Claims

1. A method for analyzing an RNA molecule comprising the following steps:
   (i) providing an RNA molecule;
   (ii) providing at least one conjugate comprised of a chemical moiety with RNA cleaving activity and an oligonucleotide, wherein the sequence of said oligonucleotide is complementary to a target sequence of the RNA molecule;
   (iii) cleaving the RNA molecule provided in step (i) to obtain RNA fragments by contacting the RNA molecule with the at least one conjugate provided in step (ii) under conditions allowing the hybridization of said oligonucleotide to said target sequence and the cleavage of the RNA molecule; and
   (iv) determining a physical property of the RNA molecule by analyzing one or more of the RNA fragments obtained in step (iii),
   wherein the RNA molecule is an mRNA molecule and comprises a 5′ cap structure and/or a 3′ homopolymeric sequence.

2. The method of claim 1, wherein cleaving the RNA molecule results in a 5′ fragment, a 3′ fragment and optionally one or more central fragments; and/or wherein the fragments are separated from each other before analyzing the one or more of the RNA fragments in step (iv),
   optionally wherein the fragments are separated
   (a) by chromatography, preferably by HPLC or by an affinity chromatography including an oligo-dT based capturing column chromatography; or
   (b) by electrophoresis.

3. The method of claim 2, wherein the 5′ fragment is analyzed and/or the 3′ fragment is analyzed,
   optionally wherein the 5′ fragment is analyzed for one or more of
   (i) presence and/or integrity of the cap structure,
   (ii) methylation pattern; and
   (iii) orientation;
   preferably by analytical HPCL and/or mass-spectrometry, and/or
   wherein the 3′ fragment is analysed, preferably wherein the 3′ fragment comprises a homopolymeric sequence, more preferably a polyA and/or polyC sequence.

4. The method of claim 3, wherein
   (a) the 5′ fragment has a length of ~~about~~ 1 to ~~about~~ 100 nucleotides, preferably ~~about~~ 1 to ~~about~~ 50 nucleotides, more preferably ~~about~~ 1 to ~~about~~ 25 nucleotides and most preferably ~~about~~ 10- to 15 nucleotides;

(b) the 3′ fragment is analyzed for its nucleotide composition and/or length, preferably by complete hydrolysis of the 3′ fragment followed by analysis of the individual nucleotides gained thereby by analytical HPLC and/or mass spectrometry;

(c) the 3′ fragment has a length of ~~about~~ 10 to ~~about~~ 500 nucleotides, preferably ~~about~~ 50 to ~~about~~ 500 nucleotides and more preferably ~~about~~ 50 to ~~about~~ 250 nucleotides; and/or

(d) the analysis of the fragments involves mass spectroscopy, preferably analytical mass spectrometry, HPLC, preferably analytical HPLC and/or sequencing.

5. The method of any one of claims 1-4, wherein the RNA molecule

(a) is single stranded RNA, preferably a therapeutic mRNA molecule; and/or

(b) is generated by RNA in vitro transcription.

6. The method of any one of claims 1-5, wherein at least two conjugates are provided and contacted

(a) at the same time with the RNA molecule to simultaneously cleave the RNA molecule, wherein the at least two conjugates comprise oligonucleotides with sequences complementary to different target sequences of the RNA molecule; or

(b) one after the other with the RNA molecule to sequentially cleave the RNA molecule, wherein the at least two conjugates comprise oligonucleotides with sequences complementary to different target sequences of the RNA molecule.

7. The method of any one of claims 1-6, wherein the target sequence is present

(a) once in the RNA molecule; or

(b) more than once in the RNA molecule.

8. A method for analyzing a population of RNA molecules comprising the following steps:

(i) providing a population of RNA molecules, wherein the population of RNA molecules comprises at least two different types of RNA molecules, wherein the different types of RNA molecules comprise an identical target sequence;

(ii) providing a conjugate comprised of a chemical moiety with RNA cleaving activity and an oligonucleotide, wherein the sequence of said oligonucleotide is complementary to the target sequence;

(iii) cleaving the population of RNA molecules provided in step (i) to obtain RNA fragments by contacting the RNA molecules with the conjugate provided in step (ii) under conditions allowing the hybridization of said oligonucleotide to said target sequence and the cleavage of the RNA molecules; and

(iv) determining a physical property of the RNA molecules in the population by analyzing one or more of the RNA fragments obtained in step (iii);

wherein the population of RNA molecules comprises RNA molecules comprising a 5′ cap structure and/or a 3′ homopolymeric sequence and wherein the RNA molecules are mRNA molecules.

9. A method for analyzing a population of RNA molecules comprising the following steps:

(i) providing a population of RNA molecules, wherein the population of RNA molecules comprises at least two different types of RNA molecules, wherein the different types of RNA molecules comprise different target sequences;

(ii) providing at least two conjugates comprised of a chemical moiety with RNA cleaving activity and an oligonucleotide, wherein the oligonucleotide sequence of each conjugate is complementary to one of the different target sequences;

(iii) cleaving the population of RNA molecules provided in step (i) to obtain RNA fragments by contacting the RNA molecules with the at least two conjugates provided in step (ii) under conditions allowing the hybridization of said oligonucleotides to said target sequences and the cleavage of the RNA molecules; and

(iv) determining a physical property of the RNA molecules in the population by analyzing one or more of the RNA fragments obtained in step (iii);

wherein the population of RNA molecules comprises RNA molecules comprising a 5′ cap structure and/or a 3′ homopolymeric sequence and wherein the RNA molecules are mRNA molecules.

10. The method of claim 8 or 9, wherein cleaving the population of RNA molecules results in 5′ fragments, 3′ fragments and optionally central fragments; and/or
wherein the fragments are separated from each other before analyzing the RNA fragments in step (iv), optionally wherein the fragments are separated
(a) by chromatography, preferably by HPLC or by an affinity chromatography including an oligo-dT based capturing column chromatography; or
(b) by electrophoresis.

11. The method of ~~any one of~~ claim~~s 8~~ 10, wherein the 5′ fragments are analyzed and/or the 3′ fragments are analyzed; optionally wherein the 5′ fragments are analyzed for one or more of
(i) presence and/or integrity of the cap structure,
(ii) methylation pattern; and
(iii) orientation,
preferably by analytical HPCL and/or mass-spectrometry; and/or
wherein the 3′ fragments are analysed, preferably wherein the 3′ fragments comprise a homopolymeric sequence, preferably a polyA and/or polyC sequence.

12. The method of claim 11, wherein
(a) the 3′ fragments are analyzed for their nucleotide composition and/or length, preferably by complete hydrolysis of the 3′ fragments followed by analysis of the individual nucleotides gained thereby by analytical HPLC and/or mass spectrometry; and/or
(b) the analysis of the fragments involves mass spectroscopy, HPLC and/or sequencing.

13. The method of any one of claims 8-12, wherein the population of RNA molecules

(a) comprises single stranded RNA molecules, preferably therapeutic mRNA molecules; and/or
(b) is generated by RNA in vitro transcription.

14. The method of any one of claims 1-13, wherein the oligonucleotide
(a) has a length of ~~about~~ 5 to ~~about~~ 100 nucleotides, preferably ~~about~~ 5 to ~~about~~ 50 nucleotides and more preferably ~~about~~ 5 to ~~about~~ 25 nucleotides;
(b) is single stranded, preferably a single stranded DNA and/or RNA oligonucleotide; and/or
(c) comprises one or more LNA and/or PNA nucleotides.

15. The method of any one of claims 1-5, 7, 8, or 10-14~~1-14~~, wherein the conjugate comprises or preferably consists of a chemical moiety with RNA cleaving activity coupled to the oligonucleotide
(a) via a covalent bond; or
(b) via a spacer, preferably wherein the spacer is an alkyl-unit, more preferably a C5 to C15 alkyl unit, even more preferably a C6 to C10 alkyl unit and most preferably a C8 alkyl unit, preferably coupled via an amide group;
optionally, wherein the chemical moiety with RNA cleaving activity
(i) is coupled to the oligonucleotide at the 5' end of said oligonucleotide;
(ii) catalyzes the hydrolysis of an RNA phosphodiester bond of an RNA backbone;
(iii) is an acid catalyst or a base catalyst; and/or
(iv) is an artificial moiety;
preferably wherein the chemical moiety with RNA cleaving activity cleaves single-stranded RNA; and/or wherein the chemical moiety with RNA cleaving activity cleaves metal-dependent or metal-independent, preferably metal-independent.

16. The method of any one of claims 1-15, wherein the chemical moiety with RNA cleaving activity is selected from the group consisting of
(i) Tris(2-aminobenzimidazol), 1H-Imidazo[1,2-a]imidazole, 5H-Benzimidazo[1,2-a]benzimidazol, Hexahydro-2H-pyrimido[1,2a]pyrimidin-2,8-dion, 2-Aminobenzimidazol, Imidazo[1,2-a]benzimidazol, 2-Aminochinolin, or
(ii) ~~moieties derived from Diethylentriamin (DETA), for example a~~ DETA-PEG, or
(iii) moieties ~~derived~~ selected from Diethylentriamin, Tetramin, 2-aminopyridin, 2-Aminoperimidin, 2-(Methyl-amino)perimidin, Bis(2-aminoperimidin), Tris(2-aminoperimidin), 2-Amino-6-bromperimidin, 2-Ammo-6-carbonsäuremethylesterperimidin-Hydrobromid, 2-Amino-6(essigsäuremethylester)perimidin-Hydrobromid, 2-Amino-6-(propionsäuremethylester)perimidin-Hydrobromid, and 2-Amino-6-methylperimidin-Hydrobromid;
preferably, wherein the chemical moiety with RNA cleaving activity is tris(2-aminobenzimidazole).

17. The method of any one of claims 1-5, 7, 8 or 10-16~~1-16~~, wherein

(a) the molar ratio of each of the at least one conjugates to the RNA molecule or the population of RNA molecules is between ~~about~~ 1:1 and ~~about~~ 10:1;

(b) the conditions allowing the hybridization and the cleavage of the RNA molecule comprise one or more temperature shifts, preferably wherein step (iii) of the method is as follows:

(iii) cleaving the RNA molecule or the population of RNA molecules, respectively, provided in step (i) to obtain RNA fragments by contacting the RNA molecule(s) with the conjugate provided in step (ii) at a first temperature between ~~about~~ 5°C and ~~about~~ 50°C, optionally at a second temperature between ~~about~~ 20°C and ~~about~~ 40°C, and at a third temperature between ~~about~~ 70°C and ~~about~~ 90°C;

followed by a step of repeating the above step (iii) at least once, wherein this step precedes step (iv);

(c) the cleavage of the RNA molecule or the population of RNA molecules occurs at the 3′ end or the 5′ end of the target sequence or 1, 2 or 3 nucleotides upstream or downstream of the respective end of the target sequence;

(d) the at least one conjugate is coupled to a support, optionally wherein said support is coupled to a column resulting in at least one conjugate immobilized to a column; and/or

(e) the one or more of the fragments are analyzed for their length.

18. Use of the method of any one of claims 1-17 in the quality control of RNA, preferably in the quality control during or following the production of the RNA; or for obtaining an RNA fingerprint.

19. A column having a conjugate comprising a chemical moiety with RNA cleaving activity and an oligonucleotide, wherein the conjugate is coupled to a support, immobilized thereto by coupling the support to the column.

20. A reactor comprising the column of claim 19.

21. Use of the column of claim 19 or the reactor of claim 20 in a method for analyzing RNA.