



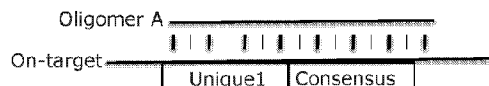
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(54) Title: OLIGOMERS WITH IMPROVED OFF-TARGET PROFILE

On-target binding with various oligomer designs

A



B



C



(57) **Abrégé/Abstract:**

The present invention provides oligomers that binds RNA that consists of a lower affinity region and a higher affinity region, wherein the monomers in the higher affinity region increases the melting temperature (i.e. affinity) of the oligomer base paired to RNA more than the monomers used in the lower affinity region, wherein said increase in melting temperature is relatively to the alternative use of DNA monomers of the same (base) sequence. The oligomers of the invention are useful for binding to target RNA such as mRNA and non-coding RNA and have the advantage that they will be less prone to off-target binding via the lower affinity region than via the higher affinity region.

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On-target binding with various oligomer designs

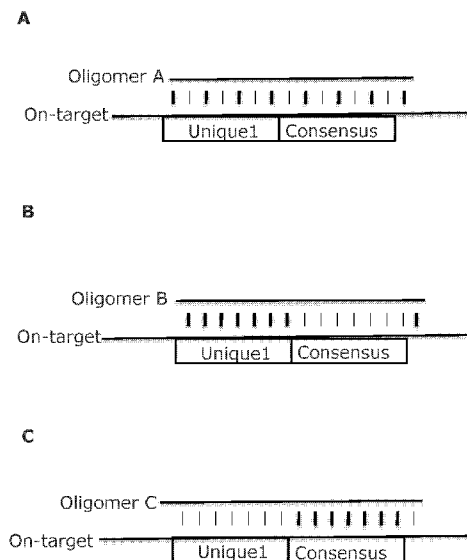


Fig. 1

(57) **Abstract:** The present invention provides oligomers that binds RNA that consists of a lower affinity region and a higher affinity region, wherein the monomers in the higher affinity region increases the melting temperature (i.e. affinity) of the oligomer base paired to RNA more than the monomers used in the lower affinity region, wherein said increase in melting temperature is relatively to the alternative use of DNA monomers of the same (base) sequence. The oligomers of the invention are useful for binding to target RNA such as mRNA and non-coding RNA and have the advantage that they will be less prone to off-target binding via the lower affinity region than via the higher affinity region.

Oligomers with improved off-target profile

Background of the invention

- 5 The present invention relate to oligomers and oligonucleotides that can bind to complementary RNA. Oligonucleotides are useful as reagents in science, research and development e.g. for detection and analysis of cellular RNA or for modulation of the activity of cellular RNA.
- 10 Oligonucleotides are also being developed as therapeutics, where they are typically also intended to bind to and modulate the activity of cellular RNA. Oligonucleotides that bind cellular RNA are herein also termed antisense oligonucleotides.
- 15 Some antisense oligonucleotides act by inducing destabilization of their target RNA e.g. by activating RNase H degradation of the target RNA or by activating the RISC pathway. Such oligonucleotides are typically described as gapmers and siRNAs respectively. Another class of oligonucleotides act as steric blocker and hence prevents the target RNA from interacting with another macromolecule. One
- 20 example is splice switching oligonucleotides that modulate splicing of pre-mRNA. Another example is microRNA inhibitors that bind to microRNA and prevent the microRNA from binding to its mRNA targets. A thorough description of the design, preparation and use of antisense oligonucleotides can be found in "Antisense Drug Technology, Principles, Strategies and Applications, 2nd Edition, Edited by Stanley
- 25 Crooke".

- It is easy to design an antisense oligonucleotide that is complementary to a given target RNA and hence is capable of binding to the target RNA. However, when attempting to develop antisense oligonucleotides as therapeutics,
- 30 complementarity to the target is not enough. The antisense oligonucleotide need to be modified to give it more drug-like properties, e.g. better potency, bioavailability and biostability. Moreover, it has turned out that a problem of many antisense oligonucleotides is off-target binding, i.e. binding to non-intended RNAs,

which may lead to undesirable effects. To minimize off-target binding, it is typically attempted to target unique sequences in the RNA transcriptome. Even so, off-target binding of antisense oligonucleotides is still a problem. One object of the present invention is to provide antisense oligonucleotides, in particular steric
 5 block oligonucleotides that have new and desirable characteristics in terms of off-target binding.

Summary of the invention

The present invention provides oligomers that can bind RNA consisting of
 10

- a lower affinity region,
- a higher affinity region,
- wherein the monomers in the higher affinity region increases the melting temperature (i.e. affinity) of the oligomer base paired to RNA more than
 15 the monomers used in the lower affinity region, wherein said increase in melting temperature is relatively to the alternative use of DNA monomers of the same (base) sequence.

The oligomers of the invention are useful for binding to target RNA such as mRNA
 20 and non-coding RNA and have the advantage that they will be less prone to off-target binding via the lower affinity region than via the higher affinity region.

Brief description of the figures

Figure 1 A show a typical steric block oligomer bound to its intended target (the
 25 on-target). The consensus sequence is a sequence that is shared with other RNAs and is preferably part of a binding site for at cellular molecule, .e.g. a microRNA. The sequence adjacent to the consensus sequence is denoted unique 1 and is sequence that differs (also herein termed "specificity sequence") among RNAs sharing the consensus sequence. Base pairs are indicated by lines between the
 30 oligomer and the target. The oligomer is uniformly modified with monomer analogues that enhance affinity toward complementary RNA and the position of these modifications is shown by a bold (base pair) line to the target. The oligomer

may e.g. be a BNA/2'O-methyl RNA oligomer and the affinity enhancing analogues is then BNA (bicyclic nucleic acids). The illustrated design could just as well have been affinity enhancing analogues in every third position or even in all positions or any other repeating pattern, which gives a relatively uniform affinity over the
 5 length of the oligomer. It is clear that G:C base pairs are stronger than A:U base pairs. However, for the sake of simplicity such sequence specific affinity variations are not considered in the present example.

Figure 1B shows a steric block oligonucleotide of same sequence as oligomer A of
 10 figure 1A, but with a non-uniform distribution of affinity increasing modifications bound to its intended target. The affinity of this oligomer toward complementary RNA will not differ dramatically from the oligomer of 1A. Depending on the monomers chosen, the overall affinity may be higher or lower.

15 Figure 1C shows a yet another steric block oligonucleotide of same sequence as oligomer A of figure 1A, but with a non-uniform distribution of affinity increasing modifications bound to its intended target. The affinity of this oligomer toward complementary RNA will not differ dramatically from the oligomer of 1A or 1B. Depending on the monomers chosen, the overall affinity may be higher or lower.

20

In theory, all 3 oligomers have a similar affinity toward complementary RNA and would therefore be expected to have a similar potency, e.g. when used in cells or organisms. However, they have a different off-target profile (binds to a different set of off-targets).

25

Figure 2 shows the same oligomers as illustrated in figure 1, however, bound to an off-target that shares the consensus sequence with the intended target.

The oligomer in 2A will have an intermediate affinity toward the off-target,
 30 whereas the oligomer in 2B will have a lowered affinity toward the off-target because the region complementary to the consensus sequence is a "lower affinity region", as seen by the lower density of affinity increasing modifications, such as BNA. I.e. the B oligomer will be less likely bind to off-targets comprising the consensus sequence.

35

On the other hand, the C oligomer will however be more likely to bind to off-targets comprising the consensus sequence, because it does so via its "higher affinity region".

5 Figure 3 shows the same oligomers as illustrated in figure 1 and 2, however, bound to an off-target that does not share the consensus sequence. It is seen that oligomer B will do so most potently, because it binds via its higher affinity region, whereas oligomer C will do so least potently because it does so via its lower affinity region. I.e. the increased specificity of oligomer B toward RNA targets
10 that share the consensus sequence is achieved at the expense of decreased specificity toward RNA targets comprising the specificity region.

Disclosure of the invention

The present invention provides oligomers for targeting RNA, e.g. mRNA and non-
15 coding RNA.

A first aspect of the invention is an oligomer of 10-40 monomers that binds RNA comprising (or more preferably consisting of)

- 20 • a lower affinity region,
- a higher affinity region,
- wherein the monomers in the higher affinity region (altogether) increases the melting temperature (i.e. affinity) of the oligomer base paired to complementary RNA more than the monomers used in the lower affinity
25 region (altogether), wherein said increase in melting temperature is relatively to the alternative use of DNA monomers of the same (base) sequence,
- and wherein the lower affinity region or higher affinity region is of 5-12 contiguous monomers, such as 5-10, 5-9, 5-8, 6-10, 7-10, 8-10, 6-9 or 6-
30 8 contiguous monomers and wherein the other region is either contiguous or consists of two sub regions flanking the region of 5-12 monomers.

It should be understood that when referring to *"the monomers in the higher affinity region.."* and *"the monomers used in the lower affinity region"* in the above, reference is to the monomers altogether in the region and not to the monomers individually. Thus, not every monomer in the higher affinity region
 5 does necessarily increase the melting temperature more than individual monomers used in the lower affinity region.

Preferably, the monomers in the higher affinity region (altogether) increases the melting temperature (i.e. affinity) of the oligomer base paired to RNA at least 5
 10 degrees Celsius more than the monomers used in the lower affinity region, such as at least 5 degrees, 10 degrees, 15 degrees, 20 degrees, 25 degrees, 30 degrees, 35 degrees, 40 degrees Celsius more than the monomers used in the lower affinity region, wherein said increase in melting temperature is relatively to the alternative use of DNA monomers of the same sequence.

15

When referring to the melting temperature (also herein termed t_m or t_m value) of the oligomer base paired to RNA, the temperature is preferably measured in 100 mM NaCl, 0.1 mM EDTA, 10 mM NaH_2PO_4 , 5 mM Na_2HPO_4 , pH 7.0), using 1.0 μM concentrations of both oligomer and complementary RNA.

20

In a preferred embodiment, the monomers in the higher affinity region on average (per monomer) increase the melting temperature more than the monomers in the lower affinity region. I.e. the increase in t_m of the lower and higher affinity region is divided by the number of monomers in the lower and higher affinity
 25 region, respectively. It should be noted that the increase in t_m of the lower affinity region may be negative, which is the case when the t_m is decreased relatively to the alternative use of DNA monomers of the same sequence.

Preferably, the monomers in the higher affinity region on average (per monomer)
 30 increase the melting temperature at least 2 degrees Celsius, such as 3, 4, 5, 6, 7 or 8 degrees Celsius more than the monomers in the lower affinity region.

The oligomer is typically an oligonucleotide and preferably comprise at least two different types of monomers, e.g. selected from the group consisting of DNA,
 35 RNA, BNA (in particular 2',4'-bicyclic nucleic acids (2'4'-BNA) such as ENA and

LNA (2'-O,4'-C-methylene-beta-D-ribofuranosyl and analogues thereof e.g., amino-LNA, thio-LNA, alfa-L-ribo-LNA, beta-D-xylo-LNA, S-cMOE (2',4'-constrained MOE), R-cMOE, cEt (2',4'-constrained ethyl), morpholino, phosphoramidate, FANA, PNA, CENA, 2'MOE, 2'F, 2'O-methyl.

5

It is particular preferred that the higher affinity region comprises 2',4'-bicyclic nucleic acids (2'4'-BNA).

In another embodiment, the lower affinity region comprises only two types of
10 monomers, preferably selected from the group consisting of DNA, RNA, morpholino, phosphoramidate, FANA, PNA, UNA, 2'MOE, 2'F and 2'O-methyl.

In another embodiment, the lower affinity region comprises only one type of monomer, preferably selected from the group consisting of morpholino,
15 phosphoramidate, FANA, PNA, UNA, 2'MOE, 2'F, and 2'O-methyl.

As is known to the skilled man, different monomers generally have different effects on tm. E.g. BNAs have a strong effect on tm and 2'-modifications have a moderate effect. Some modifications like e.g. UNA even decrease tm, i.e. have a
20 negative effect.

As used in the present context, 2',4'-bicyclic nucleic acids are herein classified as monomers that have a strong effect on tm. Morpholino, phosphoramidate-, FANA, PNA, CENA, 2'MOE, 2'F, 2'O-methyl monomers are herein classified as monomers
25 that have a moderate effect on tm. Monomers that decrease tm are e.g. UNA (unlocked nucleic acid). DNA and RNA monomers are herein classified as neutral monomers with regard to tm (DNA monomers are reference monomers).

In a preferred embodiment, the lower affinity region has a lower density (number
30 of affinity increasing monomers/total number of monomers in the given region) of monomers that have a strong and/or moderate effect on tm than does the higher affinity region. This feature can usually be used to easily identify oligomers of the invention, e.g. by identifying the lower affinity region within the oligomer.

It follows that oligomers with a uniform distribution of high affinity/and or moderate monomers are not part of the scope of this invention, because they do not comprise a lower affinity region. Also oligomers with slight deviations from a uniform distribution are not part of the scope of this invention, as slight deviations
 5 are not enough to create a region of lower affinity that differ substantially in affinity (as expressed in the melting temperature of the oligonucleotide) from the rest of the oligomer.

In many embodiments, the oligomer comprises one or more phosphorothioate
 10 linkages. The oligomer may e.g. be fully modified with phosphorothioate linkages or e.g. one, two or three phosphorothioate linkages at each end. Phosphorothioate linkages generally decrease melting temperature. However, this may be counteracted e.g. by the use of 2'4' BNA monomers. I.e. the higher affinity region may very well comprise phosphorothioate linkages.

15

Length and affinity of the oligomer

The overall affinity (as expressed by the melting temperature) of the oligomer toward complementary RNA depends on the length of the oligomer as well as the content of affinity increasing (and sometimes decreasing) monomers. It is an
 20 object of the invention to design the oligomer such that it has sufficient affinity toward its intended target, while displaying improved selectivity and/or potency toward its intended target over other targets sharing an identical sequence (which is hence a shared sequence).

25 The length of the oligomer is preferably between 10 and 40 monomers. In other embodiments, the length is between 10 and 35 monomers, between 10 and 30 monomers, between 10 and 25 monomers. Even more preferably, the length of the oligomer is between 10 and 20 monomers, such as between 11 and 19 monomers or between 12 and 18 monomers.

30

When using shorter lengths, it is necessary to use a higher density of monomers that increase the affinity of the oligomer. Particular preferred are 2',4'-bicyclic nucleic acids (2'4'-BNA) that have a strong effect on affinity. Hence, in a preferred embodiment, at least 30 % of the monomers are 2'4'-BNA monomers, e.g.

selected from the group consisting of: LNA (2'-O,4'-C-methylene-beta-D-ribofuranosyl and analogues thereof e.g., amino-LNA, thio-LNA, alfa-L-ribo-LNA, beta-D-xylo-LNA, S-cMOE (2',4'-constrained MOE), R-cMOE, cEt (2',4'-constrained ethyl). More preferably, at least 35 %, such as least 40%, 45%, 50%,
 5 55% or 60 % are 2'4'-BNA monomers.

The melting temperature of the oligomer base paired to RNA should preferably be at least 60 degrees Celsius. In other embodiments, it is preferred that the t_m of the oligomer is at least 65 degrees Celsius, at least 70 degrees Celsius, at least 75
 10 degrees Celsius, at least 80 degrees Celsius, at least 85 degrees Celsius, at least 90 degrees Celsius or at least 95 degrees Celsius.

It is even more preferred that the t_m is at least 75 degrees Celsius, such as 80, 85, 90 or 95 degrees Celsius.

15

In one embodiment, the lower affinity region is shorter than the higher affinity region. In another region, the lower affinity region is longer than the higher affinity region.

20 *The lower and higher affinity regions*

As mentioned above, it is preferred that the lower affinity region has a lower density of monomers that have a strong and/or moderate effect on t_m than does the higher affinity region.

25 When the oligomer comprise 2'4'-BNA monomers, the density of these may e.g. 100 % in the higher affinity region (full modification), 50% (mixmer with 1 BNA per 1 other monomer, e.g. 1 BNA monomer in every second position), 33 % (1 BNA per 2 other monomers, e.g. in every third position), 25 % etc. The BNAs need not necessarily be evenly dispersed the high affinity region. At any rate, the
 30 lower affinity region is easily identified by the lower density of 2'4'-BNA monomers. When the higher affinity region comprises 2'4' BNA monomers, it typically contains at least 1 BNA per 4 non-BNA monomers and also typically does not contain 4 or 5 contiguous non-BNA monomers.

The amount of 2'4'-BNA monomers in the higher affinity region is preferably at least 2 fold, or even more preferably 3, 4 or 5 fold higher than in the lower affinity region of the oligomer.

- 5 In a preferred embodiment, the lower affinity region does not comprise any affinity enhancing monomers selected from the group consisting of 2'4'-BNA, PNA, 2'MOE and 2'F.

More preferably, the lower affinity region does not comprise any 2'4'-BNA
10 monomers.

In another embodiment, the lower affinity region comprise at least 5 contiguous monomers, such as 6 or 7 contiguous monomers which are not 2'4'BNA monomers.

15

In yet another embodiment, the lower affinity region comprise at least 5 contiguous monomers, such as 6 or 7 contiguous monomers which are not DNA monomers.

- 20 In yet another embodiment, neither the 5'end of the lower affinity region nor the 3'end of the lower affinity region is a BNA monomer. Even more preferably, neither the two monomers at the 5'end of the lower affinity region nor the two monomers at the 5'end of the monomer are BNA monomers. This feature further helps define the lower affinity region within an oligomer of the invention.

25

Preferably, the melting temperature of the lower affinity region toward complementary RNA is less than 70 degrees Celsius, such as less than 65, 60, 55, 50, 45, 40 and 35 degrees Celsius. The melting temperature in this embodiment may be determined by measuring the t_m against a truncated RNA with a length
30 corresponding to the lower affinity region, i.e. where the truncated RNA cannot base pair to the higher affinity region of the oligomer. It is even more preferred that the melting temperature of the lower affinity region toward complementary RNA is less than 60 degrees Celsius, such as less than 55, 50, 45, 40 and 35 degrees Celsius.

35

Preferably, the lower affinity region is contiguous and of 5-12 monomers, such as 5-10, 5-9, 5-8, 6-10, 7-10, 8-10, 6-9 or 6-8 contiguous monomers. Even more preferred is a lower affinity region of 6-10 contiguous monomers. I.e. in a preferred embodiment, the lower affinity region of the oligomer can be identified
 5 as a region with none or just 1 BNA monomers over a region of 6-10 monomers, wherein the density of BNA monomers in the remaining part of the oligomer has a higher density of BNA monomers, and hence the remaining part of the oligomer is the higher affinity region.

10 Uses and advantages - selectivity

The oligomers of the invention are useful for binding to target RNA such as mRNA and non-coding RNA and have the advantage that they will be less prone to off-target binding via the lower affinity region than via the higher affinity region.

15

Consider e.g. two RNA sequences of 10-40 monomers (within RNA targets such as mRNA or non-coding RNA) that have identical sequences in one region and differ e.g. by 1, 2 or 3 nucleotides over a length of between 5 and 10 nucleotides in sequence in an adjacent region. The region that differs may be termed the
 20 "specificity region", whereas the identical region may be referred to as the "shared region". There could just as well be multiple target RNAs that share the shared sequence and differ in the adjacent specificity region.

When the goal is to design an oligomer that preferentially bind one RNA sequence
 25 (the intended target, herein also termed the on-target) over the other target (the off-target), it is obvious that the oligomer should be complementary to the intended target. However, before the present invention, it was not obvious to design and use an oligomer comprising a higher affinity region and a lower affinity such that the higher affinity region of the oligomer should be complementary to
 30 the specificity region of the (intended) on-target and the lower affinity region should be complementary to the shared region (shared between the off-targets and the on-target). In this way, off-target binding via the shared region will be minimized because of a lower affinity as expressed in t_m , while still maintaining high affinity to the intended target.

On the other hand, if a certain degree of off-target binding is acceptable or even desirable, the higher affinity region of the oligomer should be complementary to the shared region and the lower affinity region should be complementary to the
 5 specificity sequence. In this way, off-target binding via the shared region will be increased.

It is to be understood that oligomers of the invention do not necessarily overall have less or more binding to off-targets in the transcriptome of a cell when
 10 compared to a uniformly modified oligomer of the same sequence.

However, they will have decreased binding to off-targets sharing the same shared sequence (because the lower affinity region is complementary to the shared sequence). Potentially, this may be achieved at the expense of more off-target binding via the higher affinity region. At any rate, the oligomers of the invention
 15 have a different off-target profile as compared to uniformly modified oligomers of the same sequence.

Hence, the oligomers of the invention can be designed to have less off-target binding among off-targets of interest or more off-target binding among off-targets
 20 of interest compared to a uniformly modified oligomer of the same sequence and similar t_m value.

It should be understood that the oligomer will preferably have perfect complementarity to its intended target.
 25

The non-obvious characteristics and uses of the oligomer of the invention will be further described below.

Activity/mechanism of action of the oligomer of the invention – steric block

30 The oligomers of the invention are typically steric blockers, i.e. they bind to target RNA without mediating degradation of the target RNA. Steric block oligonucleotides are well described in the art and are e.g. used as splice switching oligonucleotides and microRNA inhibitors. As is known by the skilled man, many monomers can be used in the design of steric block oligonucleotides, since most

artificial nucleotide monomers do not allow recruitment of RNase H. See e.g. Antisense Drug Technology, Principles, Strategies and Applications, 2nd Edition, Edited by Stanley Crooke, page 93, which mentions 2-O-Me, 2-O-methoxyethyl (MOE), and 2-O-aminopropyl, phosphoramidate, and methylphosphonate derivatives, as well as locked nucleic acid (LNA/2'4'-BNA), peptide nucleic acid (PNA), and morpholino based oligomers. Also mixtures of these monomers do not allow RNase H recruitment. RNase H recruitment generally requires at least 5 contiguous DNA monomers and most often 6, 7 or 8 contiguous monomers are used. Therefore, in a preferred embodiment, the oligomer of the invention does not comprise contiguous DNA monomers, such as 5, 6, 7 or 8 contiguous DNA monomers.

In one embodiment, the oligomer does not comprise a Morpholino monomer.
 In another embodiment, the oligomer does not comprise a FANA monomer.
 15 In another embodiment, the oligomer does not comprise a PNA monomer.
 In another embodiment, the oligomer does not comprise a CENA monomer.
 In another embodiment, the oligomer does not comprise a 2'MOE monomer.
 In another embodiment, the oligomer does not comprise a 2'F monomer.
 In another embodiment, the oligomer does not comprise a 2'O-methyl monomer.
 20 In yet another embodiment, the oligomer does not comprise a phosphoramidate monomer.

Preferred target RNAs and sequences

25 Binding sites for cellular macromolecules such as RNA binding proteins and RNA (e.g. mRNA or microRNA) often comprise a sequence which is shared among other binding sites of the same cellular macromolecule. Such sequences are herein referred to as consensus sequences. As described above, it is one objective of the present invention to provide oligomers that have improved (or reduced) selectivity
 30 in terms of targeting an intended target (RNA) over other RNAs that share a sequence with the intended target. In a preferred embodiment, the shared sequence is a binding site for an RNA binding protein or RNA or and the shared sequence may be represented by a consensus sequence.

One way to avoid or reduce off-target binding to other RNAs sharing the same (consensus) sequence could be to avoid the consensus sequence as target, i.e. not included a sequence complementary to the consensus region in the oligomer. However, when the oligomer is intended to block a binding site, this may reduce the activity of the oligomer, since the binding site is then not completely blocked. Most often it will be desirable to indeed target the consensus sequence as well as adjacent sequence(s).

Oligomers with increased selectivity are most preferred. In these embodiments, the lower affinity region is preferably complementary to a consensus sequence. The length of the lower affinity region is preferably the same as the length of the consensus sequence or 1, 2, 3 or 4 monomers longer. The additional monomers may be added to the 5'end of the lower affinity region, the 3'end of the lower affinity region or both.

Preferred consensus sequences are microRNA binding sites in mRNA of a given microRNA, which typically share complementarity to position 2-7 (the so-called seed sequence) of the microRNA, but differ in sequence outside of this region. I.e. in this instance the consensus sequence of the binding site is a sequence that is complementary to the seed sequence of the microRNA.

Another preferred consensus sequence is the seed sequence shared among microRNA families that typically differ outside of the seed sequence.

Hence, in a preferred embodiment, the oligomer of the invention comprise a lower affinity region that can bind to a RNA sequence that is complementary to position 1-7, 2-7, 1-8 or 2-8 of any of SEQ ID NOs: 1-2042 or a lower affinity region that can bind to a RNA sequence that comprise position 1-7, 2-7, 1-8 or 2-8 of any SEQ ID NOs: 1-2042, wherein the positions are counted from the 5' end. The region capable of binding to the consensus sequence may be referred to as the anti-consensus sequence, which is hence comprised within the lower affinity region of the oligomer or is identical to the lower affinity region of the oligomer. In this embodiment, the oligomer improves selectivity, when targeting one microRNA over other microRNA in the same family or when targeting one microRNA binding site over other binding sites of the same microRNA.

Likewise, the oligomers may also be used for decreasing selectivity. In this embodiment, the oligomer of the invention comprise a higher affinity region can bind to a RNA sequence that is complementary to position 1-7, 2-7, 1-8 or 2-8 of any of SEQ ID NOs: 1-2042 or a lower affinity region that can bind to a RNA sequence that comprise position 1-7, 2-7, 1-8 or 2-8 of any SEQ ID NOs: 1-2042, wherein the positions are counted from the 5' end.

Oligomers with increased selectivity are most preferred.

10

Table 1. Human microRNA sequences

NO	Sequence (5' to 3')	ID and accession
1	AAGAUGUGGAAAAAUUGGAAUC	>hsa-miR-576-3p MIMAT0004796
2	CAGUGGUUUUACCCUAUGGUAG	>hsa-miR-140-5p MIMAT0000431
3	CAGGCAGUGACUGUUCAGACGUC	>hsa-miR-2682-5p MIMAT0013517
4	AAGAAGAGACUGAGUCAUCGAAU	>hsa-miR-5197-3p MIMAT0021131
5	CUCUAGAGGGAAGCGCUUUCUG	>hsa-miR-522-5p MIMAT0005451
6	UUUCUGUCUUUUCUGGUCCAG	>hsa-miR-4743-3p MIMAT0022978
7	GUUUGCACGGGUGGGCCUUGUCU	>hsa-miR-557 MIMAT0003221
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2029	AGAGUCGGCGACGCCGCCAGC	>hsa-miR-4785 MIMAT0019949

2030	UGGGGGAGCCAUGAGAUAGAGCA	>hsa-miR-4723-5p MIMAT0019838
2031	AUCAAUAAGGACUAGUCUGCA	>hsa-miR-3671 MIMAT0018094
2032	CGCCCCUCCUGCCCCACAG	>hsa-miR-4749-3p MIMAT0019886
2033	UGAGCCCCUGUGCCGCCCCAG	>hsa-miR-1225-3p MIMAT0005573
2034	GCUGAUGAUGAUGGUGCUGAAG	>hsa-miR-4502 MIMAT0019038
2035	GGGAGUCUACAGCAGGG	>hsa-miR-4294 MIMAT0016849
2036	CACUCAGCCUUGAGGGCACUUUC	>hsa-miR-512-5p MIMAT0002822
2037	GACACAUGACCAUAAAUGCUGAA	>hsa-miR-4643 MIMAT0019703
2038	UAAGUGCUUCCAUGUUUGAGUGU	>hsa-miR-302d-3p MIMAT0000718
2039	UGCACGGCACUGGGGACACGU	>hsa-miR-3177-3p MIMAT0015054
2040	CCUCCUGCCCUCUUGCUGU	>hsa-miR-1976 MIMAT0009451
2041	CAACGGAAUCCCAAAGCAGCUG	>hsa-miR-191-5p MIMAT0000440
2042	CAGCCCUCUCCCGCACCCAAA	>hsa-miR-4687-5p MIMAT0019774

In one embodiment, the oligomer does not comprise a lower affinity region that can bind to a RNA sequence that is complementary to position 2-7 of SEQ ID NO:1051.

5

In yet another embodiment, the oligomer is not complementary to any of SEQ ID NOs: 1-2041 (over its full length).

Particular preferred oligomers of the invention have a length of 10-20 monomers, and consists of

- a higher affinity region that comprise at least 25% BNA monomers
- a lower affinity region of 6-10 contiguous monomers that comprise less than 25% BNA monomers and wherein the two monomers at the 5' end of the lower affinity region and the two monomers at the 3' end of the lower affinity region are non-BNA monomers
- and wherein the oligomer does not comprise a region of 5 contiguous DNA monomers

In this embodiment, different affinities of the higher affinity region and the lower affinity region (as defined above) is ensured by a higher density of BNA

monomers in the higher affinity region as compared to the density of BNA monomers in the lower affinity region.

It is preferred that the higher affinity region consist of at least 4 monomers, more preferably, at least 5, 6, 7 or 8 monomers.

Preferably, the density of BNA monomers in the higher affinity region is at least 2-fold higher than the density of BNA monomers in the lower affinity region. Even more preferably, the density is at least 3-fold higher such as 4 or 5 fold higher.

10

In a preferred embodiment, the lower affinity region does not comprise any BNA monomers at all.

The higher affinity region preferably comprise at least 33% (1 BNA monomer/3 monomers in the higher affinity region), 50% (1 BNA/2 monomers), 67% (2 BNA/3 monomers), (3 BNA/4 monomers) 75 % or 100 % BNA monomers.

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Preferably, at least one internucleotide linkage is a phosphorothioate linkage. In another embodiment, all internucleotide linkages are phosphorothioate linkages.

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In a related embodiment, the oligomer the lower affinity region consists of 8-10 contiguous monomers and the remaining monomers of the oligomer are BNA monomers.

25 Typically, both the 5'end of the oligomer and the 3'end of the oligomer is a BNA monomer.

The oligomer may even be of 12-18 monomers or 13-17 monomers.

30 Preferably, the lower affinity region can base pair to the seed sequence of a microRNA or can base pair to the RNA complement of the seed sequence of a microRNA. Human microRNAs are preferred.

Examples

Example 1

In the present example, the affinity of various oligomers toward an intended target, as well as to an off-target RNA was measured. Finally, the affinity of the
5 oligomers toward a target RNA consisting of the consensus sequence was measured.

The employed target RNA first was:

10

(135-Hcvtarg) 5'GAUGGGGGCGACACUCCACCAUGAAU

wherein a consensus sequence is underlined.

15 The sequence of the oligomers complementary to this target RNA:

5' CAUGGUGGAGUGUC

In reverse orientation:

20 CUGUGAGGUGGUAC-5'

And when bound to each other:

Target: 5'GAUGGGGGCGACACUCCACCAUGAAU

25 .

| | | | | | | | | | |

HCV Oligomer

CUGUGAGGUGGUAC5'

First, the following oligomers were employed, wherein lower affinity regions are underlined:

30 (123-HCVtm, uniformly modified)

5'ICIAmUmGIGmUIGIGmAlGmUmGITIC

(124-HCVtm, lower affinity region complementary to consensus sequence)

5'ICIAITIGITmGmGmAmGmUmGIUIC

(125-HCV, tm lower affinity region complementary to consensus sequence)

5'ICIAITIGmUmGmGmAmGmUmGmUIC

(126-HCVtm, lower affinity region complementary to consensus sequence)

5'ICIAITIGmUmGmGuAmGmUmGmUIC

5 (127-HCVtm, lower affinity region complementary to consensus sequence)

5'ICIAmUmGIGmUmGmGmAmGmUmGITIC

(128-HCVtm, higher affinity region complementary to consensus sequence)

5'mCmAmUmGmGITIGIGIAIGITIGITmC

10 m indicates 2'O-methyl RNA

l indicate LNA (BNA)

u indicate unlocked Nucleic Acid (UNA)

The melting temperatures were measured in in medium salt concentration, i.e.

15 100 mM NaCl, 0.1 mM EDTA, 10 mM NaH₂PO₄, 5 mM Na₂HPO₄, pH 7.0), using 1.0 µM concentrations of both oligomer and complementary RNA and in low salt concentration, i.e. in 10 mM NaCl instead of 100 mM NaCl.

Table 2:

20

Oligomer	Tm, 100 mM NaCl	Tm, 10 mM NaCl
123-HCVtm	>90	86
124-HCVtm	>90	84
125-HCVtm	>90	80
126-HCVtm	79	65
127-HCVtm	>90	79
128-HCVtm	>85	83

For the measurements in high salt, only 126HCVtm sticks out with a lower tm value, which is in line with the presence of a UNA monomer that decreases affinity.

25

For the measurements in low salt, the tm values correlate well with the content of LNA monomer and again it is seen that one UNA monomer decreases affinity significantly.

Next, the affinity of another oligomer sequence (Aldo A) toward the same target RNA was measured. This other oligomer is complementary to the same consensus sequence of the target RNA, but differs in the remaining part of the sequence:

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Target: 5' GAUGGGGGCGACACUCCACCAUGAAU

|11111111

Aldo Oligomer: GUUGUGAGGUCCGG5'

1 indicate a potential G:U base pair.

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The oligomers used in these measurements were:

Lower affinity regions are underlined

(129-Aldo, uniformly modified)

15 5'1G1GmC1CmU1GmG1AmGmU1GmU1T1G

(130-Aldotm, lower affinity region complementary to consensus sequence)

5'1G1G1C1C1TmGmGmAmGmUmG1T1T1G

(131-Aldotm, lower affinity region complementary to consensus sequence)

20

5'1G1G1C1CmUmGmGmAmGmUmGmU1T1G

(133-Aldotm, higher affinity region complementary to consensus sequence)

5' mGmGmCmC1T1G1G1A1G1T1G1TmUmG

25

Table 3:

Oligomer	Tm, 100 mM NaCl
129-Aldotm	77
130-Aldotm	70
131-Aldotm	66
133-Aldotm	83

In this set of measurements, the HCV target may be seen as an off-target that shares the consensus target with the intended target of the oligomer. Again the

melting temperature correlates overall with the content of LNA. Moreover, it is seen that affinity increases when the content of LNA increases in the complementary region. When the LNA density is higher in the non-complementary region, the melting temperature decreases, see oligomer 131.

5

Next, the affinity of the oligomers toward a complementary 8 nucleotide consensus RNA was measured. The affinity of the oligomers toward this 8 nucleotide consensus RNA may be seen as a measure of the affinity of the oligomer toward to off-targets comprising the consensus sequence, but which are not complementary to the oligomer in the regions adjacent to the consensus sequence.

15 The consensus RNA was:

5'ACACUCCA

Table 4:

Oligomer	T _m , 100 mM NaCl
123-HCVtm	77
124-HCVtm	59
125-HCVtm	48
126-HCVtm	No transition
127-HCVtm	60
128-HCVtm	82
129-Aldotm	73
130-Aldotm	66
131-Aldotm	55
132-Aldotm	84

20 As seen, less off-target binding (more specific oligomers) can be designed by reducing the LNA content in the complementary region.

If the same design principles are used to design antisense oligomers toward other target sequences comprising a consensus sequence, generally improved specificity can be achieved.

- 5 Thus, the teachings of the present invention, including the findings in the above experiments, should be generally applicable and not limited to certain sequences/targets.

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Claims

1. An oligomer of 10-20 monomers that binds RNA that consists of
 - 5 • a lower affinity region of 6-10 contiguous monomers that comprise no BNA monomers
 - a higher affinity region of at least 4 monomers that comprise at least 25 % BNA monomers, said higher affinity region being either
 - 10 composed of contiguous monomers or consisting of two sub regions flanking the lower affinity region
 - wherein the oligomer does not comprise a region of 5 contiguous DNA monomers and
 - 15 • wherein the lower affinity region can base pair to the seed sequence of a microRNA or can base pair to the RNA complement of the seed sequence of microRNA, wherein the seed sequence is position 2-7 of the microRNA.
 - 20
2. The oligomer of claim 1, wherein at least one internucleotide linkage is a phosphorothioate linkage.
3. The oligomer of claim 1 or 2, wherein the lower affinity region can base
 - 25 pair to the RNA complement of the seed sequence of a microRNA.
4. The oligomer of any one of claims 1 to 3, wherein both the 5'end of the oligomer and the 3'end of the oligomer is a BNA monomer.
- 30 5. The oligomer of any one of claims 1 to 4, consisting of 12-18 monomers.
6. The oligomer of claim 1, wherein the microRNA is a human microRNA.

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On-target binding with various oligomer designs

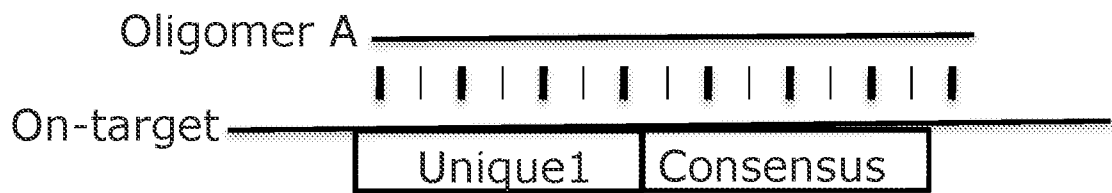
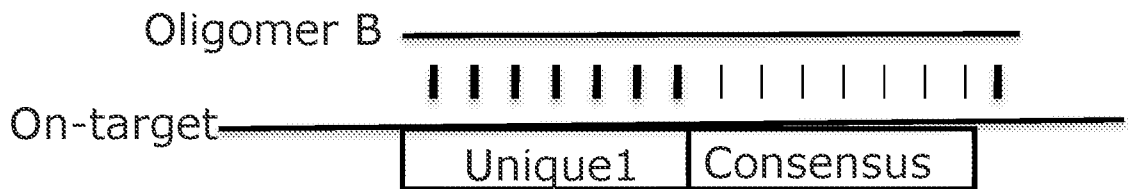
A**B****C**

Fig. 1

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Off-target binding to competing consensus sequence

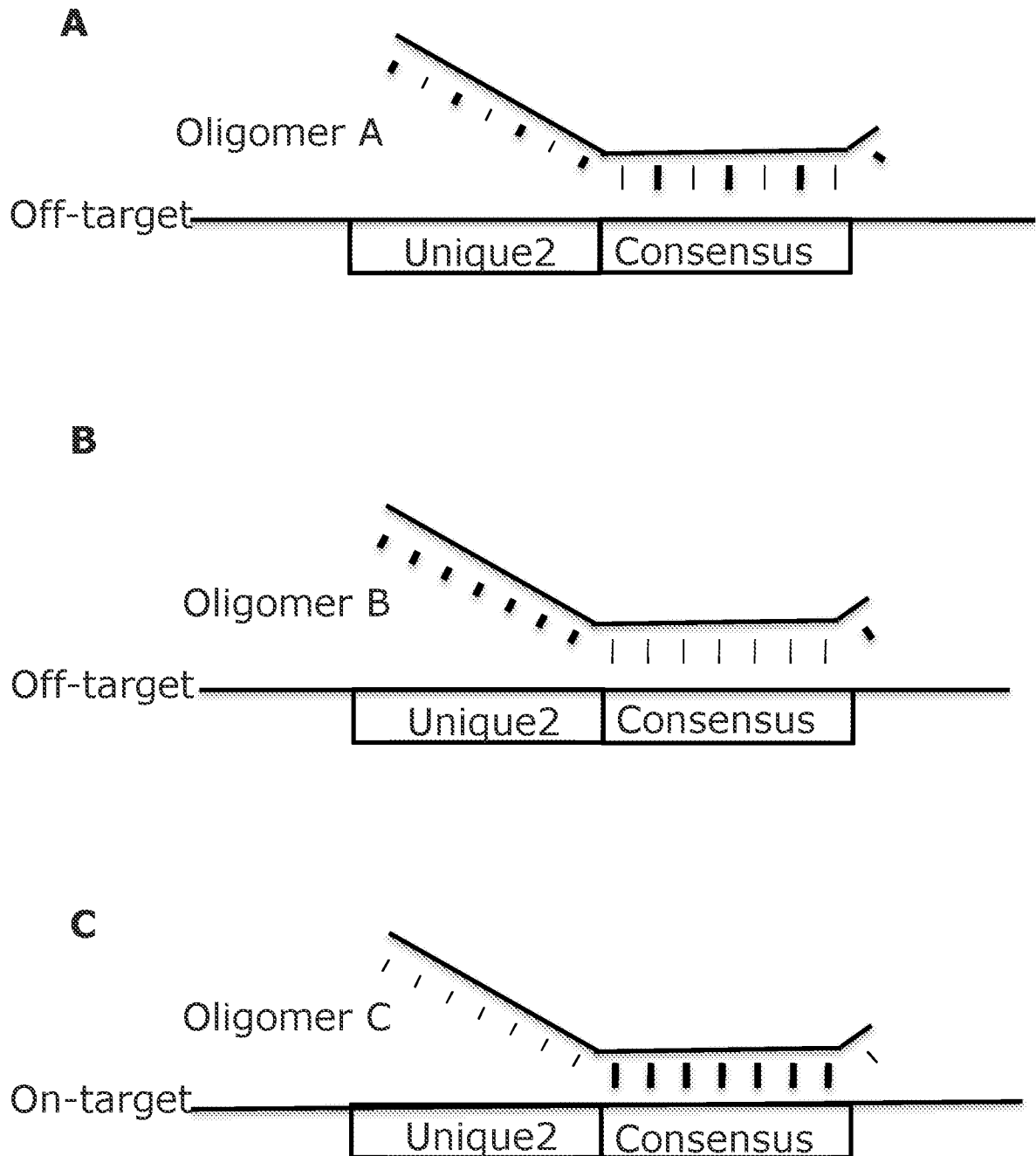


Fig. 2

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Off-target binding to non-consensus target

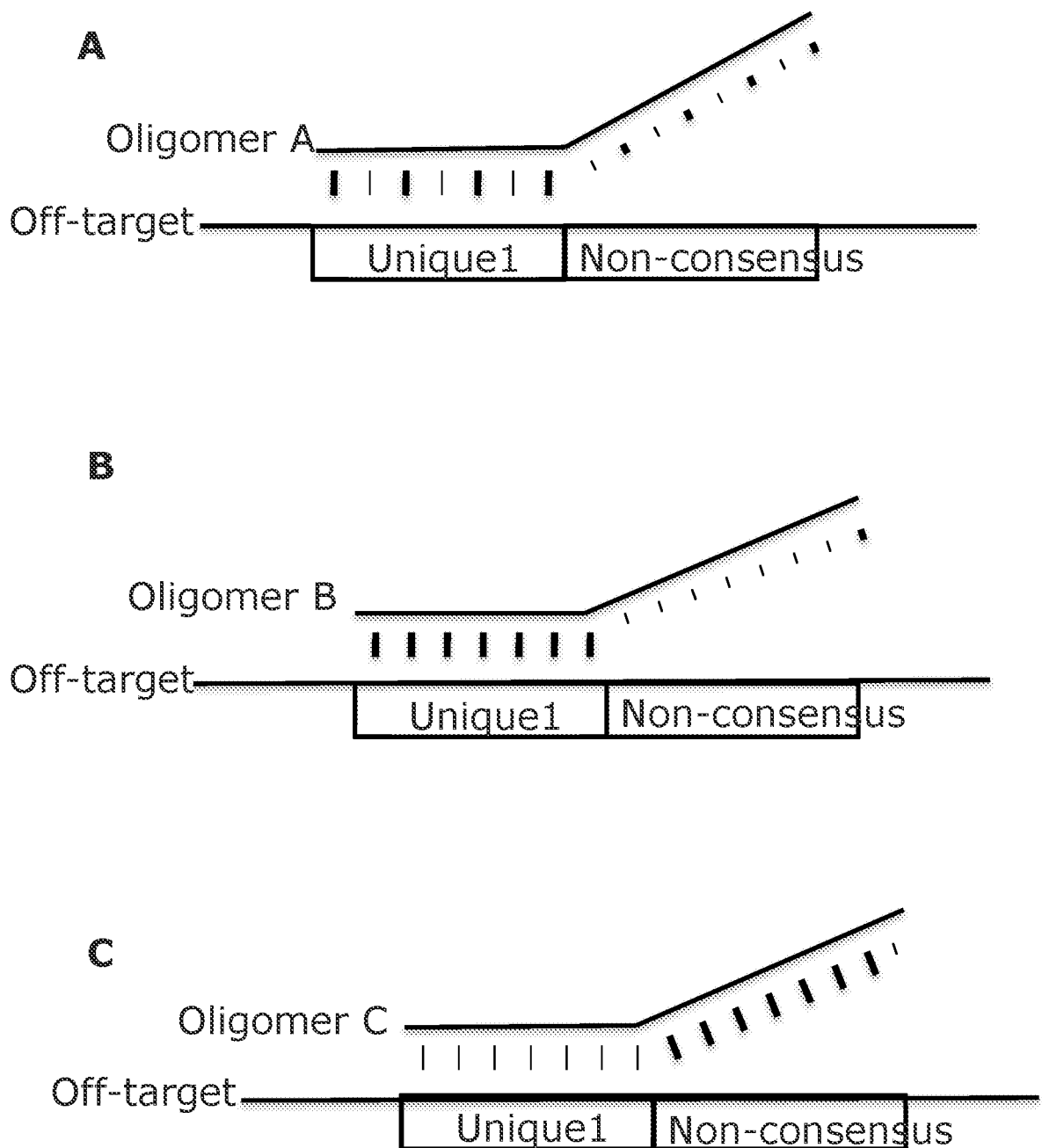


Fig. 3

On-target binding with various oligomer designs

A



B



C

