a first biotin domain. In embodiments, the second linker includes a second biotin-binding domain non-covalently attached to a second biotin domain.

[0107] The first biotin-binding domain, the second biotin-binding domain, the first biotin domain and the second biotin domain may be covalently or non-covalently attached to the first non-cell penetrating protein or the second protein (e.g., second non-cell penetrating protein), respectively. In embodiments, the first non-cell penetrating protein is covalently attached to the first biotin-binding domain (e.g., avidin, streptavidin) and the phosphorothioate nucleic acid is covalently attached to the first biotin domain (e.g., biotin). In embodiments, the first non-cell penetrating protein is covalently attached to the first biotin domain (e.g., biotin) and the phosphorothioate nucleic acid is covalently attached to the first biotin-binding domain (e.g., avidin, streptavidin). In embodiments, the second protein (e.g., second non-cell penetrating protein) is covalently attached to the second biotin-binding domain (e.g., avidin, streptavidin) and the phosphorothioate nucleic acid is covalently attached to the second biotin domain (e.g., biotin). In embodiments, the second protein (e.g., second non-cell penetrating protein) is covalently attached to the second biotin domain (e.g., biotin) and the phosphorothioate nucleic acid is covalently attached to the second biotin-binding domain (e.g., avidin, streptavidin).

[0108] The first linker and the second linker provided herein may be covalent linkers. The linkers provided herein (e.g., first linker, second linker) may covalently connect the first non-cell penetrating protein or the second protein (e.g., second non-cell penetrating protein) applying methods well known in the art and compatible with the composition of the linkers and the first non-cell penetrating protein and the second protein (e.g., second non-cell penetrating protein). The linkers provided herein may include the conjugated product of reactive groups (e.g., alkyne, azide, maleimide or thiol reactive moiety) at the point of attachment to the first non-cell penetrating protein, at the point of attachment to the phosphorothioate nucleic acid or at the point of attachment to the second protein (e.g., second non-cell penetrating protein). Thus, the linkers provided herein may be polyvalent and may be formed by conjugate chemistry techniques. Non-limiting examples of linkers useful for the compositions and methods provided herein (e.g., first linker, second linker) include alkyl

groups (including substituted alkyl groups and alkyl groups containing heteroatom moieties), with short alkyl groups, esters, amide, amine, epoxy groups and ethylene glycol or derivatives thereof. The linkers provided herein (e.g., first linker, second linker) may include a sulfone group, forming sulfonamide, an ester group or an ether group (e.g., triethyl ether).

[0109] In embodiments, the first linker includes a first biotin-binding domain non-covalently attached to a first biotin domain. In embodiments, the first biotin-binding domain is a first avidin domain. In embodiments, the first biotin-binding domain is a first streptavidin domain. In embodiments, the first streptavidin domain binds a plurality of first biotin domains. In embodiments, the first streptavidin domain binds about four first biotin domains.

[0110] In embodiments, the first biotin-binding domain is attached to the first non-cell penetrating protein. In embodiments, the first biotin-binding domain is covalently attached to the first non-cell penetrating protein. In embodiments, the first biotin-binding domain is non-covalently attached to the first non-cell penetrating protein. In embodiments, a plurality of first biotin-binding domains is attached to the first non-cell penetrating protein. In embodiments, the first biotin-binding domain is attached to the phosphorothioate nucleic acid. In embodiments, the first biotin-binding domain is covalently attached to the phosphorothioate nucleic acid. In embodiments, the first biotin-binding domain is non-covalently attached to the phosphorothioate nucleic acid. In embodiments, a plurality of phosphorothioate nucleic acids is attached to the first biotin-binding domain.

[0111] In embodiments, the first biotin domain is attached to the phosphorothioate nucleic acid. In embodiments, the first biotin domain is covalently attached to the phosphorothioate nucleic acid. In embodiments, the first biotin domain is non-covalently attached to the phosphorothioate nucleic acid. In embodiments, a plurality of phosphorothioate nucleic acids is attached to the first biotin domain. In embodiments, the first biotin domain is attached to the first non-cell penetrating protein. In embodiments, the first biotin domain is covalently attached to the first non-cell penetrating protein. In embodiments, the first biotin domain is non-covalently attached to the first non-cell penetrating protein. In embodiments, the first biotin domain is non-covalently attached to the first biotin domain, thereby forming the first linker.

[0112] In embodiments, the first linker or the second linker is a covalent linker. In embodiments, the second linker is a covalent linker. In embodiments, the first linker is a

covalent linker. In embodiments, the first linker is a bond. In embodiments, the second linker is a bond. In embodiments, the first linker is a covalent linker and the second linker is a covalent linker. In embodiments, the first linker is a non-covalent linker and the second linker is a non-covalent linker. In embodiments, the first linker is a covalent linker and the second linker is a non-covalent linker. In embodiments, the first linker is a non-covalent linker and the second linker is a covalent linker. In further embodiments, the second linker is a bond.

[0113] In embodiments, the second linker includes a second biotin-binding domain non-covalently attached to a second biotin domain. In embodiments, the second biotin-binding domain is a second avidin domain. In embodiments, the second biotin-binding domain is a second streptavidin domain. In embodiments, the second streptavidin domain binds a plurality of second biotin domains. In embodiments, the second streptavidin domain binds about four second biotin domains.

[0114] In embodiments, the second biotin-binding domain is attached to the second protein (e.g., second non-cell penetrating protein). In embodiments, the second biotin-binding domain is covalently attached to the second protein (e.g., second non-cell penetrating protein). In embodiments, the second biotin-binding domain is non-covalently attached to the second protein (e.g., second non-cell penetrating protein). In embodiments, a plurality of second biotin-binding domains is attached to the second protein (e.g., second non-cell penetrating protein). In embodiments, the second biotin-binding domain is attached to the phosphorothioate nucleic acid. In embodiments, the second biotin-binding domain is covalently attached to the phosphorothioate nucleic acid. In embodiments, the second biotin-binding domain is non-covalently attached to the phosphorothioate nucleic acid. In embodiments, a plurality of phosphorothioate nucleic acids is attached to the second biotin-binding domain.

[0115] In embodiments, the second biotin domain is attached to the phosphorothioate nucleic acid. In embodiments, the second biotin domain is covalently attached to the phosphorothioate nucleic acid. In embodiments, the second biotin domain is non-covalently attached to the phosphorothioate nucleic acid. In embodiments, a plurality of phosphorothioate nucleic acids is attached to the second biotin domain. In embodiments, the second biotin domain is attached to the second protein (e.g., second non-cell penetrating protein). In embodiments, the second biotin domain is covalently attached to the second

protein (e.g., second non-cell penetrating protein). In embodiments, the second biotin domain is non-covalently attached to the second protein (e.g., second non-cell penetrating protein). In embodiments, the second biotin-binding domain is non-covalently attached to the second biotin domain, thereby forming the second linker.

[0116] The first member of the biotin binding pair and the second member of the biotin binding pair may be covalently or non-covalently attached to the first or second non-cell penetrating protein, the phosphorothioate nucleic acid or phosphorothioate polymer backbone. In embodiments, the non-cell penetrating protein is covalently attached to the first member of the biotin binding pair (e.g., avidin, streptavidin) and the phosphorothioate nucleic acid or the phosphorothioate polymer backbone is covalently attached to the second member of the biotin binding pair (e.g., biotin).

[0117] In embodiments, the biotin-binding domain is an avidin domain. In embodiments, the biotin-binding domain is a streptavidin domain. In embodiments, the streptavidin domain binds a plurality of biotin domains. In embodiments, the streptavidin domain binds about four biotin domains. In embodiments, the biotin-binding domain is attached to the non-cell penetrating protein. In embodiments, the biotin-binding domain is covalently attached to the first non-cell penetrating protein. In embodiments, the biotin-binding domain is covalently attached to the second non-cell penetrating protein. In embodiments, the biotin-binding domain is non-covalently attached to the first non-cell penetrating protein. In embodiments, the biotin-binding domain is non-covalently attached to the second non-cell penetrating protein. In embodiments, a plurality of biotin-binding domains is attached to the first non-cell penetrating protein. In embodiments, a plurality of biotin-binding domains is attached to the second non-cell penetrating protein. In embodiments, the biotin-binding domain is attached to the phosphorothioate nucleic acid or phosphorothioate polymer backbone. In embodiments, the biotin-binding domain is covalently attached to the phosphorothioate nucleic acid or phosphorothioate polymer backbone. In embodiments, the biotin-binding domain is non-covalently attached to the phosphorothioate nucleic acid or phosphorothioate polymer backbone. In embodiments, a plurality of biotin-binding domains is attached to the phosphorothioate nucleic acid or phosphorothioate polymer backbone. In embodiments, the biotin-binding domain is non-covalently attached to the biotin domain, thereby forming the non-covalent linker.

[0118] In embodiments, the biotin domain is attached to the phosphorothioate nucleic acid or the phosphorothioate polymer backbone. In embodiments, the biotin domain is covalently attached to the phosphorothioate nucleic acid or the phosphorothioate polymer backbone. In embodiments, the biotin domain is non-covalently attached to the phosphorothioate nucleic acid or the phosphorothioate polymer backbone. In embodiments, a plurality of phosphorothioate nucleic acids or phosphorothioate polymer backbones is attached to the biotin domain. In embodiments, the biotin domain is attached to the first non-cell penetrating protein. In embodiments, the biotin domain is attached to the second non-cell penetrating protein. In embodiments, the biotin domain is covalently attached to the first non-cell penetrating protein. In embodiments, the biotin domain is covalently attached to the second non-cell penetrating protein. In embodiments, the biotin domain is non-covalently attached to the first non-cell penetrating protein. In embodiments, the biotin domain is non-covalently attached to the second non-cell penetrating protein. In embodiments, a plurality of biotin domains is attached to the first non-cell penetrating protein. In embodiments, a plurality of biotin domains is attached to the second non-cell penetrating protein. In embodiments, the biotin domain is non-covalently attached to the biotin-binding domain, thereby forming the non-covalent linker.

[0119] As discussed above, the nucleic acids, e.g., the phosphorothioate nucleic acids or phosphorothioate polymer backbones may be attached to the first or second non-cell penetrating protein through a non-covalent linker including a biotin-binding domain (e.g., avidin or streptavidin) and a biotin domain. The nucleic acids, e.g., the phosphorothioate nucleic acids or phosphorothioate polymer backbones may be attached to the biotin-binding domain or the biotin domain through a variety of mechanisms. Similarly, the first or second non-cell penetrating protein may be attached to the biotin-binding domain or the biotin domain through a variety of mechanisms. The phosphorothioate nucleic acid, phosphorothioate polymer backbone or the first or second non-cell penetrating protein can be covalently or non-covalently attached to the biotin-binding domain or the biotin domain. The first or second non-cell penetrating protein may be covalently bound to a biotin-binding domain or a biotin domain. Where the first or second non-cell penetrating protein is covalently bound to a biotin-binding domain or biotin domain, the biotin-binding domain or biotin domain covalently binds an amino acid of the protein. In embodiments, the first or second non-cell penetrating protein includes a covalent reactive moiety (as described above) and the reactive moiety is reactive with the biotin-binding domain or the biotin

domain. In embodiments, the biotin-binding domain or the biotin domain includes a covalent reactive moiety and the reactive moiety is reactive with the first or second non-cell penetrating protein.

[0120] In embodiments, the phosphorothioate nucleic acid or phosphorothioate polymer backbone includes a covalent reactive moiety and the reactive moiety is reactive with the biotin-binding domain or the biotin domain. As described above, the covalent reactive moiety may be reactive with a lysine, arginine, cysteine or histidine of the protein (e.g. with the amino acid side chains). In embodiments, the covalent reactive moiety is reactive with a cysteine. The covalent reactive moiety may be a vinyl sulfone. In embodiments, phosphorothioate nucleic acid or phosphorothioate polymer backbone includes a reactive moiety having the formula S-S-R, where R is a protecting group. In embodiments, R is a hexanol (a monovalent substituent). As used herein, the term hexanol includes compounds with the formula $C_6H_{13}OH$ and includes, 1-hexanol, 2-hexanol, 3-hexanol, 2-methyl-1-pentanol, 3-methyl-1-pentanol, 4-methyl-1-pentanol, 2-methyl-2-pentanol, 3-methyl-2-pentanol, 4-methyl-2-pentanol, 2-methyl-3-pentanol, 3-methyl-3-pentanol, 2,2-dimethyl-1-butanol, 2,3-dimethyl-1-butanol, 3,3-dimethyl-1-butanol, 2,3-dimethyl-2-butanol, 3,3-dimethyl-2-butanol, and 2-ethyl-1-butanol. In embodiments, R is 1-hexanol. In embodiments, the phosphorothioate nucleic acid is covalently bound to the biotin-binding domain or biotin domain. In embodiments, the phosphorothioate nucleic acid includes a reactive moiety. In embodiments, the reactive moiety is a vinyl sulfone or a reactive moiety with the formula S-S-R, as described above. In embodiments, R is a hexanol, for example, 1-hexanol.

[0121] In one embodiment, the first non-cell penetrating protein is a first antibody, the first linker includes a first biotin-binding domain and a first biotin domain, wherein said first biotin-binding domain is an avidin domain, the second non-cell penetrating protein is a second antibody, and the second linker includes a second biotin-binding domain and a second biotin domain, wherein said second biotin-binding domain is an avidin domain. In one further embodiment, the first antibody is an anti-STAT3 antibody and the second antibody is an anti-NFkB antibody. In one further embodiment, the phosphorothioate nucleic acid is a double-stranded nucleic acid.

[0122] In one embodiment, the first non-cell penetrating protein is a first antibody, the first linker is a covalent linker, the second non-cell penetrating protein is a second antibody, and

the second linker includes a second biotin-binding domain and a second biotin domain, wherein said second biotin-binding domain is an avidin domain. In one further embodiment, the first antibody is an anti-STAT3 antibody and the second antibody is an anti-NFkB antibody. In one further embodiment, the phosphorothioate nucleic acid is a double-stranded nucleic acid.

[0123] In one embodiment, the first non-cell penetrating protein is a first antibody, the first linker includes a first biotin-binding domain and a first biotin domain, wherein said first biotin-binding domain is an avidin domain, the second non-cell penetrating protein is a second antibody, and the second linker is a covalent linker. In one further embodiment, the first antibody is an anti-STAT3 antibody and the second antibody is an anti-NFkB antibody. In one further embodiment, the phosphorothioate nucleic acid is a double-stranded nucleic acid.

[0124] In one embodiment, the first non-cell penetrating protein is a first antibody, the first linker is a covalent linker, the second non-cell penetrating protein is a second antibody, and the second linker is a covalent linker. In one further embodiment, the first antibody is an anti-STAT3 antibody and the second antibody is an anti-NFkB antibody. In one further embodiment, the phosphorothioate nucleic acid is a double-stranded nucleic acid.

[0125] In one embodiment, the first non-cell penetrating protein is a first antibody, the first linker includes a first biotin-binding domain and a first biotin domain, wherein said first biotin-binding domain is an avidin domain, the second non-cell penetrating protein is a second antibody, and the second linker includes a second biotin-binding domain and a second biotin domain, wherein said second biotin-binding domain is an avidin domain. In one further embodiment, the first antibody is an anti-CTLA-4 antibody and the second antibody is an anti-FOXP3 antibody. In one further embodiment, the phosphorothioate nucleic acid is a double-stranded nucleic acid.

[0126] In one embodiment, the first non-cell penetrating protein is a first antibody, the first linker is a covalent linker, the second non-cell penetrating protein is a second antibody, and the second linker includes a second biotin-binding domain and a second biotin domain, wherein said second biotin-binding domain is an avidin domain. In one further embodiment, the first antibody is an anti-CTLA-4 antibody and the second antibody is an anti-FOXP3 antibody. In one further embodiment, the phosphorothioate nucleic acid is a double-stranded nucleic acid.

[0127] In one embodiment, the first non-cell penetrating protein is a first antibody, the first linker includes a first biotin-binding domain and a first biotin domain, wherein said first biotin-binding domain is an avidin domain, the second non-cell penetrating protein is a second antibody, and the second linker is a covalent linker. In one further embodiment, the first antibody is an anti-CTLA-4 antibody and the second antibody is an anti-FOXP3 antibody. In one further embodiment, the phosphorothioate nucleic acid is a double-stranded nucleic acid.

[0128] In one embodiment, the first non-cell penetrating protein is a first antibody, the first linker is a covalent linker, the second non-cell penetrating protein is a second antibody, and the second linker is a covalent linker. In one further embodiment, the first antibody is an anti-CTLA-4 antibody and the second antibody is an anti-FOXP3 antibody. In one further embodiment, the phosphorothioate nucleic acid is a double-stranded nucleic acid.

[0129] In embodiments, the phosphorothioate nucleic acid is a single-stranded nucleic acid. In embodiments, the phosphorothioate nucleic acid is a double-stranded nucleic acid. Where the phosphorothioate nucleic acid is a double-stranded nucleic acid, the phosphorothioate nucleic acid includes a first phosphorothioate nucleic acid hybridized to a second phosphorothioate nucleic acid, the first phosphorothioate nucleic acid attached to the first non-cell penetrating protein and the second phosphorothioate nucleic acid attached to the second non-cell penetrating protein. In embodiments, the first phosphorothioate nucleic acid is complementary to the second phosphorothioate nucleic acid. The first phosphorothioate nucleic acid has a sequence identity of at least 50% to a nucleic acid complementary to the second phosphorothioate nucleic acid. The first phosphorothioate nucleic acid has a sequence identity of about 50% to a nucleic acid complementary to the second phosphorothioate nucleic acid. The first phosphorothioate nucleic acid has a sequence identity of at least 60% to a nucleic acid complementary to the second phosphorothioate nucleic acid. The first phosphorothioate nucleic acid has a sequence identity of at least 70% to a nucleic acid complementary to the second phosphorothioate nucleic acid. The first phosphorothioate nucleic acid has a sequence identity of at least 80% to a nucleic acid complementary to the second phosphorothioate nucleic acid. The first phosphorothioate nucleic acid has a sequence identity of at least 90% to a nucleic acid complementary to the second phosphorothioate nucleic acid. The first phosphorothioate nucleic acid has a sequence identity of at least 95% to a nucleic acid complementary to the second

phosphorothioate nucleic acid. The first phosphorothioate nucleic acid has a sequence identity of at least 98% to a nucleic acid complementary to the second phosphorothioate nucleic acid.

[0130] In embodiments, the phosphorothioate nucleic acid or phosphorothioate polymer backbone is about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more nucleic acid residues in length. In embodiments, the phosphorothioate nucleic acid or phosphorothioate polymer backbone is from about 10 to about 30 nucleic acid residues in length. In embodiments, the phosphorothioate nucleic acid or phosphorothioate polymer backbone acid is about 20 nucleic acid residues in length. In embodiments, the length of each nucleic acid or polymer backbone can be at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or more nucleic acid residues or sugar residues in length. In embodiments, each phosphorothioate nucleic acid or phosphorothioate polymer backbone is independently from 5 to 50, 10 to 50, 15 to 50, 20 to 50, 25 to 50, 30 to 50, 35 to 50, 40 to 50, 45 to 50, 5 to 75, 10 to 75, 15 to 75, 20 to 75, 25 to 75, 30 to 75, 35 to 75, 40 to 75, 45 to 75, 50 to 75, 55 to 75, 60 to 75, 65 to 75, 70 to 75, 5 to 100, 10 to 100, 15 to 100, 20 to 100, 25 to 100, 30 to 100, 35 to 100, 40 to 100, 45 to 100, 50 to 100, 55 to 100, 60 to 100, 65 to 100, 70 to 100, 75 to 100, 80 to 100, 85 to 100, 90 to 100, 95 to 100, or more residues in length. In embodiments, each phosphorothioate nucleic acid or phosphorothioate polymer backbone is independently from 10 to 15, 10 to 20, 10 to 30, 10 to 40, or 10 to 50 residues in length.

[0131] In embodiments, the first non-cell penetrating protein and the second non-cell penetrating protein independently have a molecular weight of more than 25 kD. In embodiments, the first non-cell penetrating protein and the second non-cell penetrating protein independently have a molecular weight of about 25 kD to about 750 kD. Thus, the first non-cell penetrating protein and the second non-cell penetrating protein independently have a molecular weight of at least about 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 405, 410, 415, 420, 425, 430, 435, 440, 445, 450, 455,

460, 465, 470, 475, 480, 485, 490, 495, 500, 505, 510, 515, 520, 525, 530, 535, 540, 545, 550, 555, 560, 565, 570, 575, 580, 585, 590, 595, 600, 605, 610, 615, 620, 625, 630, 635, 640, 645, 650, 655, 660, 665, 670, 675, 680, 685, 690, 695, 700, 705, 710, 715, 720, 725, 730, 735, 740, 745, 750, or more kilodaltons (kD). In embodiments, the first non-cell penetrating protein and the second non-cell penetrating protein independently have a molecular weight from at least about 25 to 100 kD, at least about 25 to 150 kD, at least about 25 to 200 kD, at least about 25 to 250 kD, at least about 25 to 300 kD, at least about 25 to 350 kD, at least about 25 to 400 kD, at least about 25 to 450 kD, at least about 25 to 500 kD, at least about 25 to 550 kD, at least about 25 to 600 kD, at least about 25 to 650 kD, at least about 25 to 700 kD or at least above 25 to 750 kD.

[0132] In embodiments, the first non-cell penetrating protein and the second non-cell penetrating protein independently are an antibody. As discussed in more detail above, antibodies can be full length antibodies such as IgG, IgA, IgM, IgD or IgE antibodies or fragments thereof. In embodiments, the antibody is an IgG antibody or a fragment thereof. In embodiments, the antibody is an IgG antibody or a fragment thereof. In embodiments, the antibody is an scFv fragment or a humanized antibody. In embodiments, the antibody is an IgA, IgM, IgD or IgE antibody. In embodiments, the antibody is a scFv fragment. In embodiments, the antibody is a humanized antibody. Thus, provided are a first antibody and a second antibody connected through a phosphorothioate nucleic acid, wherein the phosphorothioate nucleic acid enhances delivery of the first antibody and the second antibody into a cell. In embodiments, the first antibody or the second antibody is a therapeutic antibody, i.e., an antibody used in the treatment of disease. In embodiments, the first non-cell penetrating protein (e.g., antibody) or the second non-cell penetrating protein (e.g., antibody) bind an intracellular target. In embodiments, the first non-cell penetrating protein (e.g., antibody) and the second non-cell penetrating protein (e.g., antibody) bind an intracellular target. In embodiments, the first non-cell penetrating protein binds a different intracellular target relative to the second non-cell penetrating protein. Thus, in embodiments the first non-cell penetrating protein binds a first intracellular target and the second non-cell penetrating protein binds a second intracellular target. In embodiments, the first intracellular target is a signaling molecule (e.g., a STAT3 protein). In embodiments, the second intracellular target is a transcription factor (e.g., an NFkB protein). In embodiments, the first non-cell penetrating protein binds a different epitope on the intracellular target relative to the second non-cell penetrating protein.

[0133] The intracellular target can be a therapeutic target or a diagnostic target or other target of interest located intracellularly, e.g., a target or structure, e.g., histone, to be imaged, e.g., by confocal microscopy. Thus, provided are cell penetrating conjugates bound to an intracellular target. In embodiments, the intracellular target is a target of a disease selected from the group consisting of autoimmune disease, inflammatory disease, metabolic disorder, developmental disorder, cardiovascular disease, liver disease, intestinal disease, infectious disease, endocrine disease, neurological disorder, and cancer. Examples of intracellular targets include without limitation oncogenic transcription factors including but not limited to STAT3, Myc, NF κ B, AP1, HIF, mutant p53; oncoproteins including but not limited to Ras, Raf, MAPK, PI3 kinase, AKT, BTK, JAKs, SRC family members; immunomodulatory molecules including FOXp3, T-BET, GATA3, STAT1, 2, 3, 4, 5, 6. The target of a disease can be a diagnostic target or therapeutic target or other target of interest associated with the disease. Exemplary intracellular targets of cancer include, but are not limited to, STAT (e.g., STAT3), NF κ B, PKB/Akt, Myc family members, steroid hormone receptors (e.g., estrogen receptor), ligands of steroid hormone receptors (e.g., cyclin D1), receptor tyrosine kinases (RTKs), EGFR, VEGFR, PDGFR, Src family members, Ras, Abl, BCR-Abl, NPM-Alk, Janus kinases (JAKs), Bruton's tyrosine kinase (BTK), and viral oncoproteins (e.g., an EBV protein, or an HPV protein, e.g., E6 and E7). In embodiments, the intracellular target of the infectious disease is a viral protein or viral transcript. Thus, the intracellular target can be a viral protein or viral transcript of a human immunodeficiency virus (HIV), influenza virus, herpes simplex virus, epstein barr virus, cytomegalovirus, human papilloma virus, or hepatitis virus. In embodiments, the intracellular target is a DNA binding protein including, but not limited to, a transcription factor, a transcriptional enhancer, a transcriptional repressor, a histone or post-translationally modified histone. In embodiments, the intracellular target is epigenetically modified DNA, e.g., methylated or hydroxymethylated cytosine (5mC or 5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). In embodiments, the intracellular target is a nucleic acid, e.g., an RNA transcript or a nucleic acid. For example, the intracellular target may be the nucleic acid of an infectious pathogen, e.g., a parasite, virus or bacteria.

[0134] In embodiments, the intracellular target is a signaling molecule or a transcription factor. In embodiments, the signaling molecule is a phosphatase or kinase. In embodiments, the transcription factor is a nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B) protein. In embodiments, the NF κ B protein is p65. In embodiments, the first non-

cell penetrating protein binds a STAT3 protein and the second non-cell penetrating protein binds an NF κ B protein. In embodiments, the intracellular target is a cancer target or located within a cancer cell. In embodiments, the intracellular target is a STAT protein, e.g., STAT3 or exportin 7. In embodiments, the intracellular target is selected from the group consisting of STAT3, NF κ B and Src. In embodiments, the intracellular target is STAT3. In embodiments, the intracellular target is NF κ B. In embodiments, the non-cell penetrating protein further includes a label, a small molecule or a functional nucleic acid attached to the protein. In embodiments, the cell penetrating conjugate is bound to an intracellular target.

CELL COMPOSITIONS

[0135] In another aspect, a cell including the cell penetrating conjugate provided herein including embodiments thereof is provided. Provided are cells including one or more of the provided cell penetrating conjugates, e.g., the cells may include a plurality of cell penetrating conjugates. In embodiments, the conjugate is bound within the cell to an intracellular target. By way of example, the cells can include a first non-cell penetrating protein and a second non-cell penetrating protein connected through a phosphorothioate nucleic acid or polymer backbones. The first and second non-cell penetrating protein may be bound within the cell to an intracellular target. In embodiments, the second non-cell penetrating protein binds a different epitope on the intracellular target relative to the first non-cell penetrating protein. In embodiments, the second non-cell penetrating protein binds a second intracellular target. In embodiments, the first and/or second non-cell penetrating protein is an antibody. Thus, the first and second non-cell penetrating proteins can be the same protein or a different protein.

PHARMACEUTICAL COMPOSITIONS

[0136] Provided herein are pharmaceutical compositions comprising the cell penetrating conjugates provided herein including embodiments thereof and a pharmaceutically acceptable carrier. The provided compositions are, *inter alia*, suitable for formulation and administration *in vitro* or *in vivo*. Suitable carriers and excipients and their formulations are described in Remington: The Science and Practice of Pharmacy, 21st Edition, David B. Troy, ed., Lippincott Williams & Wilkins (2005). By pharmaceutically acceptable carrier is meant a material that is not biologically or otherwise undesirable, i.e., the material is administered to a subject without causing undesirable biological effects or interacting in a deleterious manner with the other components of the pharmaceutical composition in which it is contained. If

administered to a subject, the carrier is optionally selected to minimize degradation of the active ingredient and to minimize adverse side effects in the subject.

[0137] Pharmaceutical compositions provided by the present invention include compositions wherein the active ingredient (e.g. compositions described herein, including embodiments or examples) is contained in a therapeutically effective amount, *i.e.*, in an amount effective to achieve its intended purpose. The actual amount effective for a particular application will depend, *inter alia*, on the condition being treated. When administered in methods to treat a disease, the recombinant proteins described herein will contain an amount of active ingredient effective to achieve the desired result, e.g., modulating the activity of a target molecule, and/or reducing, eliminating, or slowing the progression of disease symptoms. Determination of a therapeutically effective amount of a compound of the invention is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure herein.

[0138] In another aspect, a pharmaceutical composition including the cell penetrating conjugate provided herein including embodiments thereof and a pharmaceutically acceptable carrier is provided. In embodiments, the first non-cell penetrating protein or the second non-cell penetrating protein binds an intracellular target. In embodiments, the first non-cell penetrating protein and the second non-cell penetrating protein binds an intracellular target. In embodiments, the second non-cell penetrating protein binds a different epitope on the intracellular target relative to the first non-cell penetrating protein. In embodiments, the first non-cell penetrating protein binds a first intracellular target. In embodiments, the first non-cell penetrating protein is an antibody. In embodiments, the second non-cell penetrating protein binds a second intracellular target. In embodiments, the second non-cell penetrating protein is an antibody. The first and second non-cell penetrating proteins can be the same protein or a different protein.

[0139] The compositions for administration will commonly include an agent as described herein dissolved in a pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., buffered saline and the like. These solutions are sterile and generally free of undesirable matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like,

for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of active agent in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the subject's needs.

[0140] Solutions of the active compounds as free base or pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations can contain a preservative to prevent the growth of microorganisms.

[0141] Pharmaceutical compositions can be delivered via intranasal or inhalable solutions or sprays, aerosols or inhalants. Nasal solutions can be aqueous solutions designed to be administered to the nasal passages in drops or sprays. Nasal solutions can be prepared so that they are similar in many respects to nasal secretions. Thus, the aqueous nasal solutions usually are isotonic and slightly buffered to maintain a pH of 5.5 to 6.5. In addition, antimicrobial preservatives, similar to those used in ophthalmic preparations and appropriate drug stabilizers, if required, may be included in the formulation. Various commercial nasal preparations are known and can include, for example, antibiotics and antihistamines.

[0142] Oral formulations can include excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders. In some embodiments, oral pharmaceutical compositions will comprise an inert diluent or assimilable edible carrier, or they may be enclosed in hard or soft shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 75% of the weight of the unit, or preferably between 25-60%. The amount of active compounds in such compositions is such that a suitable dosage can be obtained.

[0143] For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered and the liquid diluent first rendered isotonic with sufficient saline or glucose. Aqueous solutions, in particular, sterile aqueous media, are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion.

[0144] Sterile injectable solutions can be prepared by incorporating the active compounds or constructs in the required amount in the appropriate solvent followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium. Vacuum-drying and freeze-drying techniques, which yield a powder of the active ingredient plus any additional desired ingredients, can be used to prepare sterile powders for reconstitution of sterile injectable solutions. The preparation of more, or highly, concentrated solutions for direct injection is also contemplated. DMSO can be used as solvent for extremely rapid penetration, delivering high concentrations of the active agents to a small area.

[0145] The formulations of compounds can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials. Thus, the composition can be in unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the active component. Thus, the compositions can be administered in a variety of unit dosage forms depending upon the method of administration. For example, unit dosage forms suitable for oral administration include, but are not limited to, powder, tablets, pills, capsules and lozenges.

[0146] The dosage and frequency (single or multiple doses) administered to a mammal can vary depending upon a variety of factors, for example, whether the mammal suffers from another disease, and its route of administration; size, age, sex, health, body weight, body mass index, and diet of the recipient; nature and extent of symptoms of the disease being treated (e.g. symptoms of cancer and severity of such symptoms), kind of concurrent treatment, complications from the disease being treated or other health-related problems. Other therapeutic regimens or agents can be used in conjunction with the methods and compounds of the invention. Adjustment and manipulation of established dosages (e.g., frequency and duration) are well within the ability of those skilled in the art.

[0132] For any composition (e.g., the cell-penetrating conjugate provided) described herein, the therapeutically effective amount can be initially determined from cell culture assays. Target concentrations will be those concentrations of active compound(s) that are capable of achieving the methods described herein, as measured using the methods described herein or known in the art. As is well known in the art, effective amounts for use in humans can also be determined from animal models. For example, a dose for humans can be formulated to achieve a concentration that has been found to be effective in animals. The dosage in humans can be adjusted by monitoring effectiveness and adjusting the dosage upwards or downwards, as described above. Adjusting the dose to achieve maximal efficacy in humans based on the methods described above and other methods is well within the capabilities of the ordinarily skilled artisan.

[0147] Dosages may be varied depending upon the requirements of the patient and the compound being employed. The dose administered to a patient, in the context of the present invention should be sufficient to affect a beneficial therapeutic response in the patient over time. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects. Determination of the proper dosage for a particular situation is within the skill of the practitioner. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under circumstances is reached.

[0148] Dosage amounts and intervals can be adjusted individually to provide levels of the administered compound effective for the particular clinical indication being treated. This will provide a therapeutic regimen that is commensurate with the severity of the individual's disease state.

[0149] Utilizing the teachings provided herein, an effective prophylactic or therapeutic treatment regimen can be planned that does not cause substantial toxicity and yet is effective to treat the clinical symptoms demonstrated by the particular patient. This planning should involve the careful choice of active compound by considering factors such as compound potency, relative bioavailability, patient body weight, presence and severity of adverse side effects, preferred

[0150] "Pharmaceutically acceptable excipient" and "pharmaceutically acceptable carrier" refer to a substance that aids the administration of an active agent to and absorption by a subject and can be included in the compositions of the present invention without causing a

significant adverse toxicological effect on the patient. Non-limiting examples of pharmaceutically acceptable excipients include water, NaCl, normal saline solutions, lactated Ringer's, normal sucrose, normal glucose, binders, fillers, disintegrants, lubricants, coatings, sweeteners, flavors, salt solutions (such as Ringer's solution), alcohols, oils, gelatins, carbohydrates such as lactose, amylose or starch, fatty acid esters, hydroxymethylcellulose, polyvinyl pyrrolidine, and colors, and the like. Such preparations can be sterilized and, if desired, mixed with auxiliary agents such as lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, and/or aromatic substances and the like that do not deleteriously react with the compounds of the invention. One of skill in the art will recognize that other pharmaceutical excipients are useful in the present invention.

[0151] The term "pharmaceutically acceptable salt" refers to salts derived from a variety of organic and inorganic counter ions well known in the art and include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium, and the like; and when the molecule contains a basic functionality, salts of organic or inorganic acids, such as hydrochloride, hydrobromide, tartrate, mesylate, acetate, maleate, oxalate and the like.

[0152] The term "preparation" is intended to include the formulation of the active compound with encapsulating material as a carrier providing a capsule in which the active component with or without other carriers, is surrounded by a carrier, which is thus in association with it. Similarly, cachets and lozenges are included. Tablets, powders, capsules, pills, cachets, and lozenges can be used as solid dosage forms suitable for oral administration.

METHODS OF FORMING CONJUGATES

[0153] In another aspect, a method of forming a cell penetrating conjugate is provided. The method includes hybridizing a first phosphorothioate nucleic acid attached to a first non-cell penetrating protein to a second phosphorothioate nucleic acid attached to a second non-cell penetrating protein, thereby forming a cell penetrating conjugate. In embodiments, the phosphorothioate nucleic acid is covalently attached to the first or the second phosphorothioate nucleic acid. In embodiments, the phosphorothioate nucleic acid is non-covalently attached to the first or the second phosphorothioate nucleic acid. In embodiments, the phosphorothioate nucleic acid is attached to the first or the second phosphorothioate nucleic acid through a non-covalent linker. In embodiments, the phosphorothioate nucleic

acid includes a covalent reactive moiety. In embodiments, the method includes prior to the hybridizing: (i) contacting the first non-cell penetrating protein with the first phosphorothioate nucleic acid, thereby forming a first protein phosphorothioate nucleic acid complex. The second non-cell penetrating protein is contacted with the second phosphorothioate nucleic acid, thereby forming a second protein- phosphorothioate nucleic acid complex. And the first protein phosphorothioate nucleic acid complex and the second protein phosphorothioate nucleic acid complex are contacted.

METHODS OF DELIVERY

[0154] In another aspect, a method of delivering a non-cell penetrating protein as provided herein including embodiments thereof into a cell is provided. In embodiments, the non-cell penetrating protein binds the nuclear protein in the cytoplasm thereby forming a first non-cell penetrating protein-nuclear protein complex. In embodiments, the non-cell penetrating protein binds the nuclear protein in the cytoplasm thereby forming a second non-cell penetrating protein-nuclear protein complex. In embodiments, the first non-cell penetrating protein-nuclear protein complex and the second non-cell penetrating protein-nuclear protein complex are not capable of entering the nucleus of the cell.

[0155] In embodiments, the cell penetrating conjugates are used for diagnosing a disease in a subject. Thus, provided is a method of diagnosing a disease in a subject comprising administering to the subject an effective amount of a cell penetrating conjugate or composition comprising a cell penetrating conjugate as described herein. Administration of the conjugate diagnoses the disease or one or more symptoms of the disease in the subject. The disclosed methods involve comparing the levels or activity of a biomarker, e.g., intracellular target of a disease, from a test sample to a control sample. As discussed above, a control sample or value refers to a sample that serves as a reference, usually a known reference, for comparison to a test sample. A control can also represent an average value gathered from a population of similar individuals, e.g., cancer patients or healthy individuals with a similar medical background, same age, weight, etc. A control value can also be obtained from the same individual, e.g., from an earlier-obtained sample, prior to disease, or prior to treatment. As also discussed above, diagnosis refers to a relative probability that a disease (e.g. an autoimmune, inflammatory autoimmune, cancer, infectious, immune, or other disease) is present in the subject.

[0156] The terms comparing, correlating and associated, in reference to determination of a disease risk factor, refers to comparing the presence or amount of the risk factor (e.g., amount of intracellular target of a disease) in an individual to its presence or amount in persons known to suffer from, or known to be at risk of disease, or in persons known to be free of disease, and assigning an increased or decreased probability of having/developing the disease to an individual based on the assay result(s).

METHODS OF DETECTING

[0157] Provided herein is also a method of detecting an intracellular target in a cell, including contacting the cell with a cell penetrating conjugate as provided herein including embodiments thereof and detecting binding of the cell penetrating conjugate to an intracellular target. As described above, the cell penetrating conjugate may bind to one intracellular target or two intracellular targets, wherein the two intracellular targets may be independently different. The cell can be a fixed cell or a live cell. In embodiments, the cell is located *in vitro* or *in vivo*. Binding can be detected directly or indirectly. It is understood and contemplated herein that numerous methods may be used to detect the binding of the cell penetrating conjugate to its intracellular target. For example, binding can be detected directly by assaying coupling between the cell penetrating conjugate and its intracellular target. Binding can be determined, for example, by selecting an assay from the group consisting of a coimmunoprecipitation assay, a colocalization assay, or a fluorescence polarizing assay, as described below. The assays are known in the art, e.g., see Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd Ed., Cold Spring Harbor Press, Cold Spring Harbor, NY (2001); Dickson, Methods Mol. Biol. 461:735-44 (2008); Nickels, Methods 47(1):53-62 (2009); and Zinchuk et al., Acta Histochem. Cytochem. 40(4):101-11 (2007).

[0158] In embodiments, binding is determined by an imaging method or system. Thus, the cell penetrating conjugates provided herein including embodiments thereof can also be used in imaging applications or other applications for analyzing intracellular target levels and/or activities. For example, the provided cell penetrating conjugates can be used for *in vitro* or *in vivo* imaging of intracellular targets of interest. In embodiments, the cell penetrating conjugates are used for live cell imaging. For example, live cell imaging can be used to monitor intracellular target distribution and/or dynamics inside living cells and is also applicable to monitoring target interactions. For example, the cell penetrating conjugates can be used in immunoprecipitation and co-immunoprecipitation assays to study protein-protein

interactions in cells, In embodiments, in living cells. In embodiments, the cell penetrating conjugates are used for analysis of intracellular targets by flow cytometry. In imaging applications, the cell penetrating conjugates are labeled as appropriate to the application being used. As described above, a label or a detectable moiety is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means. Useful labels include, but are not limited to, ³²P, fluorescent dyes, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin, digoxigenin, or haptens and proteins or other entities which can be made detectable, e.g., by incorporating a radiolabel into a peptide or antibody specifically reactive with a target peptide. Any method known in the art for conjugating an antibody to the label may be employed, e.g., using methods described in Hermanson, *Bioconjugate Techniques* 1996, Academic Press, Inc., San Diego.

METHODS OF TREATMENT

[0159] The cell penetrating conjugates provided herein including embodiments thereof and compositions including the cell penetrating conjugates as described herein including embodiments thereof are useful for both prophylactic and therapeutic treatment. For prophylactic use, a therapeutically effective amount of the agents described herein are administered to a subject prior to or during early onset (e.g., upon initial signs and symptoms of an autoimmune disease). Therapeutic treatment involves administering to a subject a therapeutically effective amount of the agents described herein after diagnosis or development of disease. Thus, in another aspect, a method of treating a disease in a subject in need thereof is provided. The method includes administering to a subject an effective amount of the cell penetrating conjugate as provided herein including embodiments thereof, thereby treating the disease in the subject.

[0160] In embodiments, the first and second non-cell penetrating protein is an antibody. In embodiments, the disease is selected from the group consisting of autoimmune disease, developmental disorder, inflammatory disease, metabolic disorder, cardiovascular disease, liver disease, intestinal disease, infectious disease, endocrine disease, neurological disorder, and cancer. In embodiments, the disease is an autoimmune disease. In embodiments, the disease is a developmental disorder. In embodiments, the disease is an inflammatory disease. In embodiments, the disease is a metabolic disorder. In embodiments, the disease is a cardiovascular disease. In embodiments, the disease is a liver disease. In embodiments, the disease is an intestinal disease. In embodiments, the disease is an infectious disease. In

embodiments, the disease is an endocrine disease. In embodiments, the disease is a neurological disorder. In embodiments, the disease is cancer. In embodiments, the cancer is lymphoma. In embodiments, the cancer is melanoma.

[0161] In embodiments, the first non-cell penetrating protein or the second non-cell penetrating protein bind an intracellular target. In embodiments, the first non-cell penetrating protein and the second non-cell penetrating protein bind an intracellular target. In embodiments, the first non-cell penetrating protein binds a different intracellular target relative to said second non-cell penetrating protein. In embodiments, the first non-cell penetrating protein binds a different epitope on the intracellular target relative to the second non-cell penetrating protein. In embodiments, the intracellular target is STAT3, an NF κ B protein or Src. In embodiments, the intracellular target is p65. In embodiments, the intracellular target is STAT3. In embodiments, the first non-cell penetrating protein binds STAT3 and the second non-cell penetrating protein binds an NF κ B protein. In embodiments, the first non-cell penetrating protein of the conjugate is an antibody that specifically binds STAT3 and the second non-cell penetrating protein is an antibody that specifically binds another epitope of STAT3.

[0162] In the provided methods of treatment, additional therapeutic agents can be used that are suitable to the disease being treated. Thus, in some embodiments, the provided methods of treatment further comprise administering a second therapeutic agent to the subject. Suitable additional therapeutic agents include, but are not limited to, therapeutic agent is selected from the group consisting of analgesics, anesthetics, analeptics, corticosteroids, anticholinergic agents, anticholinesterases, anticonvulsants, antineoplastic agents, allosteric inhibitors, anabolic steroids, antirheumatic agents, psychotherapeutic agents, neural blocking agents, anti-inflammatory agents, antihelmintics, antibiotics, anticoagulants, antifungals, antihistamines, antimuscarinic agents, antimycobacterial agents, antiprotozoal agents, antiviral agents, dopaminergics, hematological agents, immunological agents, muscarinics, protease inhibitors, vitamins, growth factors, and hormones. The choice of agent and dosage can be determined readily by one of skill in the art based on the given disease being treated.

[0163] Combinations of agents or compositions can be administered either concomitantly (e.g., as a mixture), separately but simultaneously (e.g., via separate intravenous lines) or sequentially (e.g., one agent is administered first followed by administration of the second agent). Thus, the term combination is used to refer to concomitant, simultaneous or

sequential administration of two or more agents or compositions. The course of treatment is best determined on an individual basis depending on the particular characteristics of the subject and the type of treatment selected. The treatment, such as those disclosed herein, can be administered to the subject on a daily, twice daily, bi-weekly, monthly or any applicable basis that is therapeutically effective. The treatment can be administered alone or in combination with any other treatment disclosed herein or known in the art. The additional treatment can be administered simultaneously with the first treatment, at a different time, or on an entirely different therapeutic schedule (e.g., the first treatment can be daily, while the additional treatment is weekly).

[0164] According to the methods provided herein, the subject is administered an effective amount of one or more of the agents provided herein. The terms effective amount and effective dosage are used interchangeably. The term effective amount is defined as any amount necessary to produce a desired physiologic response (e.g., reduction of inflammation). Effective amounts and schedules for administering the agent may be determined empirically by one skilled in the art. The dosage ranges for administration are those large enough to produce the desired effect in which one or more symptoms of the disease or disorder are affected (e.g., reduced or delayed). The dosage should not be so large as to cause substantial adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex, type of disease, the extent of the disease or disorder, route of administration, or whether other drugs are included in the regimen, and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any contraindications. Dosages can vary and can be administered in one or more dose administrations daily, for one or several days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products. For example, for the given parameter, an effective amount will show an increase or decrease of at least 5%, 10%, 15%, 20%, 25%, 40%, 50%, 60%, 75%, 80%, 90%, or at least 100%. Efficacy can also be expressed as “-fold” increase or decrease. For example, a therapeutically effective amount can have at least a 1.2-fold, 1.5-fold, 2-fold, 5-fold, or more effect over a control. The exact dose and formulation will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, e.g., Lieberman, *Pharmaceutical Dosage Forms* (vols. 1-3, 1992); Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding* (1999); Remington: *The*

Science and Practice of Pharmacy, 20th Edition, Gennaro, Editor (2003), and Pickar, Dosage Calculations (1999)).

[0165] Disclosed are materials, compositions, and components that can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed methods and compositions. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutations of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a method is disclosed and discussed and a number of modifications that can be made to a number of molecules including the method are discussed, each and every combination and permutation of the method, and the modifications that are possible are specifically contemplated unless specifically indicated to the contrary. Likewise, any subset or combination of these is also specifically contemplated and disclosed. This concept applies to all aspects of this disclosure including, but not limited to, steps in methods using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed, it is understood that each of these additional steps can be performed with any specific method steps or combination of method steps of the disclosed methods, and that each such combination or subset of combinations is specifically contemplated and should be considered disclosed.

[0166] The terms "subject," "patient," "individual," etc. are not intended to be limiting and can be generally interchanged. That is, an individual described as a "patient" does not necessarily have a given disease, but may be merely seeking medical advice.

[0167] As used herein, "treating" or "treatment of" a condition, disease or disorder or symptoms associated with a condition, disease or disorder refers to an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of condition, disorder or disease, stabilization of the state of condition, disorder or disease, prevention of development of condition, disorder or disease, prevention of spread of condition, disorder or disease, delay or slowing of condition, disorder or disease progression, delay or slowing of condition, disorder or disease onset, amelioration or palliation of the condition, disorder or disease state, and remission, whether partial or total. "Treating" can also mean prolonging survival of a subject beyond that expected in the

absence of treatment. "Treating" can also mean inhibiting the progression of the condition, disorder or disease, slowing the progression of the condition, disorder or disease temporarily, although in some instances, it involves halting the progression of the condition, disorder or disease permanently. As used herein the terms treatment, treat, or treating refers to a method of reducing the effects of one or more symptoms of a disease or condition characterized by expression of the protease or symptom of the disease or condition characterized by expression of the protease. Thus in the disclosed method, treatment can refer to a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% reduction in the severity of an established disease, condition, or symptom of the disease or condition. For example, a method for treating a disease is considered to be a treatment if there is a 10% reduction in one or more symptoms of the disease in a subject as compared to a control. Thus the reduction can be a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or any percent reduction in between 10% and 100% as compared to native or control levels. It is understood that treatment does not necessarily refer to a cure or complete ablation of the disease, condition, or symptoms of the disease or condition. Further, as used herein, references to decreasing, reducing, or inhibiting include a change of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater as compared to a control level and such terms can include but do not necessarily include complete elimination.

[0168] Where combination treatments are contemplated, it is not intended that the agents (i.e. ribonucleic acid compounds) described herein be limited by the particular nature of the combination. For example, the agents described herein may be administered in combination as simple mixtures as well as chemical hybrids. An example of the latter is where the agent is covalently linked to a targeting carrier or to an active pharmaceutical. Covalent binding can be accomplished in many ways, such as, though not limited to, the use of a commercially available cross-linking agent.

[0169] As used herein, the term "pharmaceutically acceptable" is used synonymously with "physiologically acceptable" and "pharmacologically acceptable". A pharmaceutical composition will generally comprise agents for buffering and preservation in storage, and can include buffers and carriers for appropriate delivery, depending on the route of administration.

[0170] An "effective amount" is an amount sufficient to accomplish a stated purpose (e.g. achieve the effect for which it is administered, treat a disease, reduce enzyme activity, reduce

one or more symptoms of a disease or condition). An example of an "effective amount" is an amount sufficient to contribute to the treatment, prevention, or reduction of a symptom or symptoms of a disease, which could also be referred to as a "therapeutically effective amount." A "reduction" of a symptom or symptoms (and grammatical equivalents of this phrase) means decreasing of the severity or frequency of the symptom(s), or elimination of the symptom(s). A "prophylactically effective amount" of a drug is an amount of a drug that, when administered to a subject, will have the intended prophylactic effect, e.g., preventing or delaying the onset (or reoccurrence) of an injury, disease, pathology or condition, or reducing the likelihood of the onset (or reoccurrence) of an injury, disease, pathology, or condition, or their symptoms. The full prophylactic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a prophylactically effective amount may be administered in one or more administrations. An "activity decreasing amount," as used herein, refers to an amount of antagonist required to decrease the activity of an enzyme or protein relative to the absence of the antagonist. A "function disrupting amount," as used herein, refers to the amount of antagonist required to disrupt the function of an enzyme or protein relative to the absence of the antagonist. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products. For example, for the given parameter, an effective amount will show an increase or decrease of at least 5%, 10%, 15%, 20%, 25%, 40%, 50%, 60%, 75%, 80%, 90%, or at least 100%. Efficacy can also be expressed as “-fold” increase or decrease. For example, a therapeutically effective amount can have at least a 1.2-fold, 1.5-fold, 2-fold, 5-fold, or more effect over a control. The exact amounts will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, e.g., Lieberman, *Pharmaceutical Dosage Forms* (vols. 1-3, 1992); Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding* (1999); Pickar, *Dosage Calculations* (1999); and Remington: *The Science and Practice of Pharmacy*, 20th Edition, 2003, Gennaro, Ed., Lippincott, Williams & Wilkins).

[0171] As used herein, the term "administering" means oral administration, administration as a suppository, topical contact, intravenous, intraperitoneal, intramuscular, intralesional, intrathecal, intranasal or subcutaneous administration, or the implantation of a slow-release device, e.g., a mini-osmotic pump, to a subject. Administration is by any route, including parenteral and transmucosal (e.g., buccal, sublingual, palatal, gingival, nasal, vaginal, rectal, or transdermal). Parenteral administration includes, e.g., intravenous, intramuscular, intra-

arteriole, intradermal, subcutaneous, intraperitoneal, intraventricular, and intracranial. Other modes of delivery include, but are not limited to, the use of liposomal formulations, intravenous infusion, transdermal patches, *etc.* By "co-administer" it is meant that a composition described herein is administered at the same time, just prior to, or just after the administration of one or more additional therapies, for example cancer therapies such as chemotherapy, hormonal therapy, radiotherapy, or immunotherapy. The compounds of the invention can be administered alone or can be coadministered to the patient.

Coadministration is meant to include simultaneous or sequential administration of the compounds individually or in combination (more than one compound). Thus, the preparations can also be combined, when desired, with other active substances (e.g. to reduce metabolic degradation). The compositions of the present invention can be delivered by transdermally, by a topical route, formulated as applicator sticks, solutions, suspensions, emulsions, gels, creams, ointments, pastes, jellies, paints, powders, and aerosols.

[0172] The compositions of the present invention may additionally include components to provide sustained release and/or comfort. Such components include high molecular weight, anionic mucomimetic polymers, gelling polysaccharides and finely-divided drug carrier substrates. These components are discussed in greater detail in U.S. Pat. Nos. 4,911,920; 5,403,841; 5,212,162; and 4,861,760. The entire contents of these patents are incorporated herein by reference in their entirety for all purposes. The compositions of the present invention can also be delivered as microspheres for slow release in the body. For example, microspheres can be administered via intradermal injection of drug-containing microspheres, which slowly release subcutaneously (see Rao, *J. Biomater. Sci. Polym. Ed.* 7:623-645, 1995; as biodegradable and injectable gel formulations (see, e.g., Gao *Pharm. Res.* 12:857-863, 1995); or, as microspheres for oral administration (see, e.g., Eyles, *J. Pharm. Pharmacol.* 49:669-674, 1997). In embodiments, the formulations of the compositions of the present invention can be delivered by the use of liposomes which fuse with the cellular membrane or are endocytosed, *i.e.*, by employing receptor ligands attached to the liposome, that bind to surface membrane protein receptors of the cell resulting in endocytosis. By using liposomes, particularly where the liposome surface carries receptor ligands specific for target cells, or are otherwise preferentially directed to a specific organ, one can focus the delivery of the compositions of the present invention into the target cells *in vivo*. (See, e.g., Al-Muhammed, *J. Microencapsul.* 13:293-306, 1996; Chonn, *Curr. Opin. Biotechnol.* 6:698-708, 1995;

Ostro, *Am. J. Hosp. Pharm.* 46:1576-1587, 1989). The compositions of the present invention can also be delivered as nanoparticles.

[0173] Utilizing the teachings provided herein, an effective prophylactic or therapeutic treatment regimen can be planned that does not cause substantial toxicity and yet is effective to treat the clinical symptoms demonstrated by the particular patient. This planning should involve the careful choice of active compound by considering factors such as compound potency, relative bioavailability, patient body weight, presence and severity of adverse side effects, preferred mode of administration and the toxicity profile of the selected agent.

[0174] "Anti-cancer agent" is used in accordance with its plain ordinary meaning and refers to a composition (e.g. compound, drug, antagonist, inhibitor, modulator) having antineoplastic properties or the ability to inhibit the growth or proliferation of cells. In embodiments, an anti-cancer agent is a chemotherapeutic. In embodiments, an anti-cancer agent is an agent identified herein having utility in methods of treating cancer. In embodiments, an anti-cancer agent is an agent approved by the FDA or similar regulatory agency of a country other than the USA, for treating cancer.

KITS

[0175] In another aspect, a kit including the cell penetrating conjugate provided herein including embodiments thereof or the pharmaceutical composition as provided herein including embodiments thereof and instructions for use are provided. In embodiments, the first non-cell penetrating protein, the second non-cell penetrating protein, the first phosphorothioate nucleic acid and the second phosphorothioate nucleic acid are in a separate container. In embodiments, the kit includes one or more additional agents for treating or preventing one or more symptoms of a disease. In embodiments, the kit includes a means of administering the composition, such as, for example, a syringe, needle, tubing, catheter, patch, and the like. The kit may also include formulations and/or materials requiring sterilization and/or dilution prior to use.

EXAMPLES

[0176] The following examples describe the generation of cell-penetrating bispecific antibody-to-antibody-conjugates by hybridization of phosphorothioated sense/antisense DNA oligonucleotides attached to antibodies for the purpose of targeting intracellular antigens and diagnostic detection/therapeutic intervention.

[0177] Example 1. Production of bispecific antibodies via hybridization of attached DNA oligos.

[0178] As described in more detail below, it is demonstrated herein that conjugating double-stranded phosphorothioated DNA oligonucleotides to antibodies enables antibodies to penetrate cells, recognize and bind to intracellular antigens. Furthermore, Applicants illustrate that this strategy enables creation of cell-penetrating bispecific antibodies, which can recognize and bind to two intended intracellular target proteins. As an example, Applicants showed that attaching phosphorothioated DNA oligos that are sense and antisense to anti-STAT3 and anti-NFkB(p65) antibodies, respectively, allow hybridization the chosen nucleic acid sequences. Once the hybridization procedure was completed, coupled antibodies were purified comparing to single IgG antibody by means of anticipated increased molecular weight (FIG. 1).

[0179] By choosing phosphorothioated oligo-sense and oligo-antisense attached to two different antibodies (e.g., anti-NFkB and anti-STAT3), the formation of anti-STAT3-anti-STAT3 or anti-NFkB-anti-NFkB can be excluded. The resulting population of bispecific antibody is pure bispecific. Excess oligo and/or antibody protein is excluded by gel filtration.

[0180] Notably, the hybridization of sense and antisense DNA oligo strands generates double-stranded (ds) phosphorothioated DNA oligos linking chosen antibodies. Phosphorothioation of DNA oligos serves as intracellular delivery entity.

[0181] Example 2. Recognition of intracellular antigens, STAT3 and NFkB(p65), by cell penetrating bi-specific antibodies.

[0182] Incubation of human lymphoma Ly3 cells with either single antibodies raised against STAT3 or NFkB(p65) modified with phosphorothioated single-stranded (ss)DNA oligo achieves recognition of intracellular targets (FIG. 2). Since NFkB(p65) IgG was modified with phosphorothioated sense ssDNA-oligo and STAT3 IgG was modified with phosphorothioated antisense ssDNA-oligo, Applicants conclude that the nucleic acid sequence is not critical for intracellular delivery which is the prerequisite for recognition of intracellular antigens.

[0183] However, incubating human lymphoma Ly3 cells with bispecific antiSTAT3-antiNFkB(p65) linked together by hybridized phosphorothioated dsDNA-oligos results in recognition of intracellular STAT3 protein and NFkB(p65) protein simultaneously.

[0184] All modified antibodies were purified by gel filtration and incubated on cells at 10 μ g/ml for 2 hrs at 37°C. Notably, 10 μ g generated bispecific antibodies bear only 50% of each single antibody at 10 μ g resulting in a reduction of target recognition as shown by the detection in Western blotting.

[0185] Flow cytometry studies further support cell penetration by modified intracellular antibodies along with images of fluorescently labeled modified antibodies (FIG. 3).

EMBODIMENTS

[0186] Embodiment 1. A cell penetrating conjugate comprising a phosphorothioate nucleic acid connecting a first non-cell penetrating protein to a second protein, wherein said phosphorothioate nucleic acid enhances intracellular delivery of said first non-cell penetrating protein and said second protein.

[0187] Embodiment 2. The cell penetrating conjugate of embodiment 1, wherein said second protein is a second non-cell penetrating protein.

[0188] Embodiment 3. The cell penetrating conjugate of embodiment 1, wherein said phosphorothioate nucleic acid is a single-stranded nucleic acid.

[0189] Embodiment 4. The cell penetrating conjugate of embodiment 1, wherein said phosphorothioate nucleic acid comprises a first phosphorothioate nucleic acid hybridized to a second phosphorothioate nucleic acid, said first phosphorothioate nucleic acid attached to said first non-cell penetrating protein and said second phosphorothioate nucleic acid attached to said second protein.

[0190] Embodiment 5. The cell penetrating conjugate of one of embodiments 1-4, wherein said phosphorothioate nucleic acid is covalently attached to said first non-cell penetrating protein and said second non-cell penetrating protein.

[0191] Embodiment 6. The cell penetrating conjugate of any one of embodiments 1 to 5, wherein said phosphorothioate nucleic acid is independently attached to a lysine, arginine, cysteine, or histidine of said first non-cell penetrating protein.

[0192] Embodiment 7. The cell penetrating conjugate of embodiment 6, wherein said phosphorothioate nucleic acid is attached to a cysteine of said first non-cell penetrating protein.

[0193] Embodiment 8. The cell penetrating conjugate of any one of embodiments 1 to 7, wherein said phosphorothioate nucleic acid is independently attached to a lysine, arginine, cysteine, or histidine of said second non-cell penetrating protein.

[0194] Embodiment 9. The cell penetrating conjugate of embodiment 8, wherein said phosphorothioate nucleic acid is attached to a cysteine of said second non-cell penetrating protein.

[0195] Embodiment 10. The cell penetrating conjugate of embodiment 1 or 4, wherein said phosphorothioate nucleic acid is attached to said first non-cell penetrating protein through a first linker and to said second protein through a second linker.

[0196] Embodiment 11. The cell penetrating conjugate of embodiment 10, wherein said first linker and said second linker are independently a non-covalent linker.

[0197] Embodiment 12. The cell-penetrating conjugate of any one of embodiments 1-11, wherein said phosphorothioate nucleic acid is about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more nucleic acid residues in length.

[0198] Embodiment 13. The cell-penetrating conjugate of embodiment 12, wherein said phosphorothioate nucleic acid is from about 10 to about 30 nucleic acid residues in length.

[0199] Embodiment 14. The cell-penetrating conjugate of embodiment 13, wherein said phosphorothioate nucleic acid is about 20 nucleic acid residues in length.

[0200] Embodiment 15. The cell penetrating conjugate of any one of embodiments 1 to 14, wherein said first non-cell penetrating protein and said second non-cell penetrating protein independently have a molecular weight of more than 25 kD.

[0201] Embodiment 16. The cell penetrating conjugate of any one of embodiments 1 to 15, wherein said first non-cell penetrating protein and said second non-cell penetrating protein independently have a molecular weight of about 25 kD to about 750 kD.

[0202] Embodiment 17. The cell penetrating conjugate of any one of embodiments 1 to 16, wherein said first non-cell penetrating protein and said second non-cell penetrating protein independently are an antibody.

[0203] Embodiment 18. The cell penetrating conjugate of embodiment 17, wherein said antibody is an IgG antibody.

[0204] Embodiment 19. The cell penetrating conjugate of embodiment 17, wherein said antibody is an IgA, IgM, IgD or IgE antibody.

[0205] Embodiment 20. The cell penetrating conjugate of embodiment 17, wherein said antibody is an scFv fragment.

[0206] Embodiment 21. The cell penetrating conjugate of any one of embodiments 17 to 20, wherein said antibody is a humanized antibody.

[0207] Embodiment 22. The cell penetrating conjugate of any one of embodiments 17 to 20, wherein said antibody is a therapeutic antibody.

[0208] Embodiment 23. The cell penetrating conjugate of any one of embodiments 1-22, wherein said first non-cell penetrating protein or said second non-cell penetrating protein bind an intracellular target.

[0209] Embodiment 24. The cell penetrating conjugate of embodiment 23, wherein said first non-cell penetrating protein and said second non-cell penetrating protein bind an intracellular target.

[0210] Embodiment 25. The cell penetrating conjugate of embodiment 24, wherein said first non-cell penetrating protein binds a different intracellular target relative to said second non-cell penetrating protein.

[0211] Embodiment 26. The cell penetrating conjugate of embodiment 24, wherein said first non-cell penetrating protein binds a different epitope on the intracellular target relative to said second non-cell penetrating protein.

[0212] Embodiment 27. The cell penetrating conjugate of any one of embodiments 23-26, wherein said intracellular target is a target of a disease selected from the group consisting of autoimmune disease, inflammatory disease, metabolic disorder, developmental disorder, cardiovascular disease, liver disease, intestinal disease, infectious disease, endocrine disease, neurological disorder, and cancer.

[0213] Embodiment 28. The cell penetrating conjugate of any one of embodiments 23-27, wherein said intracellular target is a signaling molecule or a transcription factor.

[0214] Embodiment 29. The cell penetrating conjugate of embodiment 28, wherein said signaling molecule is a phosphatase or a kinase.

[0215] Embodiment 30. The cell penetrating conjugate of embodiment 28, wherein said transcription factor is a nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B) protein.

[0216] Embodiment 31. The cell penetrating conjugate of embodiment 30, wherein said NF κ B protein is p65.

[0217] Embodiment 32. The cell penetrating conjugate of any one of embodiments 28-31, wherein said first non-cell penetrating protein binds a STAT3 protein and said second non-cell penetrating protein binds a NF κ B protein.

[0218] Embodiment 33. The cell penetrating conjugate of any one of embodiments 23-26, wherein said intracellular target is a cancer target.

[0219] Embodiment 34. The cell penetrating conjugate of any one of embodiments 23-26, wherein said intracellular target is selected from the group consisting of STAT3, NF κ B and Src.

[0220] Embodiment 35. The cell penetrating conjugate of embodiment 34, wherein said intracellular target is STAT3.

[0221] Embodiment 36. The cell penetrating conjugate of embodiment 35, wherein said intracellular target is p65.

[0222] Embodiment 37. The cell penetrating conjugate of any one of embodiments 1-36, wherein said first non-cell penetrating protein and said second non-cell penetrating protein further comprise a label, a small molecule or a functional nucleic acid attached to said protein.

[0223] Embodiment 38. The cell penetrating conjugate of any one of embodiments 1-37 bound to an intracellular target.

[0224] Embodiment 39. A method of forming a cell penetrating conjugate, said method comprising hybridizing a first phosphorothioate nucleic acid attached to a first non-cell penetrating protein to a second phosphorothioate nucleic acid attached to a second non-cell penetrating protein, thereby forming a cell penetrating conjugate.

[0225] Embodiment 40. The method of embodiment 39, further comprising prior to said hybridizing: (i) contacting said first non-cell penetrating protein with said first

phosphorothioate nucleic acid, thereby forming a first protein phosphorothioate nucleic acid complex; (ii) contacting said second non-cell penetrating protein with said second phosphorothioate nucleic acid, thereby forming a second protein- phosphorothioate nucleic acid complex; and (iii) contacting said first protein phosphorothioate nucleic acid complex and said second protein phosphorothioate nucleic acid complex.

[0226] Embodiment 41. A cell comprising the cell penetrating conjugate of any one of embodiments 1-38.

[0227] Embodiment 42. A pharmaceutical composition comprising the cell penetrating conjugate of any one of embodiments 1-38 and a pharmaceutically acceptable carrier.

[0228] Embodiment 43. A kit comprising the cell penetrating conjugate of any one of embodiments 1-38 or the pharmaceutical composition of embodiment 42 and instructions for use.

[0229] Embodiment 44. The kit of embodiment 43, wherein said first non-cell penetrating protein, said second non-cell penetrating protein, said first phosphorothioate nucleic acid and said second phosphorothioate nucleic acid are in a separate container.

[0230] Embodiment 45. A method of delivering a non-cell penetrating protein into a cell comprising contacting the cell with said cell penetrating conjugate of any one of embodiments 1-38.

[0231] Embodiment 46. The method of embodiment 45, wherein said first non-cell penetrating protein binds a nuclear protein in the cytoplasm, thereby forming a first non-cell penetrating protein-nuclear protein complex.

[0232] Embodiment 47. The method of embodiment 46, wherein said second non-cell penetrating protein binds a nuclear protein in the cytoplasm, thereby forming a second non-cell penetrating protein-nuclear protein complex.

[0233] Embodiment 48. The method of embodiment 47, wherein said first non-cell penetrating protein-nuclear protein complex and said second non-cell penetrating protein-nuclear protein complex are not capable of entering the nucleus of the cell.

[0234] Embodiment 49. A method of treating a disease in a subject in need thereof, said method comprising administering to a subject an effective amount of the cell penetrating conjugate of any one of embodiments 1-38, thereby treating the disease in said subject.

[0235] Embodiment 50. The method of embodiment 49, wherein said disease is selected from the group consisting of autoimmune disease, developmental disorder, inflammatory disease, metabolic disorder, cardiovascular disease, liver disease, intestinal disease, infectious disease, endocrine disease, neurological disorder, and cancer.

[0236] Embodiment 51. The method of embodiment 50, wherein the disease is cancer.

[0237] Embodiment 52. The method of embodiment 49, wherein said first non-cell penetrating protein or said second non-cell penetrating protein bind an intracellular target.

[0238] Embodiment 53. The method of embodiment 52, wherein said first non-cell penetrating protein and said second non-cell penetrating protein bind an intracellular target.

[0239] Embodiment 54. The method of embodiment 53, wherein said first non-cell penetrating protein binds a different intracellular target relative to said second non-cell penetrating protein.

[0240] Embodiment 55. The method of embodiment 53, wherein said first non-cell penetrating protein binds a different epitope on the intracellular target relative to said second non-cell penetrating protein.

[0241] Embodiment 56. The method of any one of embodiments 52-55, wherein said intracellular target is STAT3, a NFκB protein or Src.

[0242] Embodiment 57. The method of embodiment 56, wherein said intracellular target is p65.

[0243] Embodiment 58. The method of embodiment 57, wherein said the intracellular target is STAT3.

[0244] Embodiment 59. The method of embodiment 52, wherein said first non-cell penetrating protein binds STAT3 and said second non-cell penetrating protein binds a NFκB protein.

WHAT IS CLAIMED IS:

1. A cell penetrating conjugate comprising a phosphorothioate nucleic acid connecting a first non-cell penetrating protein to a second protein, wherein said phosphorothioate nucleic acid enhances intracellular delivery of said first non-cell penetrating protein and said second protein.
2. The cell penetrating conjugate of claim 1, wherein said second protein is a second non-cell penetrating protein.
3. The cell penetrating conjugate of claim 1, wherein said phosphorothioate nucleic acid is a single-stranded nucleic acid.
4. The cell penetrating conjugate of claim 1, wherein said phosphorothioate nucleic acid comprises a first phosphorothioate nucleic acid hybridized to a second phosphorothioate nucleic acid, said first phosphorothioate nucleic acid attached to said first non-cell penetrating protein and said second phosphorothioate nucleic acid attached to said second protein.
5. The cell penetrating conjugate of claim 1, wherein said phosphorothioate nucleic acid is covalently attached to said first non-cell penetrating protein and said second non-cell penetrating protein.
6. The cell penetrating conjugate of claim 1, wherein said phosphorothioate nucleic acid is independently attached to a lysine, arginine, cysteine, or histidine of said first non-cell penetrating protein.
7. The cell penetrating conjugate of claim 6, wherein said phosphorothioate nucleic acid is attached to a cysteine of said first non-cell penetrating protein.
8. The cell penetrating conjugate of claim 7, wherein said phosphorothioate nucleic acid is independently attached to a lysine, arginine, cysteine, or histidine of said second non-cell penetrating protein.

9. The cell penetrating conjugate of claim 8, wherein said phosphorothioate nucleic acid is attached to a cysteine of said second non-cell penetrating protein.

10. The cell penetrating conjugate of claim 1, wherein said phosphorothioate nucleic acid is attached to said first non-cell penetrating protein through a first linker and to said second protein through a second linker.

11. The cell penetrating conjugate of claim 10, wherein said first linker and said second linker are independently a non-covalent linker.

12. The cell-penetrating conjugate of claim 1, wherein said phosphorothioate nucleic acid is about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more nucleic acid residues in length.

13. The cell-penetrating conjugate of claim 12, wherein said phosphorothioate nucleic acid is from about 10 to about 30 nucleic acid residues in length.

14. The cell-penetrating conjugate of claim 13, wherein said phosphorothioate nucleic acid is about 20 nucleic acid residues in length.

15. The cell penetrating conjugate of claim 1, wherein said first non-cell penetrating protein and said second non-cell penetrating protein independently have a molecular weight of more than 25 kD.

16. The cell penetrating conjugate of claim 1, wherein said first non-cell penetrating protein and said second non-cell penetrating protein independently have a molecular weight of about 25 kD to about 750 kD.

17. The cell penetrating conjugate of claim 1, wherein said first non-cell penetrating protein and said second non-cell penetrating protein independently are an antibody.

18. The cell penetrating conjugate of claim 17, wherein said antibody is an IgG antibody.

19. The cell penetrating conjugate of claim 17, wherein said antibody is an IgA, IgM, IgD or IgE antibody.

20. The cell penetrating conjugate of claim 17, wherein said antibody is an scFv fragment.

21. The cell penetrating conjugate of claim 17, wherein said antibody is a humanized antibody.

22. The cell penetrating conjugate of claim 17, wherein said antibody is a therapeutic antibody.

23. The cell penetrating conjugate of claim 1, wherein said first non-cell penetrating protein or said second non-cell penetrating protein bind an intracellular target.

24. The cell penetrating conjugate of claim 23, wherein said first non-cell penetrating protein and said second non-cell penetrating protein bind an intracellular target.

25. The cell penetrating conjugate of claim 24, wherein said first non-cell penetrating protein binds a different intracellular target relative to said second non-cell penetrating protein.

26. The cell penetrating conjugate of claim 24, wherein said first non-cell penetrating protein binds a different epitope on the intracellular target relative to said second non-cell penetrating protein.

27. The cell penetrating conjugate of claim 23, wherein said intracellular target is a target of a disease selected from the group consisting of autoimmune disease, inflammatory disease, metabolic disorder, developmental disorder, cardiovascular disease, liver disease, intestinal disease, infectious disease, endocrine disease, neurological disorder, and cancer.

28. The cell penetrating conjugate of claim 23, wherein said intracellular target is a signaling molecule or a transcription factor.

29. The cell penetrating conjugate of claim 28, wherein said signaling molecule is a phosphatase or a kinase.

30. The cell penetrating conjugate of claim 28, wherein said transcription factor is a nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) protein.

31. The cell penetrating conjugate of claim 30, wherein said NFκB protein is p65.

32. The cell penetrating conjugate of claim 28, wherein said first non-cell penetrating protein binds a STAT3 protein and said second non-cell penetrating protein binds a NFκB protein.

33. The cell penetrating conjugate of claim 23, wherein said intracellular target is a cancer target.

34. The cell penetrating conjugate of claim 23, wherein said intracellular target is selected from the group consisting of STAT3, NFκB and Src.

35. The cell penetrating conjugate of claim 34, wherein said intracellular target is STAT3.

36. The cell penetrating conjugate of claim 35, wherein said intracellular target is p65.

37. The cell penetrating conjugate of claim 1, wherein said first non-cell penetrating protein and said second non-cell penetrating protein further comprise a label, a small molecule or a functional nucleic acid attached to said protein.

38. The cell penetrating conjugate of claim 1 bound to an intracellular target.

39. A method of forming a cell penetrating conjugate, said method comprising hybridizing a first phosphorothioate nucleic acid attached to a first non-cell penetrating protein to a second phosphorothioate nucleic acid attached to a second non-cell penetrating protein, thereby forming a cell penetrating conjugate.

40. The method of claim 39, further comprising prior to said hybridizing:

(i) contacting said first non-cell penetrating protein with said first phosphorothioate nucleic acid, thereby forming a first protein phosphorothioate nucleic acid complex;

(ii) contacting said second non-cell penetrating protein with said second phosphorothioate nucleic acid, thereby forming a second protein- phosphorothioate nucleic acid complex; and

(iii) contacting said first protein phosphorothioate nucleic acid complex and said second protein phosphorothioate nucleic acid complex.

41. A cell comprising the cell penetrating conjugate of claim 1.

42. A pharmaceutical composition comprising the cell penetrating conjugate of claim 1 and a pharmaceutically acceptable carrier.

43. A kit comprising the cell penetrating conjugate of claim 1 or the pharmaceutical composition of claim 42 and instructions for use.

44. The kit of claim 43, wherein said first non-cell penetrating protein, said second non-cell penetrating protein, said first phosphorothioate nucleic acid and said second phosphorothioate nucleic acid are in a separate container.

45. A method of delivering a non-cell penetrating protein into a cell comprising contacting the cell with said cell penetrating conjugate of claim 1.

46. The method of claim 45, wherein said first non-cell penetrating protein binds a nuclear protein in the cytoplasm, thereby forming a first non-cell penetrating protein-nuclear protein complex.

47. The method of claim 46, wherein said second non-cell penetrating protein binds a nuclear protein in the cytoplasm, thereby forming a second non-cell penetrating protein-nuclear protein complex.

48. The method of claim 47, wherein said first non-cell penetrating protein-nuclear protein complex and said second non-cell penetrating protein-nuclear protein complex are not capable of entering the nucleus of the cell.

49. A method of treating a disease in a subject in need thereof, said method comprising administering to a subject an effective amount of the cell penetrating conjugate of claim 1, thereby treating the disease in said subject.

50. The method of claim 49, wherein said disease is selected from the group consisting of autoimmune disease, developmental disorder, inflammatory disease, metabolic disorder, cardiovascular disease, liver disease, intestinal disease, infectious disease, endocrine disease, neurological disorder, and cancer.

51. The method of claim 50, wherein the disease is cancer.

52. The method of claim 49, wherein said first non-cell penetrating protein or said second non-cell penetrating protein bind an intracellular target.

53. The method of claim 52, wherein said first non-cell penetrating protein and said second non-cell penetrating protein bind an intracellular target.

54. The method of claim 53, wherein said first non-cell penetrating protein binds a different intracellular target relative to said second non-cell penetrating protein.

55. The method of claim 53, wherein said first non-cell penetrating protein binds a different epitope on the intracellular target relative to said second non-cell penetrating protein.

56. The method of claim 52, wherein said intracellular target is STAT3, a NFkB protein or Src.

57. The method of claim 56, wherein said intracellular target is p65.

58. The method of claim 57, wherein said the intracellular target is STAT3.

59. The method of claim 52, wherein said first non-cell penetrating protein binds STAT3 and said second non-cell penetrating protein binds a NFkB protein.

FIG. 1

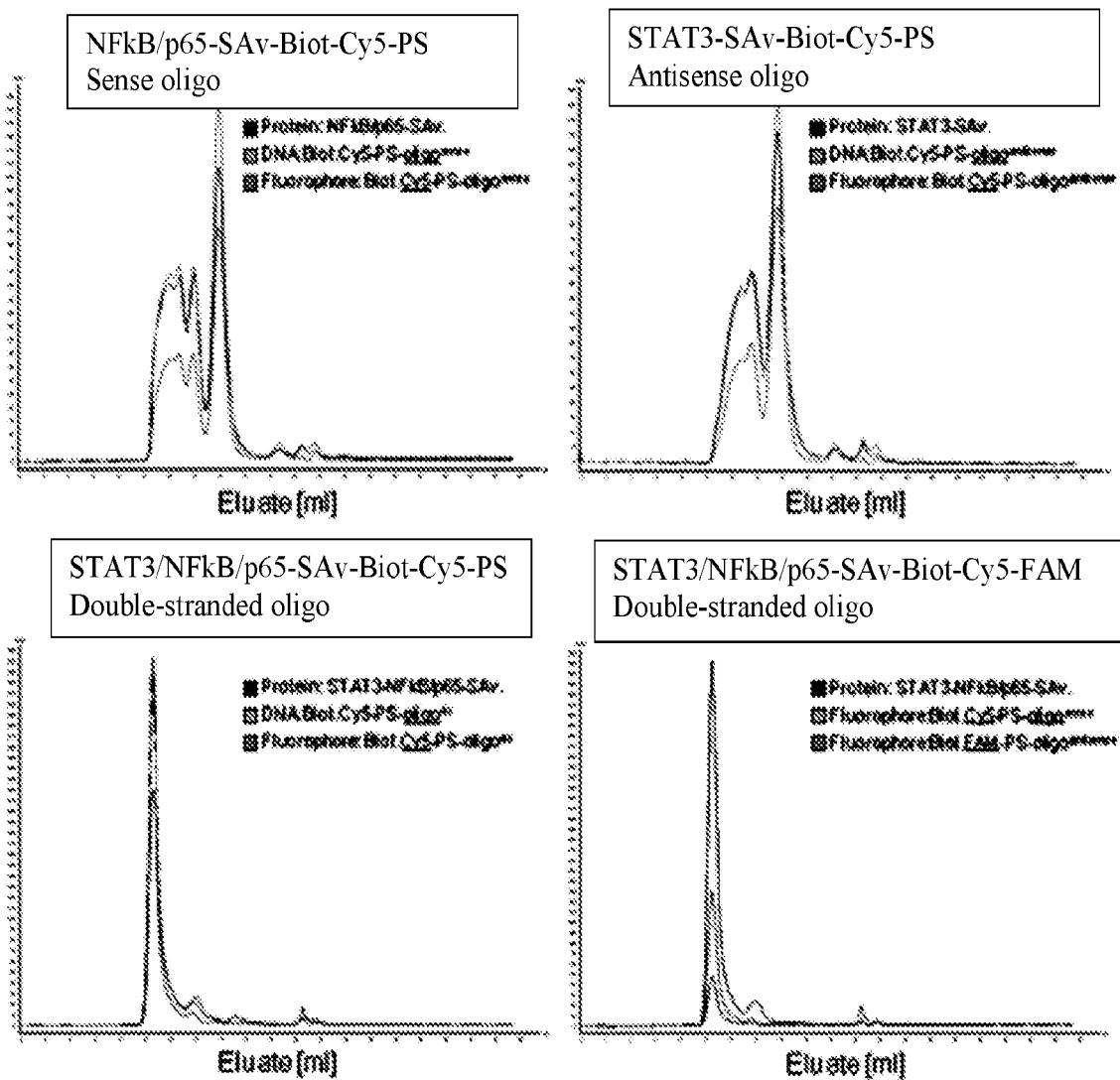


FIG. 2

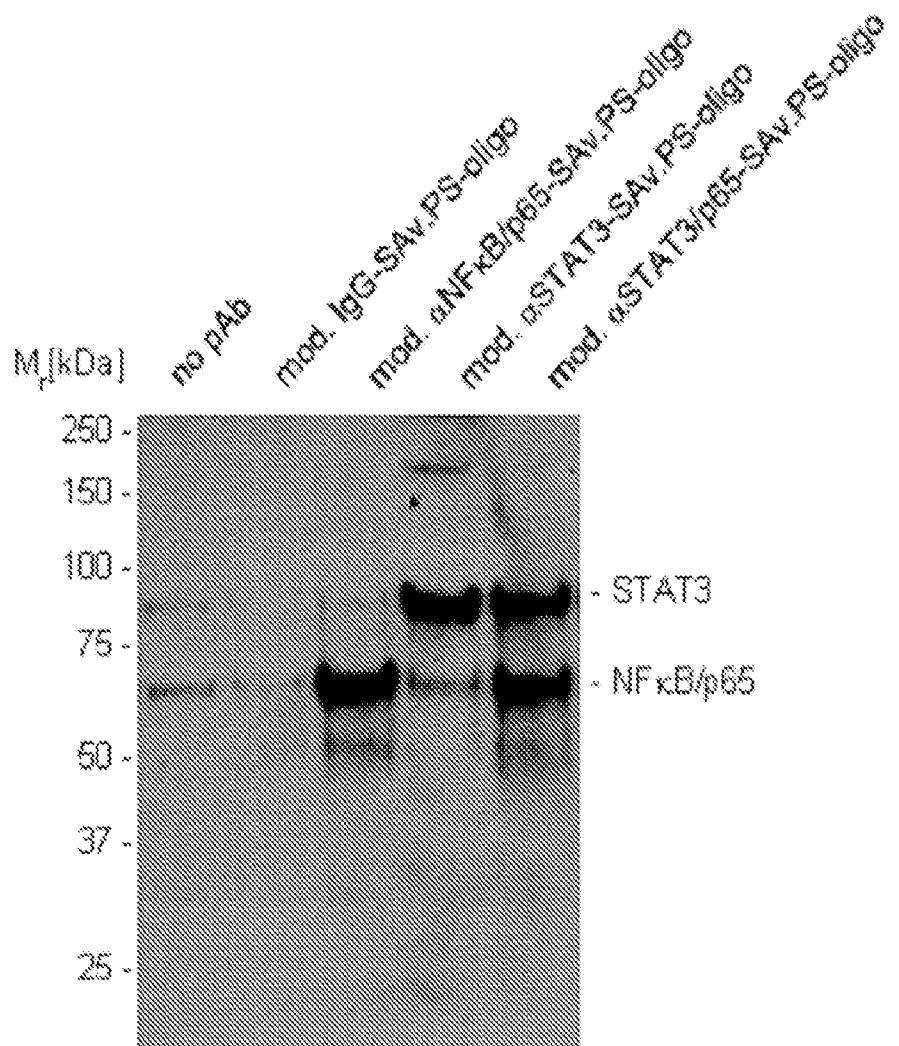


FIG. 3

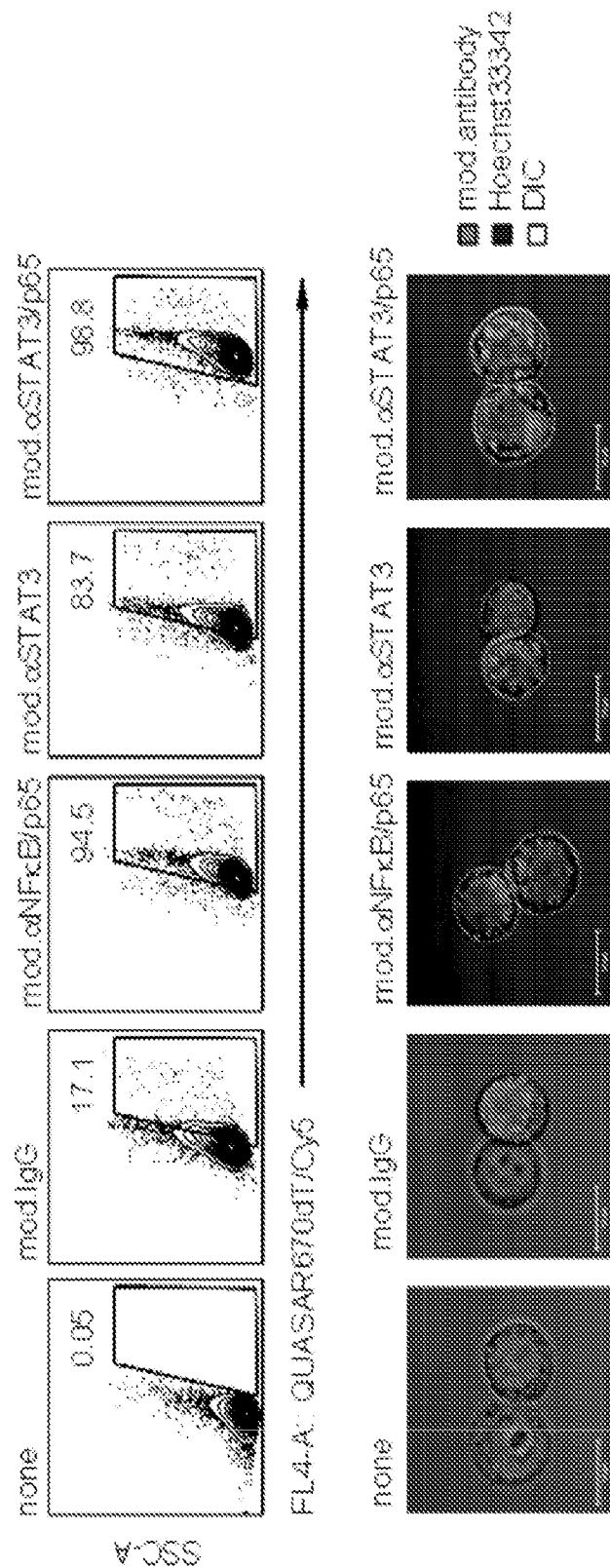


FIG. 4A

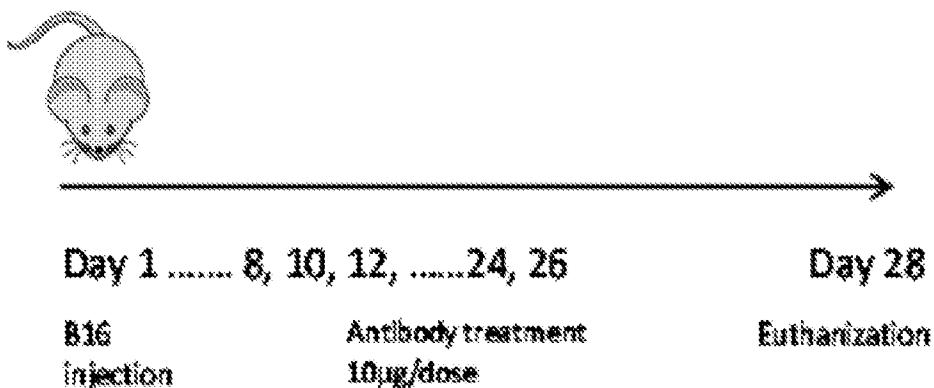
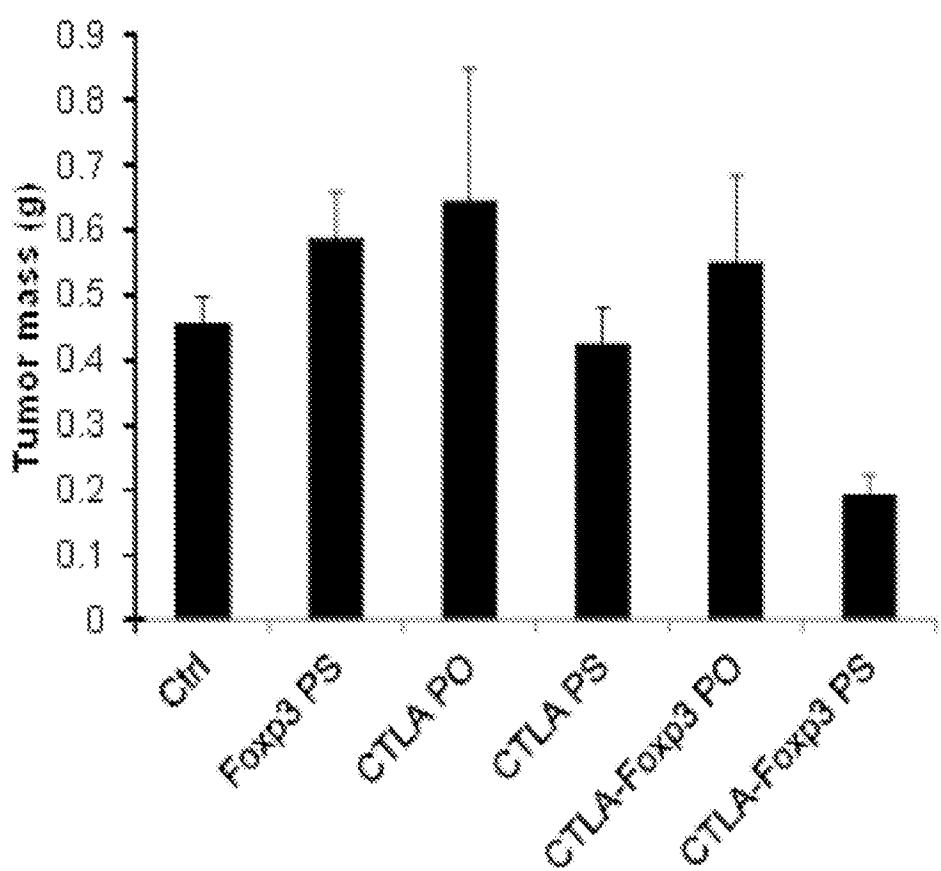


FIG. 4B



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/045817

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/00; A61K 38/14; A61K 39/00; A61K 47/00; A61K 47/48 (2016.01)

CPC - A61K 38/00; A61K 38/14; A61K 39/00; A61K 47/00; A61K 47/48 (2016.08)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC - A61K 38/00; A61K 38/14; A61K 39/00; A61K 47/00; A61K 47/48

CPC - A61K 38/00; A61K 38/14; A61K 39/00; A61K 47/00; A61K 47/48

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

USPC - 514/1; 514/1.1; 514/20.9; 530/300; 530/322 (keyword delimited)

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

Orbit, Google Patents, PubMed, Google

Search terms used: cell penetrating conjugate phosphorothioate nucleic acid connect second non-cell penetrating protein enhance intracellular delivery antibody treat cancer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2015/031837 A1 (CITY OF HOPE et al) 05 March 2015 (05.03.2015) entire document	1-59
A	US 2013/0137644 A1 (CELLECTIS) 30 May 2013 (30.05.2013) entire document	1-59
A	US 2014/0371302 A1 (MODEMA THERAPEUTICS, INC.) 18 December 2014 (18.12.2014) entire document	1-59
A	US 2011/0027300 A1 (KAMIL et al) 03 February 2011 (03.02.2011) entire document	1-59
P, X	WO 2016/115500 A1 (CITY OF HOPE) 21 July 2016 (21.07.2016) entire document	1-59

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:

“A” document defining the general state of the art which is not considered to be of particular relevance

“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

“E” earlier application or patent but published on or after the international filing date

“X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

“L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

“Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

“O” document referring to an oral disclosure, use, exhibition or other means

“&” document member of the same patent family

“P” document published prior to the international filing date but later than the priority date claimed

Date of the actual completion of the international search

02 October 2016

Date of mailing of the international search report

25 OCT 2016

Name and mailing address of the ISA/
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450
Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774