

CLAIMS

1. A nucleic acid complex, comprising:
 - a first nucleic acid strand comprising 20-60 linked nucleosides;
 - a second nucleic acid strand binding to a first region of the first nucleic acid strand to form a first nucleic acid duplex; and
 - a third nucleic acid strand binding to a second region of the first nucleic acid strand to form a second nucleic acid duplex, wherein the third nucleic acid strand comprises a overhang, wherein the overhang is not complementary to the first nucleic acid strand and is capable of binding to an input nucleic acid strand to cause the displacement of the third nucleic acid strand from the first nucleic acid strand,wherein
 - the first region of the first nucleic acid strand is 3' of the second region of the first nucleic acid strand,
 - the third nucleic acid strand does not bind to any region of the first nucleic acid strand that is 3' of the first region of the first nucleic acid strand,
 - and the first region of the first nucleic acid strand comprises a sequence complementary to a target RNA, wherein the sequence is 10-35 nucleosides in length.
2. The nucleic acid complex of claim 1, wherein the sequence complementary to the target RNA is 10-21 nucleotides in length; and/or wherein the first nucleic acid duplex does not comprise a Dicer cleavage site or wherein the nucleic acid complex does not comprise a Dicer cleavage site.
3. The nucleic acid complex of any one of claims 1-2, wherein the second nucleic acid strand binds to 17-22 linked nucleotides in the first region of the first nucleic acid strand to form the first nucleic acid duplex, wherein the third nucleic acid strand binds to 10-30 linked nucleotides in the second region of the first nucleic acid strand to form the second nucleic acid duplex or wherein the third nucleic acid strand binds to about 14 linked nucleotides in the second region of the first nucleic acid strand to form the second nucleic acid duplex.
4. The nucleic acid complex of any one of claims 1-3, wherein the first region of the first nucleic acid strand is linked to the second region of the first nucleic acid strand via a linker; optionally, wherein the linker comprises a C₃ 3-carbon linker, a nucleotide, a modified nucleotide, or a exonuclease cleavage-resistant moiety, or a combination thereof; further optionally wherein the modified nucleotide is a 2'-O-methyl nucleotide or a 2'-F nucleotide; and optionally wherein the

2'-O-methyl nucleotide is 2'-O-methyladenosine, 2'-O-methylguanosine, 2'-O-methyluridine, or 2'-O-methylcytidine and/or wherein the 2'-F nucleotide is 2'-F adenosine, 2'-F guanosine, 2'-F uridine, or 2'-F cytidine; and/or

wherein the 5' terminus of the second nucleic acid strand comprises a blocking moiety; and optionally the blocking moiety comprises, or is, a fluorophore, an inverted-dT, a tri-ethylene-glycol, a fatty acid, a Cy3, or a combination thereof; and further optionally wherein the fluorophore is attached to the 5' terminus of the second nucleic strand via a phosphorothioate linkage.

5. The nucleic acid complex of any one of claims 1-4, wherein the first nucleic acid strand comprises a 3' overhang in the first nucleic acid duplex; optionally wherein the 3' overhang of the first nucleic acid is one, two, or three nucleotides in length; further optionally, wherein the 3' overhang of the first nucleic acid comprises one or more phosphorothioate internucleoside linkages or wherein all of the internucleoside linkages in the 3' overhang of the first nucleic acid are phosphorothioate internucleoside linkages; further optionally wherein the internucleoside linkage(s) between the last two, three or four nucleosides at the 3' terminus of the first nucleic acid strand is phosphorothioate internucleoside linkage(s).

6. The nucleic acid complex of any one of claims 1-5, wherein the first region of the first nucleic acid strand does not comprise phosphorothioate internucleoside linkages except for the internucleoside linkage(s) between the last two or three nucleosides at the 5' terminus, 3' terminus, or both; or wherein the first region of the first nucleic acid strand does not comprise phosphorothioate internucleoside linkages except for the internucleoside linkage(s) between the last three nucleosides at the 5' terminus and the last three nucleosides at 3' terminus; and/or wherein the second region of the first nucleic acid strand does not comprise phosphorothioate internucleoside linkages.

7. The nucleic acid complex of any one of claims 1-6, wherein the second nucleic strand is fully complementary to the first region of the first nucleic acid strand, thereby forming no overhang at the 5' and 3' termini of the second nucleic acid strand in the first nucleic acid duplex; and/or wherein the second nucleic acid strand does not have an overhang at 3' terminus, or 5' terminus, or both in the first nucleic acid duplex; optionally

wherein the second nucleic acid strand comprises one or more phosphorothioate internucleoside linkages or wherein the second nucleic acid strand does not comprise phosphorothioate internucleoside linkages except for the internucleoside linkage(s) between the last two to three nucleosides at the 5' terminus and the last two to three nucleosides at 3' terminus or wherein the internucleoside linkage(s) between the last two, three or four nucleosides at the 5'

terminus of the second nucleic acid strand, the 3' terminus of the second nucleic acid strand, or both, are phosphorothioate internucleoside linkages.

8. The nucleic acid complex of any one of claims 1-7, wherein the 5' terminus of the third nucleic acid strand comprises at least one phosphorothioate internucleoside linkage; and/or wherein the last two, three or four nucleosides at the 5' terminus of the third nucleic acid strand are phosphorothioate internucleoside linkages; and/or

wherein less than 5%, less than 10%, less than 25%, less than 50% of the internucleoside linkages in the first nucleic acid strand are phosphorothioate internucleoside linkages; and/or wherein the first nucleic acid strand comprises no more than two phosphorothioate internucleoside linkages, or does not comprise phosphorothioate internucleoside linkages.

9. The nucleic acid complex of any one of claims 1-8, wherein at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, or all of the nucleosides of the first region of the first nucleic acid strand, the second region of the first nucleic strand, or both, are chemically modified; and/or wherein at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, or all of the nucleosides of one or more of the first nucleic acid strand, the second nucleic strand and the third nucleic strand are chemically modified; and/or wherein at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, or all of the nucleosides of the nucleic acid complex are chemically modified; optionally the chemical modifications are to resist nuclease degradation, to increase melting temperature (T_m), or both, of the nucleic acid complex; and/or

wherein at least 90%, at least 95%, or all of the nucleotides of the nucleic acid complex are non-DNA and non-RNA nucleotides; optionally wherein at most 5%, at most 10%, or at most 15% of the nucleosides of the second nucleic strand are LNA or wherein about 10%-50% of the bases of the nucleic acid complex are locked nucleic acid (LNA) or analogues thereof; optionally wherein about 10%-50% of the bases of the nucleic acid complex have a 2'-4' bridging modifications, further optionally wherein about 10%-50% of the bases of the nucleic acid complex comprises 2'-O-methyl modification, 2'-F modification, or both.

10. The nucleic acid complex of any one of claims 1-9, wherein the input nucleic acid strand is an RNA and/or wherein the target RNA is an mRNA, a miRNA, a non-coding RNA, a viral RNA transcript, a cellular RNA transcript, or a combination thereof; optionally

wherein the overhang of the third nucleic acid strand is capable of binding to the input nucleic acid strand to form a toehold, thereby causing the displacement of the third nucleic acid

strand from the first nucleic acid strand; optionally, wherein the overhang of the third nucleic acid strand is 5 to 20 nucleosides in length, preferably 8 to 16 nucleosides in length, more preferably 12 nucleosides in length; and optionally wherein the overhang of the third nucleic acid strand is 12 nucleotides in length; further optionally, wherein all internucleoside linkages of the overhang of the third nucleic acid strand are phosphorothioate internucleoside linkages; and/or

wherein the 5' terminus, the 3' terminus, or both of the third nucleic acid strand comprises a terminal moiety; optionally wherein the terminal moiety comprises a ligand, a fluorophore, a exonuclease, a fatty acid, a Cy3, an inverted dT attached to a tri-ethylene glycol, or a combination thereof, further optionally wherein the terminal moiety comprises a palmitic acid, optionally the palmitic acid is attached to the 3' terminus of the third nucleic acid strand.

11. An *in vitro* and/or *ex vivo* method of modulating a target RNA, comprising:

contacting a cell comprising a target RNA with the nucleic acid complex of any one of claims 1 to 10, wherein an input strand binds to the overhang of the third nucleic acid strand to cause displacement of the third nucleic acid strand from the first nucleic acid strand to release the sequence complementary to the target RNA into the cell, thereby modulating the target RNA.

12. The nucleic acid complex of any one of claims 1 to 10, for use in contacting a cell to modulate a target RNA, wherein an input strand binds to the overhang of the third nucleic acid strand to cause displacement of the third nucleic acid strand from the first nucleic acid strand to release the sequence complementary to the target RNA into the cell, thereby modulating the target RNA.

13. The method of claim 11 or the use of claim 12, wherein the cell is a cancer cell or a neuron.

14. The nucleic acid complex of any one of claims 1-10 for use in treating a disease or a condition, wherein the nucleic acid complex is to be administered to a subject in need thereof, wherein the input strand binds to the overhang of the third nucleic acid strand to cause displacement of the third nucleic acid strand from the first nucleic acid strand to release the sequence complementary to a target RNA, thereby reducing the activity of the target RNA or protein expression from the target RNA in the subject to treat the disease or condition.

15. The nucleic acid complex for use of claim 14, wherein the disease or condition is cancer; or

the disease or condition is a neurological disease, optionally a central nervous system (CNS) disease or disorder; and optionally the CNS disease or disorder is selected from the group consisting

of: Adrenoleukodystrophy, Alzheimer disease, Amyotrophic lateral sclerosis, Angelman syndrome, Ataxia telangiectasia, Charcot-Marie-Tooth syndrome, Cockayne syndrome, Deafness, Duchenne muscular dystrophy, Epilepsy, Essential tremor, Fragile X syndrome, Friedreich's ataxia, Gaucher disease, Huntington disease, Lesch-Nyhan syndrome, Maple syrup urine disease, Menkes syndrome, Myotonic dystrophy, Narcolepsy, Neurofibromatosis, Niemann-Pick disease, Parkinson disease, Phenylketonuria, Prader-Willi syndrome, Refsum disease, Rett syndrome, Spinal muscular atrophy, Spinocerebellar ataxia, Tangier disease, Tay-Sachs disease, Tuberous sclerosis, Von Hippel-Lindau syndrome, Williams syndrome, Wilson's disease, Zellweger syndrome or a combination thereof.

16. Use of the nucleic acid complex of any one of claims 1-10 in the preparation of a medicament for treating cancer; or

a neurological disease, optionally a central nervous system (CNS) disease or disorder; and optionally the CNS disease or disorder is selected from the group consisting of: Adrenoleukodystrophy, Alzheimer disease, Amyotrophic lateral sclerosis, Angelman syndrome, Ataxia telangiectasia, Charcot-Marie-Tooth syndrome, Cockayne syndrome, Deafness, Duchenne muscular dystrophy, Epilepsy, Essential tremor, Fragile X syndrome, Friedreich's ataxia, Gaucher disease, Huntington disease, Lesch-Nyhan syndrome, Maple syrup urine disease, Menkes syndrome, Myotonic dystrophy, Narcolepsy, Neurofibromatosis, Niemann-Pick disease, Parkinson disease, Phenylketonuria, Prader-Willi syndrome, Refsum disease, Rett syndrome, Spinal muscular atrophy, Spinocerebellar ataxia, Tangier disease, Tay-Sachs disease, Tuberous sclerosis, Von Hippel-Lindau syndrome, Williams syndrome, Wilson's disease, Zellweger syndrome or a combination thereof

17. The nucleic acid complex for use of claim 14 or 15 or the use of claim 16, wherein the use results in the distribution of the nucleic acid complex into one or more regions selected from the group consisting of: right cortex, prefrontal cortex, sensory cortex, visual cortex, striatum, dorsal hippocampus, ventral hippocampus, thalamus, cerebellum, midbrain, left hemisphere, right hemisphere, dorsal root ganglia, or a combination thereof.

18. The method or use of any one of claims 11-17, wherein the target RNA is a mRNA or a miRNA.

19. The nucleic acid complex for use or use of any one of claims 15-18, wherein the nucleic acid complex is to be administered to a subject (i) via a lipid-mediated delivery system, optionally via liposomes, nanoparticles, or micelles; (ii) via nanoparticles, inorganic nanoparticles, nucleic acid lipid particles, polymeric nanoparticles, lipid nanoparticles (LNPs), chitosan and inulin

nanoparticles, cyclodextrins nanoparticles, carbon nanotubes, liposomes, micellar structures, capsids, polymers, polymer matrices, hydrogels, dendrimers, nucleic acid nanostructure, exosomes, GalNAc-conjugated melittin-like peptides, or combinations thereof.

20. The nucleic acid complex for use or use of any one of claims 15-19, wherein the nucleic acid complex is to be administered to a subject in need thereof via a subcutaneous injection, an intravenous injection, or an intracerebroventricular injection; and/or the nucleic acid complex is administered to a subject in need thereof at a concentration about 0.1-10 nM, optionally about 0.1-1 nM.

21. The nucleic acid complex for use or use of any one of claims 15-20, wherein the nucleic acid complex is to be administered two or more times wherein two administrations of the nucleic acid complex are to be separated by at least 6 months.

22. The nucleic acid complex for use or use of any one of claims 15-21, wherein the nucleic acid complex does not result in an unintended inflammatory response.