OLIGONUCLEOTIDE COMPOSITIONS AND METHODS THEREOF

Cross-Reference to Related Applications

[0001] This application claims priority to United States Provisional Application Nos. 62/195,779, filed July 22, 2015, 62/236,847, filed October 2, 2015, and 62/331,960, filed May 4, 2016, the entirety of each of which is incorporated herein by reference.

Background

[0002] Oligonucleotides are useful in therapeutic, diagnostic, research and nanomaterials applications. The use of naturally occurring nucleic acids (*e.g.,* unmodified DNA or RNA) for therapeutics can be limited, for example, because of their instability against extra- and intracellular nucleases and/or their poor cell penetration and distribution. There is a need for new and improved oligonucleotides and oligonucleotide compositions, such as, *e.g.,* new antisense and siRNA oligonucleotides and oligonucleotide compositions.

Summary

[0003] Among other things, the present disclosure encompasses the recognition that structural elements of oligonucleotides, such as base sequence, chemical modifications (*e.g.,* modifications of sugar, base, and/or internucleotidic linkages, and patterns thereof), and/or stereochemistry (*e.g.,* stereochemistry of backbone chiral centers (chiral internucleotidic linkages), and/or patterns thereof), can have significant impact on properties, *e.g.,* activities, of oligonucleotides. In some examples, the present disclosure demonstrates that oligonucleotide compositions comprising oligonucleotides with controlled structural elements, *e.g.,* controlled chemical modification and/or controlled backbone stereochemistry patterns, provide unexpected properties, including but not limited to those described herein. In some examples, the present disclosure demonstrates that combinations of chemical modifications and stereochemistry can provide unexpected, greatly improved properties (*e.g.,* bioactivity, selectivity, *etc.*). In some examples, the present disclosure provides an oligonucleotide composition having a particular sequence of bases, and/or pattern of sugar modifications (e.g., 2'-OMe, 2'-F, 2'-MOE, etc.), and/or pattern or base modifications (e.g., 5-methylcytosine), and/or pattern of backbone modifications (phosphate or phosphorothioate), and/or pattern of

stereochemistry of backbone modifications (e.g., each phosphorothioate is *S*p or *R*p).

[0003a] In one aspect, the present disclosure refers to an oligonucleotide having the structure of:

mG * SmGmCmAmC * SA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SmAmCmUmU * SmC (SEQ ID NO: 360), or a pharmaceutically acceptable salt thereof, wherein:

*S represents a *S*p phosphorothioate;

*R represents a *R*p phosphorothioate; and

m represents a 2'-OMe modification to a nucleoside.

[0003b] In another aspect, the present disclosure refers to an oligonucleotide, wherein the oligonucleotide is a sodium salt of:

mG * SmGmCmAmC * SA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SmAmCmUmU * SmC (SEQ ID NO: 360), wherein:

*S represents a *S*p phosphorothioate;

*R represents a *R*p phosphorothioate; and

m represents a 2'-OMe modification to a nucleoside.

[0003c] In a further aspect, the present disclosure refers to a chirally controlled composition of an oligonucleotide having the structure of:

mG * SmGmCmAmC * SA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SmAmCmUmU * SmC (SEQ ID NO: 360), or a pharmaceutically acceptable salt thereof, wherein:

*S represents a *S*p phosphorothioate;

*R represents a *R*p phosphorothioate; and

m represents a 2'-OMe modification to a nucleoside.

[0003d] In another aspect, the present disclosure refers to a pharmaceutical composition, comprising a therapeutically effective amount of an oligonucleotide and at least one pharmaceutically acceptable inactive ingredient selected from pharmaceutically acceptable diluents, pharmaceutically acceptable excipients, and pharmaceutically acceptable carriers, wherein the oligonucleotide has the structure of:

mG * SmGmCmAmC * SA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SmAmCmUmU * SmC (SEQ ID NO: 360), or a pharmaceutically acceptable salt thereof,

wherein:

*S represents a *S*p phosphorothioate;

*R represents a *R*p phosphorothioate; and

m represents a 2'-OMe modification to a nucleoside.

[0004] In some examples, modifications of internucleotidic linkages can convert phosphorus atoms in modified linkages into chiral centers. For example, in a phosphorothioate (PS) modification, one of the non-bridging oxygen (O) atoms bonded to a phosphorus (P) atom is replaced with a sulfur (S) atom. A consequence of using PS modification in oligonucleotide synthesis is that it creates a chiral center at phosphorus, which can have either an "Sp" or "Rp" configuration. For instance, a conventional stereorandom PS-modified oligonucleotide composition having 19 PS linkages [e.g., having 20 nucleotides in length, 19 PS modifications, each with two possible stereochemistries (*S*p or *R*p) at each PS modification] is a random mixture of over 500,000 (2^{19}) stereoisomers, each having the same nucleotide sequence (e.g., sequence of bases) but differing in the stereochemistry along their backbones; such a composition is a "stereorandom" oligonucleotide composition. In some examples, in contrast to stereorandom compositions, a chirally controlled oligonucleotide composition is a substantially pure preparation of a single oligonucleotide in that a predetermined level of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some examples, some oligonucleotide compositions are stereopure (i.e., a chirally controlled oligonucleotide composition), wherein the stereochemistry at each PS is defined (*S*p or *R*p). In some examples, in a stereorandom compositions of oligonucleotides, the various oligonucleotides can have the same base sequence, same pattern of sugar modifications (e.g., 2'-OMe, 2'-F, 2'-OME, etc.), same pattern of base modifications (e.g., 5-methylcytosine), and same pattern of backbone modifications (phosphate or PS), but different patterns of backbone chiral centers, and their levels are random from non-stereocontrolled synthesis (not pre-determined as through stereocontrolled synthesis as certain methods exemplified herein using chiral auxilier). A chirally controlled oligonucleotide composition can be selected to have greater desired biological activity (e.g., greater activities, efficiency in RNA interference or RNAse H-mediated pathways, *etc.*) and decreased undesired activity (e.g., undesired immunogenicity, toxicity, *etc.*) than a stereorandom preparation of oligonucleotides of the same

base sequence. In some examples, a chirally controlled oligonucleotide composition is better able to differentiate between a mutant (mu) and a wild-type (wt) HTT sequence (with a single nt difference).

[0005] Among other things, the present disclosure encompasses the recognition that

stereorandom oligonucleotide preparations contain a plurality of distinct chemical entities that differ from one another, *e.g.,* in the stereochemical structure of individual backbone chiral centers within the oligonucleotide chain. Without control of stereochemistry of backbone chiral centers, stereorandom oligonucleotide preparations provide uncontrolled compositions comprising undetermined levels of oligonucleotide stereoisomers. Even though these stereoisomers may have the same base sequence, they are different chemical entities at least due to their different backbone stereochemistry, and they can have, as demonstrated herein, different properties, *e.g.,* bioactivities. Among other things, the present disclosure provides new compositions that are or contain particular stereoisomers of oligonucleotides of interest. In some examples, a particular stereoisomer may be defined, for example, by its base sequence, its length, its pattern of backbone linkages, and its pattern of backbone chiral centers. As is understood in the art, in some examples, base sequence may refer to the identity and/or modification status of nucleoside residues (*e.g.,* of sugar and/or base components, relative to standard naturally occurring nucleotides such as adenine, cytosine, guanosine, thymine, and uracil) in an oligonucleotide and/or to the hybridization character (i.e., the ability to hybridize with particular complementary residues) of such residues.

[0006] The present disclosure demonstrates, among other things, that individual stereoisomers of a particular oligonucleotide can show different stability and/or activity (*e.g.,* functional and/or toxicity properties) from each other. Moreover, the present disclosure demonstrates that stability and/or activity improvements achieved through inclusion and/or location of particular chiral structures within an oligonucleotide can be comparable to, or even better than those achieved through use of particular backbone linkages, residue modifications, *etc*. (*e.g.,* through use of certain types of modified phosphates [*e.g.,* phosphorothioate, substituted phosphorothioate, *etc*.], sugar modifications [*e.g.,* 2'- modifications, *etc*.], and/or base modifications [*e.g.,* methylation, *etc*.]).

[0007] Among other things, the present disclosure recognizes that, in some examples, properties (*e.g.,* stability and/or activities) of an oligonucleotide can be adjusted by optimizing its pattern of backbone chiral centers, optionally in combination with adjustment/optimization of one or more other features (*e.g.,* linkage pattern, nucleoside modification pattern, *etc*.) of the oligonucleotide.

[0008] In some examples, the present disclosure provides compositions of

oligonucleotides, wherein the oligonucleotides have a common pattern of backbone chiral centers which, unexpectedly, greatly enhances the stability and/or biological activity of the oligonucleotides. In some examples, a pattern of backbone chiral centers provides increased stability. In some examples, a pattern of backbone chiral centers provides surprisingly increased activity. In some examples, a pattern of backbone chiral centers provides increased stability and activity. In some examples, when an oligonucleotide is utilized to cleave a nucleic acid polymer, a pattern of backbone chiral centers, surprisingly by itself, changes the cleavage pattern of a target nucleic acid polymer. In some examples, a pattern of backbone chiral centers effectively prevents cleavage at secondary sites. In some examples, a pattern of backbone chiral centers creates new cleavage sites. In some examples, a pattern of backbone chiral centers minimizes the number of cleavage sites. In some examples, a pattern of backbone chiral centers minimizes the number of cleavage sites so that a target nucleic acid polymer is cleaved at only one site within the sequence of the target nucleic acid polymer that is complementary to the oligonucleotide. In some examples, a pattern of backbone chiral centers enhances cleavage efficiency at a cleavage site. In some examples, a pattern of backbone chiral centers of the oligonucleotide improves cleavage of a target nucleic acid polymer. In some examples, a pattern of backbone chiral centers increases selectivity. In some examples, a pattern of backbone chiral centers minimizes off-target effect. In some examples, a pattern of backbone chiral centers increase selectivity, *e.g.,* cleavage selectivity between two target sequences differing only by a single nucleotide polymorphism (SNP). In some examples, a pattern of backbone chiral centers comprises, comprises one or more repeats of, or is $(Sp)_m(Rp)_n$, $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$. In some examples described herein, m is 1-50; and n is 1-10; and t is 1-50. In some examples, a pattern of backbone chiral centers comprises or is (*S*p)m(*R*p)n, (*R*p)n(*S*p)m, (*N*p)t(*R*p)n(*S*p)m, or (*S*p)t(*R*p)n(*S*p)m. In some examples, a pattern of backbone chiral centers comprises or is $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$, wherein m > 2. In some examples, a pattern of backbone chiral centers is a sequence comprising at least 5, 6, 7, 8, 9, or 10 or more consecutive (*S*p) positions. In some examples, a pattern of backbone chiral centers is a sequence comprising at least 5 consecutive (*S*p) positions. In some examples, a pattern of backbone chiral centers is a sequence comprising at least 8 consecutive (*S*p) positions. In some examples, a pattern of backbone chiral centers is a

sequence comprising at least 10 consecutive (*S*p) positions. In some examples, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p). In some examples, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p) at or adjacent to the position of a SNP. In some examples, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p), wherein the molecule has a wing-core-wing format. In some examples, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p), wherein the molecule has a wing-core-wing format, wherein the wing on the 5' end is 1-9 nt long, the core is 1-15 nt long, and the wing on the 3' end is 1-9 nt long. In some examples, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p), wherein the molecule has a wing-core-wing format, wherein the wing on the 5' end is 5 nt long, the core is 1-15 nt long, and the wing on the 3' end is 5 nt long. In some examples, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p), wherein the molecule has a wing-core-wing format, wherein the wing on the 5' end is 1-9 nt long, the core is 10 nt long, and the wing on the 3' end is 1-9 nt long. In some examples, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p), wherein the molecule has a wing-core-wing format, wherein the wing on the 5' end is 5 nt long, the core is 10 nt long, and the wing on the 3' end is 5 nt long. In some examples, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p), wherein the molecule has a wing-core-wing format, wherein the wing on the 5' end is 5 nt long, the core is 10 nt long, and the wing on the 3' end is 5 nt long, and at least one wing comprises a nucleotide with a 2'-OMe modification. In some examples, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p), wherein the molecule has a wing-core-wing format, wherein each wing comprises at least one nucleotide with a 2'-OMe modification. In some examples, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p), wherein the molecule has a wing-core-wing format, wherein each nucleotide in both wings has a 2'-OMe modification. In some examples, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p), wherein the molecule has a wing-core-wing format, wherein the wing on the 5' end is 5 nt long, the core is 10 nt long, and the wing on the 3' end is 5 nt long, and each nucleotide in each wing has a 2'-OMe modification. In some examples, the oligonucleotide is single-stranded and has a wing-core-wing format, wherein the wing on the 5' end of the molecule

comprises 4 to 8 nt, each of which has a 2'-OMe modification and wherein the nt at the 5' end of the molecule has a phosphorothioate in the Sp conformation; the core comprises 8 to 12 nt, each of which is DNA (2'-H), wherein each has a phosphorothioate in the Sp position except one nt which has the phosphorothioate in the Rp position; and wherein the wing on the 3' end of the molecule comprises 4 to 8 nt, each of which has a 2'-OMe modification, and wherein the nt at the 3' end of the molecule comprises a phosphorothioate in the Sp conformation. In some examples, the oligonucleotide is single-stranded and has a wing-core-wing format, wherein the wing on the 5' end of the molecule comprises 6 nt, each of which has a 2'-OMe modification and wherein the nt at the 5' end of the molecule has a phosphorothioate in the Sp conformation; the core comprises 10 nt, each of which is DNA (2'-H), wherein each has a phosphorothioate in the Sp position except one nt which has the phosphorothioate in the Rp position; and wherein the wing on the 3' end of the molecule comprises 6 nt, each of which has a 2'-OMe modification, and wherein the nt at the 3' end of the molecule comprises a phosphorothioate in the Sp conformation.

[0009] In some examples, the present disclosure recognizes that chemical modifications, such as modifications of nucleosides and internucleotidic linkages, can provide enhanced properties. In some examples, the present disclosure demonstrates that combinations of chemical modifications and stereochemistry can provide unexpected, greatly improved properties (*e.g.,* bioactivity, selectivity, *etc.*). In some examples, chemical combinations, such as modifications of sugars, bases, and/or internucleotidic linkages, are combined with stereochemistry patterns, *e.g.,* $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$, to provide oligonucleotides and compositions thereof with surprisingly enhanced properties. In some examples, a provided oligonucleotide composition is chirally controlled, and comprises a combination of 2'-modification of one or more sugar moieties, one or more natural phosphate linkages, one or more phosphorothioate linkages, and a stereochemistry pattern of $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$, wherein $m > 2$.

[0010] In some examples, the present disclosure provides a chirally controlled oligonucleotide composition comprising oligonucleotides defined by having:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers, which composition is a substantially pure preparation of a single oligonucleotide in that a predetermined level of the oligonucleotides

in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers.

[0011] In some examples, the present disclosure provides a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type.

[0012] In some examples, the present disclosure provides a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers, which composition is a substantially pure preparation of a single oligonucleotide in that at least about 10% of the oligonucleotides in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers.

[0013] Among other things, the present disclosure recognizes that combinations of oligonucleotide structural elements (*e.g.*, patterns of chemical modifications, backbone linkages, backbone chiral centers, and/or backbone phosphorus modifications) can provide surprisingly improved properties such as bioactivities. In some examples, the present disclosure provides an oligonucleotide composition comprising a predetermined level of oligonucleotides which comprise one or more wing regions and a common core region, wherein:

each wing region independently has a length of two or more bases, and independently and optionally comprises one or more chiral internucleotidic linkages;

the core region independently has a length of two or more bases, and independently comprises one or more chiral internucleotidic linkages, and the common core region has:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers.

[0014] In some examples, in an oligonucleotide comprising a wing-core-wing format, a "wing" is a portion of the oligonucleotide on the 5' or 3' end of the core, with the "core" (alternatively designated a "gap") between the two wings. In some examples, an oligonucleotide can have a single wing and a single core; in such cases, the wing is on the 5' or the 3' end of the oligonucleotide. A wing and core can be defined by any of several structural elements (e.g., modifications or patterns of modifications of sugar, base, backbone or backbone stereochemistry, *etc.*). In some examples, a wing and core is defined by nucleoside modifications, wherein a wing comprises a nucleoside modification that the core region does not have. In some examples, oligonucleotides in provided compositions have a wing-core structure of nucleoside modification. In some examples, oligonucleotides in provided compositions have a core-wing structure of nucleoside modification. In some examples, oligonucleotides in provided compositions have a wing-core-wing structure of nucleoside modification. In some examples, a wing and core is defined by modifications of the sugar moieties. In some examples, a wing and core is defined by modifications of the base moieties. In some examples, each sugar moiety in the wing region has the same 2'-modification which is not found in the core region. In some examples, each sugar moiety in the wing region has the same 2'-modification which is different than any sugar modifications in the core region. In some examples, each sugar moiety in the wing region has the same 2'-modification, and the core region has no 2'-modifications. In some examples, when two or more wings are present, each sugar moiety in a wing region has the same 2'-modification, yet the common 2'-modification in a first wing region can either be the same as or different from the common 2'-modification in a second wing region.

[0015] In some examples, each wing comprises at least one chiral internucleotidic linkage and at least one natural phosphate linkage. In some examples, each wing comprises at least one modified sugar moiety. In some examples, each wing sugar moiety is modified. In some examples, a wing sugar moiety is modified by a modification that is absent from the core region. In some examples, a wing region only has modified internucleotidic linkages at one or both of its ends. In some examples, a wing region only has a modified

internucleotidic linkage at its 5'-end. In some examples, a wing region only has a modified internucleotidic linkage at its 3'-end. In some examples, a wing region only has modified internucleotidic linkages at its 5'- and 3'-ends. In some examples, a wing is to the 5'-end of a core, and the wing only has a modified internucleotidic linkage at its 5'-end. In some examples, a wing is to the 5'-end of a core, and the wing only has a modified internucleotidic linkage at its 3'-end. In some examples, a wing is to the 5'-end of a core, and the wing only has modified internucleotidic linkages at both its 5'- and 3'-ends. In some examples, a wing is to the 3'-end of a core, and the wing only has a modified internucleotidic linkage at its 5'-end. In some examples, a wing is to the 3'-end of a core, and the wing only has a modified internucleotidic linkage at its 3'-end. In some examples, a wing is to the 3'-end of a core, and the wing only has modified internucleotidic linkages at both its 5'- and 3'-ends. In some examples, the modification(s) to the sugar moiety or internucleotidic linkage or other modifications in one wing can differ from those in another wing.

[0016] In some examples, each internucleotidic linkage within a core region is modified. In some examples, each internucleotidic linkage within a core region is chiral. In some examples, a core region has a pattern of backbone chiral centers of $(Sp)_m(Rp)_n$, $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$. In some examples, a core region has a pattern of backbone chiral centers of $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$, wherein $m > 2$. Among other things, the present disclosure demonstrates that, in some examples, such patterns can provide or enhance controlled cleavage of a target sequence, *e.g.,* an RNA sequence.

[0017] In some examples, oligonucleotides in provided compositions have a common pattern of backbone phosphorus modifications. In some examples, a provided composition is an oligonucleotide composition that is chirally controlled in that the composition contains a predetermined level of oligonucleotides of an individual oligonucleotide type, wherein an oligonucleotide type is defined by:

1) base sequence;

2) pattern of backbone linkages;

3) pattern of backbone chiral centers; and

4) pattern of backbone phosphorus modifications.

[0018] As noted above and understood in the art, in some examples, the base sequence of an oligonucleotide may refer to the identity and/or modification status of nucleoside residues (*e.g.,* of sugar and/or base components, relative to standard naturally occurring

nucleotides such as adenine, cytosine, guanosine, thymine, and uracil) in the oligonucleotide and/or to the hybridization character (i.e., the ability to hybridize with particular complementary residues) of such residues.

[0019] In some examples, a particular oligonucleotide type may be defined by

1A) base identity;

1B) pattern of base modification;

1C) pattern of sugar modification;

2) pattern of backbone linkages;

3) pattern of backbone chiral centers; and

4) pattern of backbone phosphorus modifications.

Thus, in some examples, oligonucleotides of a particular type may share identical bases but differ in their pattern of base modifications and/or sugar modifications. In some examples, oligonucleotides of a particular type may share identical bases and pattern of base modifications (including, *e.g.,* absence of base modification), but differ in pattern of sugar modifications.

[0020] In some examples, oligonucleotides of a particular type are chemically identical in that they have the same base sequence (including length), the same pattern of chemical modifications to sugar and base moieties, the same pattern of backbone linkages (*e.g.,* pattern of natural phosphate linkages, phosphorothioate linkages, phosphorothioate triester linkages, and combinations thereof), the same pattern of backbone chiral centers (*e.g.,* pattern of stereochemistry (*R*p/*S*p) of chiral internucleotidic linkages), and the same pattern of backbone phosphorus modifications (*e.g.,* pattern of modifications on the internucleotidic phosphorus atom, such as $-S^-$, and $-L-R^1$ of formula I).

[0021] In some examples, the sequence of the oligonucleotide comprises or consists of the sequence of any oligonucleotide disclosed herein. In some examples, the sequence of the oligonucleotide comprises or consists of the sequence of any oligonucleotide selected from Tables N1, N2, N3, N4 and 8. In some examples, the sequence of the oligonucleotide comprises or consists of the sequence of any oligonucleotide selected from Tables N1A, N2A, N3A, N4A and 8. In some examples, the sequence of the oligonucleotide in a stereopure (chirally controlled) oligonucleotide composition comprises or consists of the sequence of WV-1092, WVE120101, WV-2603 or WV-2595. In some examples, a sequence of an oligonucleotide includes any one or more of: base sequence (including length); pattern of

chemical modifications to sugar and base moieties; pattern of backbone linkages; pattern of natural phosphate linkages, phosphorothioate linkages, phosphorothioate triester linkages, and combinations thereof; pattern of backbone chiral centers; pattern of stereochemistry (*R*p/*S*p) of chiral internucleotidic linkages; pattern of backbone phosphorus modifications; pattern of modifications on the internucleotidic phosphorus atom, such as $-S^-$, and $-L-R^1$ of formula I.

[0022] Among other things, the present disclosure recognizes the challenge of stereoselective (rather than stereorandom or racemic) preparation of oligonucleotides. Among other things, the present disclosure provides methods and reagents for stereoselective preparation of oligonucleotides comprising multiple (*e.g.,* more than 5, 6, 7, 8, 9, or 10) internucleotidic linkages, and particularly for oligonucleotides comprising multiple (*e.g.,* more than 5, 6, 7, 8, 9, or 10) chiral internucleotidic linkages. In some examples, in a stereorandom or racemic preparation of oligonucleotides, at least one chiral internucleotidic linkage is formed with less than 90:10, 95:5, 96:4, 97:3, or 98:2 diastereoselectivity. In some examples, for a stereoselective or chirally controlled preparation of oligonucleotides, each chiral internucleotidic linkage is formed with greater than 90:10, 95:5, 96:4, 97:3, or 98:2 diastereoselectivity. In some examples, for a stereoselective or chirally controlled preparation of oligonucleotides, each chiral internucleotidic linkage is formed with greater than 95:5 diastereoselectivity. In some examples, for a stereoselective or chirally controlled preparation of oligonucleotides, each chiral internucleotidic linkage is formed with greater than 96:4 diastereoselectivity. In some examples, for a stereoselective or chirally controlled preparation of oligonucleotides, each chiral internucleotidic linkage is formed with greater than 97:3 diastereoselectivity. In some examples, for a stereoselective or chirally controlled preparation of oligonucleotides, each chiral internucleotidic linkage is formed with greater than 98:2 diastereoselectivity. In some examples, for a stereoselective or chirally controlled preparation of oligonucleotides, each chiral internucleotidic linkage is formed with greater than 99:1 diastereoselectivity. In some examples, diastereoselectivity of a chiral internucleotidic linkage in an oligonucleotide may be measured through a model reaction, *e.g.* formation of a dimer under essentially the same or comparable conditions wherein the dimer has the same internucleotidic linkage as the chiral internucleotidic linkage, the 5'-nucleoside of the dimer is the same as the nucleoside to the 5'-end of the chiral internucleotidic linkage, and the 3'-nucleoside of the dimer is the same as the nucleoside to the 3'-end of the chiral internucleotidic linkage.

[0023] Among other things, it is surprisingly found that certain provided oligonucleotide compositions achieve unprecedented control of cleavage of target sequences, *e.g.,* cleavage of target RNA by RNase H. In some examples, the present disclosure demonstrates that precise control of chemical and stereochemical attributes of oligonucleotides achieves improved activity of oligonucleotide preparations as compared with otherwise comparable preparations for which stereochemical attributes are not controlled. Among other things, the present disclosure specifically demonstrates improved rate, degree, and or specificity of cleavage of nucleic acid targets to which provided oligonucleotides hybridize.

[0024] In some examples, the present disclosure provides various uses of oligonucleotide compositions. Among other things, the present disclosure demonstrates that by controlling structural elements of oligonucleotides, such as base sequence, chemical modifications, stereochemistry, *etc*., properties of oligonucleotides can be greatly improved. For example, in some examples, the present disclosure provides methods for highly selective suppression of transcripts of a target nucleic acid sequence. In some examples, the present disclosure provides methods for treating a subject by suppressing transcripts from a disease-causing copy (*e.g.,* a disease-causing allele). In some examples, the present disclosure provides methods for designing and preparing oligonucleotide compositions with surprisingly enhanced activity and/or selectivity when suppressing a transcript of a target sequence. In some examples, the present disclosure provides methods for designing and/or preparing oligonucleotide compositions which provide allele-specific suppression of a transcript from a target nucleic acid sequence.

[0025] In some examples, the present disclosure provides a method for controlled cleavage of a nucleic acid polymer, the method comprising steps of:

contacting a nucleic acid polymer whose nucleotide sequence comprises a target sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is complementary to a target sequence found in the nucleic acid polymer;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the particular base sequence and length, for oligonucleotides of the particular oligonucleotide type.

[0026] In some examples, the present disclosure provides a method for altering a cleavage pattern observed when a nucleic acid polymer whose nucleotide sequence includes a target sequence is contacted with a reference oligonucleotide composition that comprises oligonucleotides having a particular base sequence and length, which particular base sequence is or comprises a sequence that is complementary to the target sequence, the method comprising:

contacting the nucleic acid polymer with a chirally controlled oligonucleotide composition of oligonucleotides having the particular base sequence and length, which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the particular base sequence and length, for oligonucleotides of a single oligonucleotide type characterized by:

1) the particular base sequence and length;

2) a particular pattern of backbone linkages; and

3) a particular pattern of backbone chiral centers.

[0027] In some examples, the present disclosure provides a method for suppression of a transcript from a target nucleic acid sequence for which one or more similar nucleic acid sequences exist within a population, each of the target and similar sequences contains a specific nucleotide characteristic sequence element that defines the target sequence relative to the similar sequences, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length; and

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines the target nucleic acid sequence, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target nucleic acid sequence and a similar nucleic acid sequences, transcripts of the target nucleic acid sequence are suppressed at a greater level than a level of suppression observed for a similar nucleic acid sequence.

[0028] In some examples, the present disclosure provides a method for suppression of a transcript from a target nucleic acid sequence for which one or more similar nucleic acid sequences exist within a population, each of the target and similar sequences contains a specific nucleotide characteristic sequence element that defines the target sequence relative to the similar sequences, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length; and

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines the target nucleic acid sequence, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target nucleic acid sequence and a similar nucleic acid sequences, transcripts of the target nucleic acid sequence are suppressed at a greater level than a level of suppression observed for a similar nucleic acid sequence.

[0029] In some examples, transcripts of the target nucleic acid sequence are suppressed at a greater level than a level of suppression observed for any one of the similar nucleic acid sequence.

[0030] In some examples, the present disclosure provides a method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length; and

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target

allele and another allele of the same nucleic acid sequence, transcripts of the particular allele are suppressed at a greater level than a level of suppression observed for another allele of the same nucleic acid sequence.

[0031] In some examples, the present disclosure provides a method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same nucleic acid sequence, transcripts of the particular allele are suppressed at a greater level than a level of suppression observed for another allele of the same nucleic acid sequence.

[0032] In some examples, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

[0033] In some examples, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

[0034] In some examples, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that

defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

[0035] In some examples, the present disclosure provides a method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of the same target nucleic acid sequence, it shows suppression of transcripts of the particular allele at a level that is:

a) greater than when the composition is absent;

b) greater than a level of suppression observed for another allele of the same nucleic acid

sequence; or

c) both greater than when the composition is absent, and greater than a level of suppression observed for another allele of the same nucleic acid sequence.

[0036] In some examples, the present disclosure provides a method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of the same target nucleic acid sequence, it shows suppression of transcripts of the particular allele at a level that is:

a) greater than when the composition is absent;

b) greater than a level of suppression observed for another allele of the same nucleic acid sequence; or

c) both greater than when the composition is absent, and greater than a level of suppression observed for another allele of the same nucleic acid sequence.

[0037] In some examples, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length; and

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene.

[0038] In some examples, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that,

when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene.

[0039] In some examples, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with an oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene.

[0040] In some examples, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that

defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene.

[0041] In some examples, a nucleotide characteristic sequence comprises a mutation that defines the target sequence relative to other similar sequences. In some examples, a nucleotide characteristic sequence comprises a point mutation that defines the target sequence relative to other similar sequences. In some examples, a nucleotide characteristic sequence comprises a SNP that defines the target sequence relative to other similar sequences.

[0042] In some examples, the present disclosure provides a method for preparing an oligonucleotide composition comprising oligonucleotides of a particular sequence, which composition provides selective suppression of a transcript of a target sequence, comprising providing a chirally controlled oligonucleotide composition comprising oligonucleotides of a

particular oligonucleotide type characterized by:

1) a common base sequence which is the same as the particular sequence;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers, which pattern comprises $(Sp)_m(Rp)_n$, $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$, wherein:

m is 1-50;

n is 1-10;

t is 1-50; and

each *N*p is independent *R*p or *S*p.

[0043] In general, activities of oligonucleotide compositions as described herein can be assessed using any appropriate assay. Relative activities for different compositions (*e.g.,* stereocontrolled vs non-stereocontrolled, and/or different stereocontrolled compositions) are typically desirably determined in the same assay, in some examples substantially simultaneously and in some examples with reference to historical results.

[0044] Those of skill in the art will be aware of and/or will readily be able to develop appropriate assays for particular oligonucleotide compositions. The present disclosure provides descriptions of certain particular assays, for example that may be useful in assessing one or more features of oligonucleotide composition behavior with respect to RNAse H cleavage of a target sequence.

[0045] For example, certain assays that may be useful in the assessment of one or more features (*e.g.,* rate, extent, and/or selectivity of cleavage) of RNase H cleavage may include an assay as described in any assay described and/or exemplified herein (*e.g.,* in one or more of Examples 4, 9-10, 12, 14, 17-20, *etc.*).

[0046] In some examples, the present disclosure recognizes that a base sequence can impact properties of oligonucleotides. The present disclosure demonstrates that chemical and stereochemical modifications, combined with designed base sequences, can provide oligonucleotide compositions with unexpectedly improved properties (*e.g.,* surprisingly higher activity, and/or selectivity, *etc.*). In some examples, oligonucleotides having a common base sequence complementary to a characteristic sequence element of a target nucleic acid sequence provide better activity compared to another common base sequence complementary to the characteristic sequence element of a target nucleic acid sequence. In some examples,

oligonucleotides having a common base sequence complementary to a characteristic sequence element of a target nucleic acid sequence provide better selectivity compared to another common base sequence complementary to the characteristic sequence element of a target nucleic acid sequence.

[0047] In some examples, a composition of oligonucleotides having a common base sequence complementary to a characteristic sequence element of a target nucleic acid sequence, when compared to another composition of oligonucleotides having another common base sequence complementary to the characteristic sequence element of the target nucleic acid sequence, provides higher cleavage rate of a transcript from the target nucleic acid sequence, and/or a cleavage pattern which has only one major cleavage site, and the major cleavage site is within or close to the nucleotide characteristic sequence. In some examples, a composition of oligonucleotides having a complementary common base sequence, when compared to another composition of oligonucleotides having another complementary common base sequence, provide higher cleavage rate of a transcript from the target nucleic acid sequence, and a cleavage pattern which has only one major cleavage site, and the major cleavage site is within or close to a nucleotide characteristic sequence. In some examples, greater than 50%, 60%, 70%, 80% or 90% of cleavage occurs at the one major cleavage site, for example, when measured by a suitable method, *e.g.,* an RNase H assay. In some examples, a composition of oligonucleotides having a complementary common base sequence, when compared to another composition of oligonucleotides having another complementary common base sequence, provides higher cleavage rate of a transcript from the target nucleic acid sequence, and a cleavage pattern which has only one major cleavage site, and the major cleavage site is within or close to a mutation or a SNP that defines the target sequence relative to other similar sequences. In some examples, a mutation is a point mutation. In some examples, a major cleavage site is next to a mutation or a SNP that defines the target sequence relative to other similar sequences. In some examples, each common base sequence is 100% complementary to the characteristic sequence element of the target nucleic acid sequence. In some examples, a major cleavage site is within less than 5, 4, 3, or 1 internucleotidic linkage from a mutation or a SNP that defines the target sequence relative to other similar sequences. In some examples, a major cleavage site is within less than 5, 4, 3, or 1

internucleotidic linkage from a mutation or a SNP that defines the target sequence relative to other similar sequences, and is within less than 5, 4, 3, or 1 internucleotidic linkage from a cleavage site when a stereorandom composition of oligonucleotides having the same common sequence, and/or a composition of DNA oligonucleotides having the same common sequence, is used. In some examples, a major cleavage site is a cleavage site when a stereorandom composition of oligonucleotides having the same common sequence is used. In some examples, a major cleavage site is a major cleavage site when a stereorandom composition of oligonucleotides having the same common sequence is used. In some examples, a major cleavage site is a cleavage site when a composition of DNA oligonucleotides having the same common sequence is used. In some examples, a major cleavage site is a major cleavage site when a composition of DNA oligonucleotides having the same common sequence is used.

[0048] In some examples, when comparing effects of a first and a second common base sequences, a stereorandom composition of oligonucleotides having a first common base sequence may be compared to a stereorandom composition of oligonucleotides having a second common base sequence. In some examples, a stereorandom composition is a composition of oligonucleotides having a common base sequence, a common pattern of nucleoside modifications, and a common pattern of backbone linkages. In some examples, a stereorandom composition is a composition of oligonucleotides having a common base sequence, a common pattern of nucleoside modifications, wherein each internucleotidic linkage is phosphorothioate. In some examples, when comparing effects of a first and a second common base sequences, a chirally controlled oligonucleotide composition of oligonucleotides having a first common base sequence may be compared to a chirally controlled oligonucleotide composition of oligonucleotides having a second common base sequence. In some examples, oligonucleotides in a chirally controlled oligonucleotide composition have a common base sequence, a common pattern of nucleoside modifications, a common pattern of backbone linkages, a common pattern of backbone chiral centers, and a common pattern of backbone phosphorus modifications. In some examples, each internucleotidic linkage is phosphorothioate.

[0049] In some examples, oligonucleotide compositions and technologies described herein are particularly useful in the treatment of Huntington's disease. For example, in some examples, the present disclosure defines stereochemically controlled oligonucleotide compositions that direct cleavage (*e.g.,* RNase H-mediated cleavage) of nucleic acids associated

with Huntington's disease. In some examples, such compositions direct preferential cleavage of a Huntington's disease-associated allele of a particular target sequence, relative to one or more (*e.g.*, all non-Huntington's disease-associated) other alleles of the sequence.

[0050] Huntington's disease is an inherited disease that can cause progressive degeneration of nerve cells in the brain and affect a subject's motor and cognitive abilities. In some examples, Huntington's disease is an autosomal dominant disorder. In some examples, it is caused by mutations in the Huntingtin gene. Normal HTT gene contains 10 to 35 CAG tri-nucleotide repeats. People with 40 or more repeats often develop the disorder. In some examples, the expanded CAG segment on the first exon of HTT gene leads to the production of an abnormally long version of the Huntingtin protein (expanded polyglutamine tract) which is cut into smaller, toxic fragments that bind together and accumulate in neurons, disrupting the normal functions of these cells. Warby et al. (Am J Hum Genet. 2009, 84(3), 351–366) reported many SNPs that are associated with disease chromosomes and have stronger linkage associations with CAG expansion than those reported before. Many SNPs highly associated with CAG expansion do not segregate independently and are in Linkage Disequilibrium with each other. Among other things, the present disclosure recognizes that strong association between specific SNPs and CAG expanded chromosomes provides an attractive therapeutic opportunity for the treatment of Huntington Disease, *e.g.*, through antisense therapy. Furthermore, the association of specific SNPs combined with high rates of heterozygosity in HD patients provides suitable targets for allele-specific knockdown of the mutant gene product. For example references, see Liu et al. Journal of Huntington's Disease 2, 2013, 491–500; Aronin, Neil and Pfister, Edith WO 2010/118263 A1; Pfister et al. Current Biology 2009,19, 774–778.

[0051] In some examples, a targeted SNP of the present disclosure has high frequency of heterozygosity in HD and has a particular variant associated with the mutant HTT allele. In some examples, a SNP is rs362307. In some examples, a SNP is rs7685686. In some examples, a SNP may not be linked but may have a high heterozygous frequency. In some examples, a SNP is rs362268 (3'-UTR region). In some examples, a SNP is rs362306 (3'-UTR region). In some examples, a SNP is rs2530595. In some examples, a SNP is rs362331.

[0052] In some examples, a provided method for treating or preventing Huntington's disease in a subject, comprising administering to the subject a provided oligonucleotide compositions. In some examples, a provided method for treating or preventing Huntington's disease in a subject, comprising administering to the subject a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type.

[0053] In some examples, a provided method ameliorates a symptom of Huntington's disease. In some examples, a provided method slows onset of Huntington's disease. In some examples, a provided method slows progression of Huntington's disease.

[0054] In some examples, the present disclosure provides methods for identifying patients for a given oligonucleotide composition. In some examples, the present disclosure provides methods for patient stratification. In some examples, a provided method comprises identifying a mutation and/or SNP associated with a disease-causing allele. For example, in some examples, a provided method comprises identifying in a subject a SNP associated with expanded CAG repeats that are associated with or causing Huntington’s disease.

[0055] In some examples, a subject has a SNP in the subject’s Huntingtin gene. In some examples, a subject has a SNP, wherein one allele is mutant Huntingtin associated with expanded CAG repeats. In some examples, a subject has a SNP selected from rs362307, rs7685686, rs362268, rs2530595, rs362331, or rs362306. In some examples, oligonucleotides of a provided composition have a sequence complementary to a sequence comprising a SNP from the disease-causing allele (mutant), and the composition selectively suppresses expression from the diseasing-causing allele.

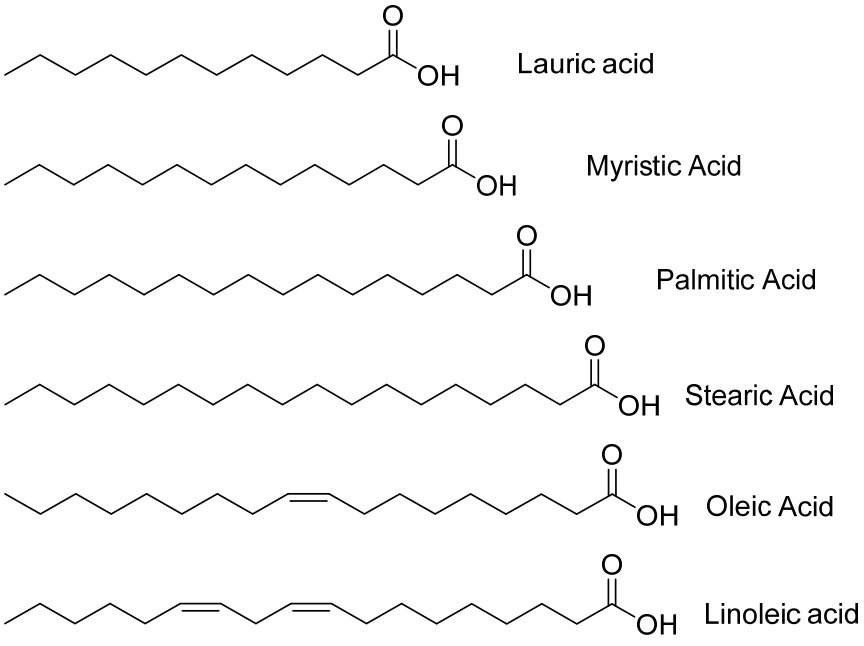
[0056] In some examples, the sequence of oligonucleotides in provided technologies (compounds, compositions, methods, *etc.*) comprises, consists of, or is the sequence of any oligonucleotide described herein. In some examples, a sequence is selected from Tables N1A, N2A, N3A, N4A or 8; or WV-1092, WVE120101, WV-2603 or WV-2595. In some

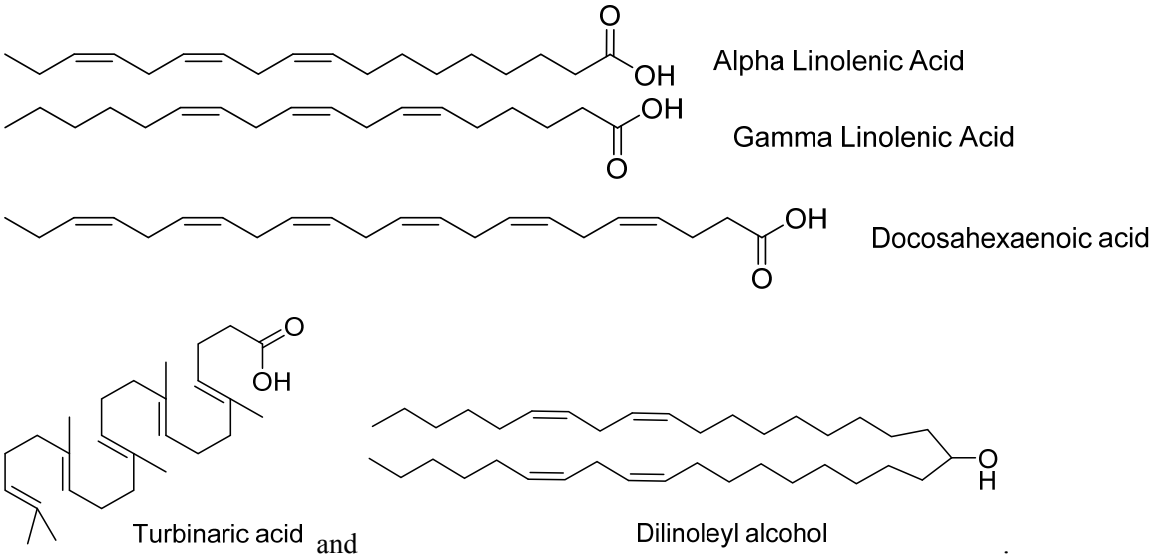
examples, a sequence is selected from the sequence of WV-1092, WVE120101, WV-2603 or WV-2595. In some examples, provided oligonucleotides are of the type defined by WV-1092, WVE120101, WV-2603 or WV-2595. In some examples, provided oligonucleotides are of the type defined by WV-1092. In some examples, provided oligonucleotides are of the type defined by WVE120101. In some examples, provided oligonucleotides are of the type defined by WV-2603. In some examples, provided oligonucleotides are of the type defined by WV-2595.

[0057] In some examples, provided oligonucleotide compositions comprises a lipid and an oligonucleotide. In some examples, a lipid is conjugated to an oligonucleotide.

[0058] In some examples, a composition comprises an oligonucleotide and a lipid selected from the list of: lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, docosahexaenoic acid (cis-DHA), turbinaric acid, arachidonic acid, and dilinoleyl. In some examples, a composition comprises an oligonucleotide and a lipid selected from the list of: lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, docosahexaenoic acid (cis-DHA), turbinaric acid, and dilinoleyl.

[0059] In some examples, a composition comprises an oligonucleotide and a lipid selected from:





[0060] In some examples, a composition comprises an oligonucleotide and a lipid, wherein the lipid comprises a C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-4} aliphatic group.

[0061] In some examples, an oligonucleotide composition comprises a plurality of oligonucleotides, which share:

1) a common base sequence;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone phosphorus modifications;

wherein one or more oligonucleotides of the plurality are individually conjugated to a lipid.

[0062] In some examples, a chirally controlled oligonucleotide composition comprises a plurality of oligonucleotides, which share:

1) a common base sequence;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone phosphorus modifications;

wherein:

the composition is chirally controlled in that the plurality of oligonucleotides share the same stereochemistry at one or more chiral internucleotidic linkages;

one or more oligonucleotides of the plurality are individually conjugated to a lipid; and

one or more oligonucleotides of the plurality are optionally and individually conjugated to a targeting compound or moiety.

[0063] In some examples, a method of delivering an oligonucleotide to a cell or tissue in a human subject, comprises:

(a) Providing a composition of any one of the examples described herein; and

(b) Administering the composition to the human subject such that the oligonucleotide is delivered to a cell or tissue in the subject.

[0064] In some examples, a method for delivering an oligonucleotide to a cell or tissue comprises preparing a composition according to any one of the examples described herein and contacting the cell or tissue with the composition.

[0065] In some examples, a method of modulating the level of a transcript or gene product of a gene in a cell, the method comprises the step of contacting the cell with a composition according to any one of the examples described herein, wherein the oligonucleotide is capable of modulating the level of the transcript or gene product.

[0066] In some examples, a method for inhibiting expression of a gene in a cell or tissue comprises preparing a composition according to any one of the examples described herein and treating the cell or tissue with the composition.

[0067] In some examples, a method for inhibiting expression of a gene in a cell or tissue in a mammal comprises preparing a composition according to any one of the examples described herein and administering the composition to the mammal.

[0068] In some examples, a method of treating a disease that is caused by the over-expression of one or several proteins in a cell or tissue in a subject, said method comprises the administration of a composition according to any one of the examples described herein to the subject.

[0069] In some examples, a method of treating a disease that is caused by a reduced, suppressed or missing expression of one or several proteins in a subject, said method comprises the administration of a composition according to any one of the examples described herein to the subject.

[0070] In some examples, a method for generating an immune response in a subject, said method comprises the administration of a composition according to any one of the

examples described herein to the subject, wherein the biologically active compound is an immunomodulating nucleic acid.

[0071] In some examples, a method for treating a sign and/or symptom of Huntington'sDisease by providing a composition of any one of the examples described herein and administering the composition to the subject.

[0072] In some examples, a method of modulating the amount of RNaseH-mediated cleavage in a cell, the method comprises the step of contacting the cell with a composition according to any one of the examples described herein, wherein the oligonucleotide is capable of modulating the amount of RNaseH-mediated cleavage.

[0073] In some examples, a method of administering an oligonucleotide to a subject in need thereof, comprises steps of providing a composition comprises the agent a lipid, and administering the composition to the subject, wherein the agent is any agent disclosed herein, and wherein the lipid is any lipid disclosed herein.

[0074] In some examples, a method of treating a disease in a subject, the method comprises steps of providing a composition comprises the agent a lipid, and administering a therapeutically effective amount of the composition to the subject, wherein the agent is any agent disclosed herein, and wherein the lipid is any lipid disclosed herein, and wherein the disease is any disease disclosed herein.

[0075] In some examples, a lipid comprises an optionally substituted C_{10}-C_{40} saturated or partially unsaturated aliphatic chain.

[0076] In some examples, a lipid comprises an optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

[0077] In some examples, a lipid comprises a C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-4} aliphatic group.

[0078] In some examples, a lipid comprises an unsubstituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

[0079] In some examples, a lipid comprises no more than one optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

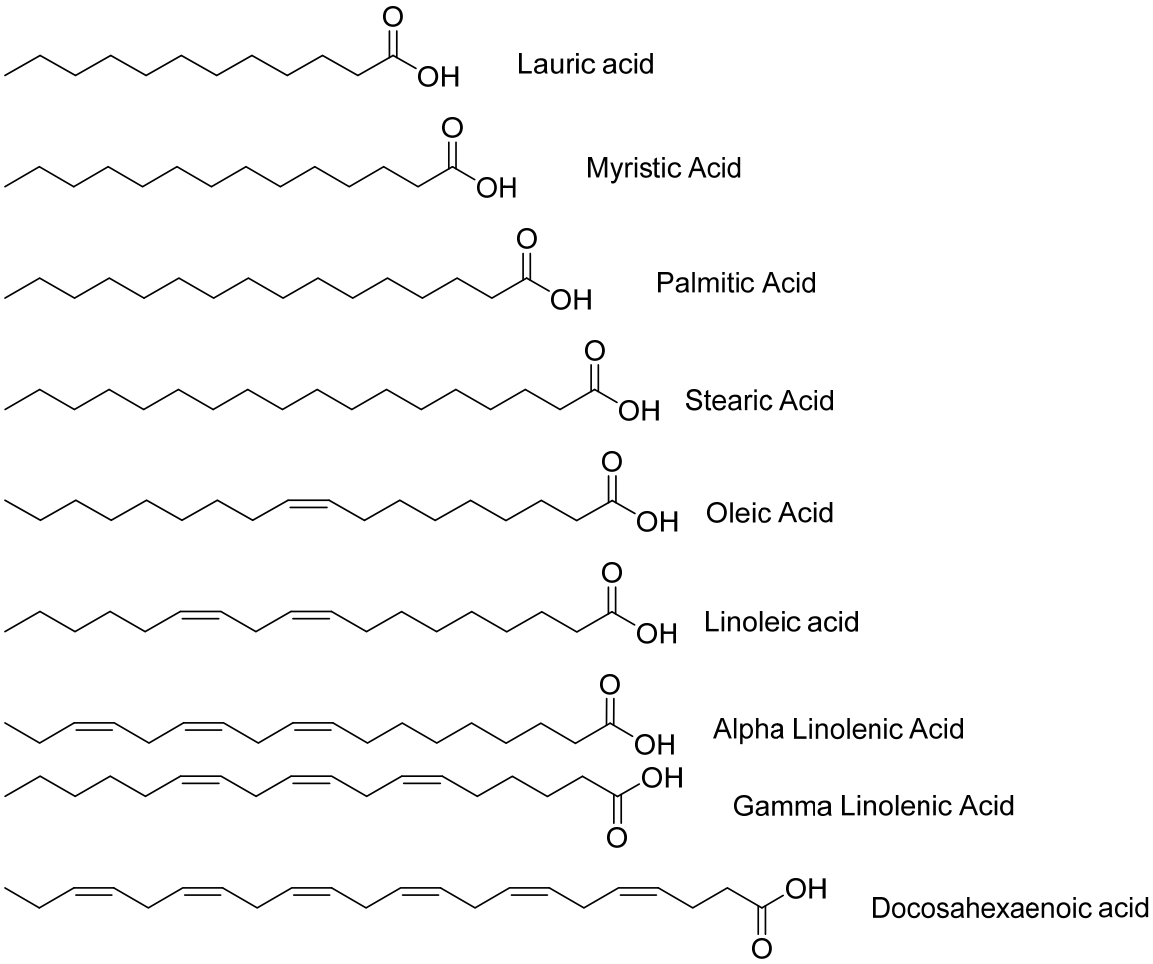
[0080] In some examples, a lipid comprises two or more optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

[0081] In some examples, a lipid comprises no tricyclic or polycyclic moiety.

[0082] In some examples, a lipid has the structure of R^1-COOH, wherein R^1 is an optionally substituted C_{10}-C_{40} saturated or partially unsaturated aliphatic chain.

[0083] The composition or method of any one of claim 16, wherein the lipid is conjugated through its carboxyl group.

[0084] The composition or method according to any one of the examples described herein, wherein the lipid is selected from:



[0085] In some examples, a lipid is conjugated to the oligonucleotide.

[0086] In some examples, a lipid is directly conjugated to the oligonucleotide.

[0087] In some examples, a lipid is conjugated to the oligonucleotide via a linker.

[0088] In some examples, a linker is selected from: an uncharged linker; a charged linker; a linker comprises an alkyl; a linker comprises a phosphate; a branched linker; an unbranched linker; a linker comprises at least one cleavage group; a linker comprises at least one redox cleavage group; a linker comprises at least one phosphate-based cleavage group; a linker comprises at least one acid-cleavage group; a linker comprises at least one ester-based cleavage group; and a linker comprises at least one peptide-based cleavage group.

[0089] In some examples, each oligonucleotide of the plurality is individually conjugated to the same lipid at the same location.

[0090] In some examples, a lipid is conjugated to an oligonucleotide through a linker.

[0091] In some examples, one or more oligonucleotides of the plurality are independently conjugated to a targeting compound or moiety.

[0092] In some examples, one or more oligonucleotides of the plurality are independently conjugated to a lipid and a targeting compound or moiety.

[0093] In some examples, one or more oligonucleotides of the plurality are independently conjugated to a lipid at one end and a targeting compound or moiety at the other.

[0094] In some examples, oligonucleotides of the plurality share the same chemical modification patterns.

[0095] In some examples, oligonucleotides of the plurality share the same chemical modification patterns comprises one or more base modifications.

[0096] In some examples, oligonucleotides of the plurality share the same chemical modification patterns comprises one or more sugar modifications.

[0097] In some examples, a common base sequence is capable of hybridizing with a transcript in a cell, which transcript contains a mutation that is linked to Huntington's Disease, or whose level, activity and/or distribution is linked to Huntington's Disease.

[0098] In some examples, an oligonucleotide is a nucleic acid.

[0099] In some examples, an oligonucleotide is an oligonucleotide.

[00100] In some examples, an oligonucleotide is an oligonucleotide which participates in RNaseH-mediated cleavage of a mutant Huntingtin gene mRNA.

[00101] In some examples, a disease or disorder is Huntington's Disease.

[00102] In some examples, a lipid comprises an optionally substituted, C_{10}-C_{80} saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, a C_1–C_6 heteroaliphatic moiety, −C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, −N(R′)–, −C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, −N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, −OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, −S(O)$_2$N(R′)–, −N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, and −C(O)O–, wherein each variable is independently as defined and described herein.

[00103] In some examples, a lipid comprises an optionally substituted C_{10}-C_{80} saturated or partially unsaturated, aliphatic chain.

[00104] In some examples, a lipid comprises an optionally substituted C_{10}-C_{80} linear, saturated or partially unsaturated, aliphatic chain.

[00105] In some examples, a lipid comprises an optionally substituted C_{10}-C_{60} saturated or partially unsaturated, aliphatic chain.

[00106] In some examples, a lipid comprises an optionally substituted C_{10}-C_{60} linear, saturated or partially unsaturated, aliphatic chain.

[00107] In some examples, a lipid comprises an optionally substituted C_{10}-C_{40} saturated or partially unsaturated, aliphatic chain.

[00108] In some examples, a lipid comprises an optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

[00109] In some examples, a lipid comprises an optionally substituted, C_{10}-C_{60} saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, a C_1–C_6 heteroaliphatic moiety, −C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, −N(R′)–, −C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, −N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, −OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, −S(O)$_2$N(R′)–, −N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, and −C(O)O–, wherein each variable is independently as defined and described herein.

[00110] In some examples, a lipid comprises an optionally substituted C_{10}-C_{80} saturated or partially unsaturated, aliphatic chain.

[00111] In some examples, a lipid comprises an optionally substituted C_{10}-C_{60} linear, saturated or partially unsaturated, aliphatic chain.

[00112] In some examples, a lipid comprises an optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

[00113] In some examples, a lipid comprises an optionally substituted, C_{10}-C_{40} saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, a C_1–C_6 heteroaliphatic moiety, −C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, −N(R′)–, −C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, −N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, −OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, −S(O)$_2$N(R′)–, −N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, and −C(O)O–, wherein each variable is independently as defined and described herein.

[00114] In some examples, a lipid comprises an optionally substituted C_{10}-C_{40} saturated or partially unsaturated, aliphatic chain.

[00115] In some examples, a lipid comprises an optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

[00116] In some examples, a composition further comprises one or more additional components selected from: a polynucleotide, carbonic anhydrase inhibitor, a dye, an intercalating agent, an acridine, a cross-linker, psoralene, mitomycin C, a porphyrin, TPPC4, texaphyrin, Sapphyrin, a polycyclic aromatic hydrocarbon phenazine, dihydrophenazine, an artificial endonuclease, a chelating agent, EDTA, an alkylating agent, a phosphate, an amino, a mercapto, a PEG, PEG-40K, MPEG, [MPEG]$_2$, a polyamino, an alkyl, a substituted alkyl, a radiolabeled marker, an enzyme, a hapten biotin, a transport/absorption facilitator, aspirin, vitamin E, folic acid, a synthetic ribonuclease, a protein, a glycoprotein, a peptide, a molecule having a specific affinity for a co-ligand, an antibody, a hormone, a hormone receptor, a non-peptidic species, a lipid, a lectin, a carbohydrate, a vitamin, a cofactor, selectivity agent, or a drug. In some examples, a composition further comprises one or more additional components selected from: a polynucleotide, carbonic anhydrase inhibitor, a dye, an intercalating agent, an acridine, a cross-linker, psoralene, mitomycin C, a porphyrin, TPPC4, texaphyrin, Sapphyrin, a polycyclic aromatic hydrocarbon phenazine, dihydrophenazine, an artificial endonuclease, a chelating agent, EDTA, an alkylating agent, a phosphate, an amino, a mercapto, a PEG, PEG-40K, MPEG,

$[MPEG]_2$, a polyamino, an alkyl, a substituted alkyl, a radiolabeled marker, an enzyme, a hapten biotin, a transport/absorption facilitator, aspirin, vitamin E, folic acid, a synthetic ribonuclease, a protein, a glycoprotein, a peptide, a molecule having a specific affinity for a co-ligand, an antibody, a hormone, a hormone receptor, a non-peptidic species, a lipid, a lectin, a carbohydrate, a vitamin, a cofactor, or a drug.

[00117] In some examples, the present disclosure provides an oligonucleotide conjugated to a selectivity agent. In some examples, the present disclosure provides a composition comprising an oligonucleotide or oligonucleotide type comprising a selectivity agent. In some examples, a selectivity agent binds specifically to one or more neurotransmitter transporters selected from the group consisting of a dopamine transporter (DAT), a serotonin transporter (SERT), and a norepinephrine transporter (NET). In some examples, a selectivity agent is selected from the group consisting of a dopamine reuptake inhibitor (DRI), a selective serotonin reuptake inhibitor (SSRI), a noradrenaline reuptake inhibitor (NRI), a norepinephrine-dopamine reuptake inhibitor (NDRI), and a serotonin-norepinephrine-dopamine reuptake inhibitor (SNDRI). In some examples, a selectivity agent is selected from the group consisting of a triple reuptake inhibitor, a noradrenaline dopamine double reuptake inhibitor, a serotonin single reuptake inhibitor, a noradrenaline single reuptake inhibitor, and a dopamine single reuptake inhibitor. In some examples, a selectivity agent is selected from the group consisting of a dopamine reuptake inhibitor (DRI), a Norepinephrine-Dopamine Reuptake Inhibitor (NDRI) and a serotonin-Norepinephrine-Dopamine Reuptake Inhibitor (SNDRI). In some examples, a selectivity agent is selected from the selectivity agents which are described in U.S. Pat. Nos. 9,084,825; and 9,193,969; and WO2011131693, WO2014064258.

[00118] In some examples, a lipid comprises a C_{10}-C_{80} linear, saturated or partially unsaturated, aliphatic chain.

[00119] In some examples, a composition further comprises a linker linking the oligonucleotide and the lipid, wherein the linker is selected from: an uncharged linker; a charged linker; a linker comprises an alkyl; a linker comprises a phosphate; a branched linker; an unbranched linker; a linker comprises at least one cleavage group; a linker comprises at least one redox cleavage group; a linker comprises at least one phosphate-based cleavage group; a linker

comprises at least one acid-cleavage group; a linker comprises at least one ester-based cleavage group; a linker comprises at least one peptide-based cleavage group.

[00120] In some examples, an oligonucleotide comprises or consists of or is an oligonucleotide or oligonucleotide composition or chirally controlled oligonucleotide composition.

[00121] In some examples, an oligonucleotide comprises or consists of or is an oligonucleotide composition or chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of any oligonucleotide described herein.

[00122] In some examples, an oligonucleotide comprises or consists of or is an oligonucleotide composition or chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of any oligonucleotide listed in Table 4.

[00123] In some examples, an oligonucleotide comprises or consists of or is an oligonucleotide composition or chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of a splice-switching oligonucleotide.

[00124] The composition or method of any of the examples described herein, wherein the oligonucleotide is a chirally controlled oligonucleotide composition.

[00125] The composition or method of any of the examples described herein, wherein the disease or disorder is Huntington's Disease.

[00126] The composition or method of any of the examples described herein, wherein the oligonucleotide is capable of participating in RNaseH-mediated cleavage of a mutant Huntingtin gene mRNA.

[00127] The composition or method of any of the examples described herein, wherein the oligonucleotide comprises, consists of or is the sequence of any oligonucleotide disclosed herein.

[00128] The composition or method of any of the examples described herein, wherein the oligonucleotide is capable of differentiating between a wild-type and a mutant Huntingtin allele.

[00129] The composition or method of any of the examples described herein, wherein the oligonucleotide is capable of participating in RNaseH-mediated cleavage of a mutant Huntingtin gene mRNA.

[00130] The composition or method of any of the examples described herein, wherein the oligonucleotide comprises, consists of or is the sequence of any oligonucleotide disclosed in Table 4.

[00131] In some examples, an oligonucleotide comprises or consists of or is an oligonucleotide or oligonucleotide composition or chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of any of: WV-1092, WV-2595, or WV-2603.

[00132] In some examples, a sequence of an oligonucleotide includes any one or more of: base sequence (including length); pattern of chemical modifications to sugar and base moieties; pattern of backbone linkages; pattern of natural phosphate linkages, phosphorothioate linkages, phosphorothioate triester linkages, and combinations thereof; pattern of backbone chiral centers; pattern of stereochemistry (Rp/Sp) of chiral internucleotidic linkages; pattern of backbone phosphorus modifications; pattern of modifications on the internucleotidic phosphorus atom, such as $-S^-$, and $-L-R^1$ of formula I.

[00133] In some examples, the present disclosure provides a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type, wherein the oligonucleotides target a mutant Huntingtin gene, and the length is from about 10 to about 50 nucleotides, wherein the backbone linkages comprise at least one phosphorothioate, and wherein the pattern of backbone chiral centers comprises at least one chiral center in a *R*p conformation and at least one chiral center in a *S*p conformation.

[00134] In some examples, the present disclosure provides a method for cleavage of a nucleic acid having a base sequence comprising a target sequence, the method comprising steps of:

(a) contacting a nucleic acid having a base sequence comprising a target sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is complementary to the target sequence in the nucleic acid;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the particular base sequence and length, for oligonucleotides of the particular oligonucleotide type, wherein the oligonucleotide targets a mutant Huntingtin gene, and the length is from about 10 to about 50 nucleotides, wherein the backbone linkages comprise at least one phosphorothioate, and wherein the pattern of backbone chiral centers comprises at least one chiral center in a Rp conformation and at least one chiral center in a Sp conformation.

[00135] In some examples, the present disclosure provides a method for cleavage of a nucleic acid having a base sequence comprising a target sequence, the method comprising steps of:

(a) contacting a nucleic acid having a base sequence comprising a target sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is complementary to the target sequence in the nucleic acid;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the particular base sequence and length, for oligonucleotides of the particular oligonucleotide type, wherein the oligonucleotide targets a mutant Huntingtin gene, and the length is from about 10 to about 50 nucleotides, wherein the

backbone linkages comprise at least one phosphorothioate, and wherein the pattern of backbone chiral centers comprises at least one chiral center in a Rp conformation and at least one chiral center in a Sp conformation; and

(b) cleavage of the nucleic acid mediated by a RNAseH or RNA interference mechanism.

[00136] In some examples, a provided composition further comprises a selectivity agent selected from: the group of compounds which binds specifically to one or more neurotransmitter transporters selected from the group consisting of a dopamine transporter (DAT), a serotonin transporter (SERT), and a norepinephrine transporter (NET); the group consisting of a dopamine reuptake inhibitor (DRI), a selective serotonin reuptake inhibitor (SSRI), a noradrenaline reuptake inhibitor (NRI), a norepinephrine-dopamine reuptake inhibitor (NDRI), and a serotonin-norepinephrine-dopamine reuptake inhibitor (SNDRI); the group consisting of a triple reuptake inhibitor, a noradrenaline dopamine double reuptake inhibitor, a serotonin single reuptake inhibitor, a noradrenaline single reuptake inhibitor, and a dopamine single reuptake inhibitor; and the group consisting of a dopamine reuptake inhibitor (DRI), a Norepinephrine-Dopamine Reuptake Inhibitor (NDRI) and a serotonin-Norepinephrine-Dopamine Reuptake Inhibitor (SNDRI).

[00137] In some examples, a provided composition comprises oligonucleotides wherein the base sequence, pattern of backbone linkages and/or pattern of backbone chiral centers of the oligonucleotides comprises or consists of the base sequence, pattern of backbone linkages and/or pattern of backbone chiral centers of any of any oligonucleotide selected from Tables N1A, N2A, N3A, N4A and 8; and WV-1092, WV-2595, and WV-2603.

[00138] In some examples, a provided composition comprises oligonucleotides wherein the base sequence, pattern of backbone linkages and/or pattern of backbone chiral centers of the oligonucleotides comprises or consists of the base sequence, and pattern of backbone linkages, and/or pattern of backbone chiral centers of any of any oligonucleotide selected from Tables N1A, N2A, N3A, N4A and 8; and WV-1092, WV-2595, and WV-2603.

[00139] In some examples, a provided composition comprises oligonucleotides wherein the base sequence, pattern of backbone linkages and/or pattern of backbone chiral centers of the oligonucleotides comprises or consists of the base sequence, and pattern of backbone linkages, and pattern of backbone chiral centers of any of any oligonucleotide selected from Tables N1A, N2A, N3A, N4A and 8; and WV-1092, WV-2595, and WV-2603.

Claims

1. An oligonucleotide having the structure of:

mG * SmGmCmAmC * SA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SmAmCmUmU * SmC (SEQ ID NO: 360), or a pharmaceutically acceptable salt thereof, wherein:

*S represents a *S*p phosphorothioate;

*R represents a *R*p phosphorothioate; and

m represents a 2'-OMe modification to a nucleoside.

2. The oligonucleotide of claim 1, wherein the oligonucleotide is in a salt form.

3. An oligonucleotide, wherein the oligonucleotide is a sodium salt of:

mG * SmGmCmAmC * SA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SmAmCmUmU * SmC (SEQ ID NO: 360), wherein:

*S represents a *S*p phosphorothioate;

*R represents a *R*p phosphorothioate; and

m represents a 2'-OMe modification to a nucleoside.

4. A chirally controlled composition of an oligonucleotide having the structure of:

mG * SmGmCmAmC * SA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SmAmCmUmU * SmC (SEQ ID NO: 360), or a pharmaceutically acceptable salt thereof, wherein:

*S represents a *S*p phosphorothioate;

*R represents a *R*p phosphorothioate; and

m represents a 2'-OMe modification to a nucleoside.

5. The composition of claim 4, wherein the oligonucleotide is in a salt form.

6. The composition of claim 4, wherein the oligonucleotide is a sodium salt.

7. A pharmaceutical composition, comprising a therapeutically effective amount of an oligonucleotide and at least one pharmaceutically acceptable inactive ingredient selected from pharmaceutically acceptable diluents, pharmaceutically acceptable excipients, and pharmaceutically acceptable carriers, wherein the oligonucleotide has the structure of:

mG * SmGmCmAmC * SA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SmAmCmUmU * SmC (SEQ ID NO: 360), or a pharmaceutically acceptable salt thereof, wherein:

*S represents a *S*p phosphorothioate;

*R represents a *R*p phosphorothioate; and

m represents a 2'-OMe modification to a nucleoside.

8. The composition of claim 7, wherein the oligonucleotide is in a salt form.

9. The composition of claim 7, wherein the oligonucleotide is a sodium salt.