

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
18 October 2007 (18.10.2007)

PCT

(10) International Publication Number
WO 2007/118065 A2

(51) International Patent Classification:
C12Q 1/68 (2006.01)

(21) International Application Number:
PCT/US2007/065834

(22) International Filing Date: 3 April 2007 (03.04.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/789,494 5 April 2006 (05.04.2006) US

(71) Applicant (for all designated States except US):
WILLIAM MARSH RICE UNIVERSITY [US/US];
Office Of Technology Transfer MS 705, P.O. Box 1892,
Houston, TX 77152-1892 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **DREZEK, Re-
bekah, Anne** [US/US]; 7550 Kirby Drive, Apt. 831,
Houston, TX 77030 (US). **CHANG, Emmanuel,
Yih-herng** [US/US]; 2626 Holly Hall Street, Unit
714, Houston, TX 77054 (US).

(74) Agent: **MORICO, Paul, R.**; Baker Botts L.L.P., 910
Louisiana Street, Houston, TX 77002-4995 (US).

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

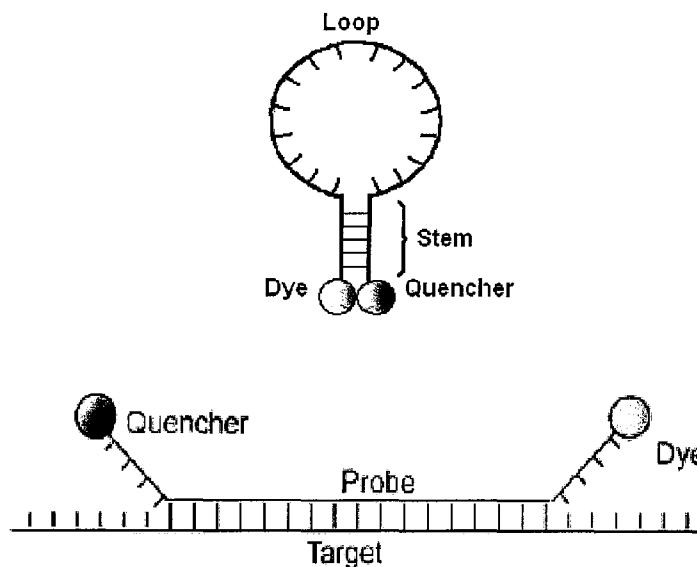
(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,
FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL,
PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM,
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished
upon receipt of that report

[Continued on next page]

(54) Title: siRNA NANOPROBES



(57) Abstract: siRNA nanoprobes for monitoring the expression of proteins intracellularly by mRNA expression while simulta-
neously silencing the expression of proteins by RNA interference and associated methods of use in biomedical applications are
provided. In one example, an siRNA nanoprobe comprising a sense strand and an antisense strand coupled by a polymer linker, and
a reporter-indicator pair formed from a reporter and an indicator, wherein the reporter and indicator are coupled to a different strand,
is provided.

WO 2007/118065 A2



— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

siRNA NANOPROBES

STATEMENT OF GOVERNMENT INTEREST

This disclosure was developed at least in part using funding from the Nanoscale
5 Science and Engineering Initiative of the National Science Foundation under NSF Award
Number EEC-0118007. The U.S. government may have certain rights in the invention.

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/789,494,
filed April 5, 2006, incorporated herein by reference.

10 SEQUENCE LISTING

This disclosure includes a sequence listing submitted as a text file pursuant to 37
C.F.R. § 1.52(e)(v) named sequence listing.txt, created on March 23, 2007 with a size of 820
bytes, which is incorporated herein by reference. The attached sequence descriptions and
Sequence Listing comply with the rules governing nucleotide and/or amino acid sequence
15 disclosures in patent applications as set forth in 37 C.F.R. §§ 1.821-1.825. The Sequence
Listing contains the one letter code for nucleotide sequence characters and the three letter
codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described
in *Nucleic Acids Res.* 13:3021-3030 (1985) and in the *Biochemical J.* 219 (No. 2):345-373
(1984). The symbols and format used for nucleotide and amino acid sequence data comply
20 with the rules set forth in 37 C.F.R. §1.822.

BACKGROUND

Small interfering RNAs (siRNAs) are one of the recently discovered nucleic acid
molecules used to silence gene expression. siRNAs utilize RNA interference (RNAi), which
may be used to study and silence gene expression. RNAi is triggered by dsRNA arising from
25 exogenous sources such as siRNAs or endogenous noncoding RNA genes that produce
microRNAs. Exogenous introduction of siRNAs can silence genes with high specificity.
These small RNAs join an effector complex, known as RNA-induced silencing complex
(RISC) which mediates mRNA cleavage. The guide strand (antisense) incorporates into RISC
and directs activated RISC to the mRNA target by Watson-Crick base pairing. The activated
30 RISC then induces gene silencing through mRNA cleavage. With a potent RNAi effector

sequence and efficient siRNA delivery into cell, highly specific post-transcriptional inhibition can be attained with minimal off target effects.

While end labeling siRNA duplexes with fluorophores have been used to monitor delivery of siRNA into cells, currently there is no way to monitor whether or not the target mRNA of interest is being expressed in the cell during RNA interference.

Fluorescence is a widely used tool in biology for detection and monitoring. Molecular beacons (MBs) are fluorescent nucleic acid probes utilizing a fluorescence resonant energy transfer (FRET) fluorophore pair or dye-quencher pair to generate a signal upon binding to complementary sequences. They are highly sensitive and specific for monitoring the expression of nucleic acids at the single-cell level. MBs typically consist of four key components: loop, stem (typically 4-6 bases), a donor fluorophore, and an acceptor fluorophore. This hairpin structure containing a FRET pair undergoes conformational change upon hybridization to a complementary nucleic acid target. During hybridization, the MB is activated by FRET pair separation, and the donor fluorescence increases while the acceptor fluorescence decreases. Studies have shown that MBs can discriminate between targets that differ by only a single nucleotide.

SUMMARY

The present disclosure, according to specific embodiments, generally relates to RNA interference. More particularly, the present disclosure relates to siRNA nanoprobe for monitoring the expression of proteins intracellularly by mRNA expression while simultaneously silencing the expression of proteins by RNA interference and associated methods of use in biomedical applications. For example, the siRNA nanoprobe of certain embodiments of the present disclosure can be used to detect and treat any disease of interest that arises from an overexpression of a targeted protein, including for example, Alzheimer's disease, cancer, and diabetes.

Furthermore, whereas the vast majority of MBs are constructed using an oligonucleotide backbone comprising the loop and stem, the siRNA-based probes of the present disclosure utilize a synthetic polymer linker as the loop of the MBs. The advantage of using a synthetic polymer linker as a loop is the resistance to Dicer cleavage since Dicer has

been shown to cleave oligonucleotide hairpin beacons such as shRNAs into two separate strands during RNA interference.

The ability to monitor the mRNA expression profile of a single cell and to compare it against another single cell would provide a powerful tool for many biological or medical applications involving gene expression, such as in the detection of cancerous cells.

The features and advantages of the present disclosure will be readily apparent to those skilled in the art upon a reading of the description of the embodiments that follows.

FIGURES

Some specific example embodiments of the disclosure may be understood by referring, in part, to the following description and the accompanying drawings.

FIGURE 1 A shows a conventional molecular beacon.

FIGURE 1B shows a siRNA nanoprobe, according to an embodiment of the present disclosure.

FIGURE 2 is a graph demonstrating separation and re-annealing of a siRNA nanoprobe as temperature cycles between 15°C to 90°C, according to an embodiment of the present disclosure. FRET efficiency was calculated by $\text{Cy5 fluorescence} / (\text{Cy3} + \text{Cy5 fluorescence})$ at 488 nm excitation.

FIGURE 3 shows confocal images at 10 and 14 hours post-transfection with Lipofectamine 2000 in normal human breast fibroblast and human breast cancer cells transfected with 3400 MW and 5000 MW siRNA nanoprobe targeted against the hTR sequence in telomerase, according to an embodiment of the present disclosure.

FIGURE 4 is a graph illustrating telomerase activity relative to untreated SK-BR-3 human breast cancer cells after transfection of 100 nM siRNA nanoprobe concentration at 44 hours ($n=3$), according to an embodiment of the present disclosure.

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

While the present disclosure is susceptible to various modifications and alternative forms, specific example embodiments have been shown in the figures and are herein described in more detail. It should be understood, however, that the description of specific

example embodiments is not intended to limit the invention to the particular forms disclosed, but on the contrary, this disclosure is to cover all modifications and equivalents as illustrated, in part, by the appended claims.

DESCRIPTION

5 The present disclosure, according to specific example embodiments, generally relates to RNA interference. More particularly, the present disclosure relates to siRNA nanoprobe for monitoring the expression of proteins intracellularly by mRNA expression while simultaneously silencing the expression of proteins by RNA interference and associated methods of use in biomedical applications. As used herein, "siRNA" is defined as a short
10 interfering ribonucleic acid.

 The siRNA nanoprobe of the present disclosure generally comprise a stem, a polymer linker, and a reporter-quencher pair.

 The stem of the siRNA based nanoprobe comprises a sense strand and an antisense strand. The strands may be modified to accept the polymer linker and are coupled to the
15 reporter-quencher pair. The sense and antisense strands may form a double-stranded structure that may result from two separate nucleic acids that are partially or completely complementary, referred to as a siRNA duplex. The sequence of the strands is chosen based on the sequence of the mRNA target of interest. The strands will generally have a size in the range for from about 7 to about 500 nucleotides in length. The size may depend on the
20 particular target chosen, as well as on the efficiency of inhibition, specificity, including absence of cross-reactivity, and the like. The strands of the siRNA duplex may comprise a nucleic acid and/or a nucleic acid analog.

 The sense and/or antisense strands of the siRNA duplex may be modified to allow attachment of a polymer linker between the sense and antisense strands. The term "modified"
25 refers to the addition of a chemical group or moiety to a sense or antisense strand. The sense strand may be modified at the 5' terminus, for example, by addition of a sulfhydryl group. The antisense strand may be modified at the 3' terminus, for example, by addition of an amino group.

 The polymer linker joins the sense and antisense strands. The polymer linker
30 generally comprises a flexible polymer that is at least long enough to prevent a detectable signal from the reporter when the siRNA duplex is unwound, or activated. The length of the

polymer linker may also affect the activation time of the siRNA probe. The polymer linker may comprise, for example, polyethylene glycol (PEG), such as a 3400 MW PEG or a 5000 MW PEG. The polymer linker may be adapted to react with the modified sense and antisense strands. For example, the polymer linker may be made heterobifunctional with the addition
5 of a maleimide and an *N*-Hydroxysuccinimide(NHS)-ester to the ends of the polymer linker.

A reporter-quencher pair is conjugated to the stem. The term "reporter" refers to any fluorescent molecule that produces a detectable signal upon spatial separation from the quencher. The term "quencher" refers to any molecule that prevents the emission of a detectable signal from the reporter by absorbing the fluorescent energy emitted by the
10 reporter. The term "conjugated" refers to covalent linkage between components. In particular embodiments, the reporter is conjugated to the antisense strand, and the quencher is conjugated to the sense strand. The sense and antisense strands may be purchased commercially in modified form with a conjugated reporter-quencher pair, for example from Dharmacon RNA Technologies, Lafayette, CO.

15 In some embodiments, the reporter-quencher pair may be a FRET fluorophore pair. The FRET fluorophore pair comprises an acceptor fluorophore and a donor fluorophore. The acceptor fluorophore may be conjugated to a nucleotide base toward the 5' terminus of the sense strand. The donor fluorophore may be conjugated to a nucleotide base toward the 3' terminus of the antisense strand. An example of a FRET fluorophore pair includes, but is not
20 limited to, Cy5 and Cy3.

In other embodiments, the reporter-quencher pair may be a quantum dot pair or other luminescent nanoparticle pair. Such reporter-quencher pairs may allow for, among other things, siRNA nanoprobe with multiplexing capabilities.

In other embodiments, the reporter-quencher pair may be a dye-quencher pair.
25 Examples of suitable dye-quencher pairs include, but are not limited to, a near infrared fluorophore and the black hole quencher class of dyes.

In other embodiments, the reporter-quencher pair may comprise a reporter that is a dye or quantum dot and a quencher that is a gold nanoparticle.

In a specific examples of siRNA nanoprobe of the present disclosure, the stem
30 comprises the sense strand 5' HS-U*UGUCUAACCCUAACUGAGTT-3' where U* is a Cy5-dUTP fluorophore conjugated base, and an antisense strand 3' NH₂-

T*TAACAGAUUGGGAUUGACUC-5' where T* is a Cy3-dTTP fluorophore conjugated base.

According to some embodiments, the siRNA nanoprobe of the present disclosure may be formed by allowing a polymer linker and siRNA duplex to react. For example, a maleimide and NHS-ester of a polymer linker can react with a sulfhydryl and an amine group of a modified siRNA duplex, respectively, to conjugate the sense and antisense strand of the siRNA duplex to the polymer linker. The final product is an antisense RNA-polymer-sense RNA loop siRNA strand (FIGURE 1 A).

When the siRNA nanoprobe of the present disclosure are in an inactive state, the sense and antisense strands are annealed forming a duplex, thereby causing the reporter and the quencher to be in close proximity. The energy emitted by the reporter is thus absorbed by the quencher, resulting in internal quenching of the fluorophore. When the siRNA nanoprobe of the present disclosure bind a target sequence they are in an active state, and the reporter and the quencher become spatially separated and the reporter can fluoresce, thereby producing a detectable signal.

If no targeted mRNA is present to compete for hybridization and activate the siRNA nanoprobe, the sense and antisense strands are able to reanneal. This is due, at least in part, to the polymer linker, which tethers the sense and antisense strands. Two mechanisms have been presented in literature where either Dicer unwinds the duplex siRNA to allow incorporation of the antisense strand into RISC, or the entire siRNA duplex can be incorporated into the RISC complex and the sense strand serves as the first target cleaved by RISC. In either case, the antisense strand of the siRNA nanoprobe generally should be able to re-anneal to the sense strand of the siRNA probe if no target mRNA sequence is present.

Accordingly, the siRNA nanoprobe of the present disclosure may be used in methods for treating and/or detecting a disease. For example, the siRNA nanoprobe of the present disclosure may be used to treat and/or diagnose cancer, such as a solid tumor, metastatic cancer, or non-metastatic cancer. Nonetheless, it is also recognized that the methods of the present disclosure may also be used to treat a non-cancerous disease resulting from the overexpression of proteins (e.g., Alzheimer's disease and diabetes). In methods for treating and/or detecting a disease, the siRNA nanoprobe may be delivered to a cell using any method, for example, via a non-charged liposome or direct injection.

In one example in which a siRNA-based MB is used for the simultaneous detection and treatment of cancer, the siRNA duplex is targeted to a telomerase. All cancer cells must express telomerase in order to continuously divide. Telomerase is a ribonucleoprotein that extends onto the 3' end of existing telomeres using an RNA template located within the enzyme. Telomerase expression is an essential step for tumor progression making it an ideal
5 general marker for cancer detection.

Such a siRNA-based MB would allow the detection of cancer by detecting telomerase mRNA expression while simultaneously silencing the expression of telomerase, thereby preventing cancer cells to continuously divide. One suitable target for such a siRNA-based
10 MB is the telomerase template sequence (hTR) repeat unit.

To facilitate a better understanding of the present disclosure, the following examples of specific embodiments are given. In no way should the following examples be read to limit or define the entire scope of the invention.

EXAMPLES

15 Example 1: Formation and Isolation of the siRNA probes

Modified 21-mer oligoribonucleotide (ss/siRNAs) were purchased commercially (Dharmacon RNA Technologies, Lafayette, CO) utilizing a previously published and validated sequence targeting the telomere repeat template sequence (hTR) as provided in Dorsett, Y. & Tuschl, T. siRNAs: Applications in functional genomics and potential as
20 therapeutics. *Nature Reviews* 3, 318-329 (2004). The modified sense strand sequence is: 5' HS-U*UGUCUAACCCUAACUGAG-TT-3' where U* is a Cy5-dUTP fluorophore conjugated base. (SEQ ID NO. 1) The modified antisense strand is: 3' NH₂-T*-AACAGAUUGGGAUUGACUC-5' where T* is a Cy3-dTTP fluorophore conjugated base (SEQ ID NO. 2). Cy3 and Cy5 is a commonly used fluorophore FRET pair. The sense and
25 anti-sense oligoribonucleotides were reacted with a maleimide-PEG-NHS ester heterobifunctional linker (3400 and 5000 MW PEG) (Nektar Therapeutics, Huntsville, AL). During the coupling reaction, siRNA antisense and sense strands were added in a 2-fold molar excess to ensure sufficient coupling onto the heterobifunctional PEG linker. The reaction was allowed to proceed overnight in the dark in RNase-free phosphate buffered
30 saline, pH 7.4 at 4°C.

The conjugated ssRNA-PEG-ssRNA was then isolated from free ssRNA and PEG by 15% denaturing polyacrylamide gel electrophoresis. The sample was heated at 90°C for 2 minutes prior to gel loading, mixed with gel loading buffer, and electrophoresed at 35 mA. The PAGE gel was post-stained with a 0.5 ug/ml ethidium bromide solution. Two distinct
5 bands were seen for each probe. A reference nucleotide sizing marker (Decade Markers, Ambion, Austin, TX) was run in parallel with the bands to determine which band contained the conjugated siRNA probe. The siRNA probe band (determined by nucleotide size) was then carefully excised from the gel and isolated by the crush and soak method. After 48 hour elution at 4°C in the dark with 10 mL of 1M NaCl, the sample was centrifuged at 2000×g for
10 5 min. The elution buffer underwent ethanol precipitation with 4 volume excess of cold 90% ethanol/10% 3M sodium acetate solution at -20°C over 48 hours to isolate the siRNA probe. The solution was centrifuged at 20,000×g for 30 min. at 4°C and resuspended in annealing buffer (20 mM KCl, 6 mM HEPES-KOH pH 7.5). Annealing was performed by heating to 60°C for 5 min. and slow cooled to room temperature over 30 min. Extinction measurements
15 at 260 nm ($\epsilon=414600$ L/mol cm) were performed in a quartz cuvette to determine final siRNA conjugate concentration on a Varian Cary 50 Bio spectrophotometer (Walnut Creek, CA). The probe was then aliquoted into RNase free tubes and stored at -80°C.

Example 2: Spectroscopy measurements of the siRNA probes

With the aim of assessing whether or not the siRNA-probe fluorescence changes upon
20 binding to the target sequence, the hybridization of the siRNA-based probes upon addition of complementary target strands was examined. All measurements were performed with a constant temperature 1.5 mL stoppered quartz fluorescence cuvette (Starna Cells, Atascadero, CA) on a Horiba Jobin Yvon SPEX FL3-22 Fluorimeter (Edison, NJ) with dual excitation and emission monochromators. Sample temperature was controlled by circulating
25 temperature water bath through the quartz cuvette. Time-integrated photoluminescence was measured using 488 nm excitation light and emission scans from 525 to 800 nm. Bandpass slits and integration time were set to 3 nm/ 3 nm and 100 ms, respectively on the fluorimeter. All values were normalized over time to a rhodamine 6G standard to avoid any artifacts that could arise from possible lamp fluctuations. FRET efficiency between Cy3 and Cy5
30 fluorophores was calculated by intensity of Cy5 fluorescence / (intensity of Cy3 fluorescence + intensity of Cy5 fluorescence).

After a 20 minute incubation at 30°C, a 9.3-fold and 8.2-fold decrease in Cy5 fluorescence occurred for 3400 MW siRNA probe and 5000 MW siRNA probe, respectively. In addition to measuring the change in Cy5 fluorescence due to loss of FRET, temperature studies were performed to examine the separation and re-annealing of the siRNA probe based on FRET efficiency. FIGURE 2 demonstrates separation and re-annealing of the siRNA probes as temperature cycles between 15°C to 90°C.

Example 3: Cell Studies

The human breast cancer cell line SK-BR-3 and normal breast fibroblast cell line CCD-1059Sk were ordered from American Type Culture Collection (ATCC, Manassas, VA). SK-BR-3 cells were cultured (37°C, 5% CO₂) in McCoy's 5A medium with 10% (v/v) fetal bovine serum (Invitrogen Corp., Carlsbad, CA). CCD-1059Sk cells were cultured (37°C, 5% CO₂) in Minimum essential medium (Eagle) with 10% (v/v) fetal bovine serum. Both SK-BR-3 cells and CCD-1059Sk cells were plated onto glass chamber slides (Nalge Nunc International, Rochester, NY) for microscopy studies. Cells were plated into 6 well tissue culture plates at a concentration to provide 80% confluence 24 hours later prior to transfection using Lipofectamine 2000 (Invitrogen Corp, Carlsbad, CA). At that time, the siRNA probe was diluted to a final volume of 500 µL with Opti-Mem Reduced Serum Media. In a separate tube, 30 µL of Lipofectamine was diluted with 470 µL of Optimem. The two solutions were mixed gently and incubated at room temperature for 5 min. The contents of both tubes were then combined and mixed gently by pipetting and incubated at room temperature for 30 min. The liposome complexes were then added to the culture medium and mixed gently for 30 seconds. At 44 hours, the cells were trypsinized, counted, and 2000 cells removed for assay of telomerase activity. Cell studies were performed with the 3400 MW siRNA probe, 5000 MW siRNA probe, non-targeting Silencer Negative Control #1 siRNA (Ambion, Austin, TX), and mock transfection at 100 nM siRNA concentrations.

All images of the cells were collected using a Zeiss LSM 510 META NLO confocal system mounted on an Axiovert 200M inverted microscope and Plan-Apochromat 63x objective lens (Carl Zeiss Microimaging, Inc., Thornwood, New York). siRNA probes were excited with a 488 nm Argon laser source in live cell imaging. Spectrofluorimeter measurements demonstrated minimal excitation of the Cy5 fluorophore with a 488 nm excitation source, indicating that Cy5 emission generation is due to fluorescence resonance

energy transfer from the Cy3 fluorophore. All images were acquired with the same detector, gain, pinhole, and power settings at 1024x1024 pixels to allow direct comparison between normal and cancer cells. Lambda emission scanning from 510-700nm was performed using the META detector to verify fluorescence intensity changes between Cy3 and Cy5
5 fluorophores.

The sequence of the siRNA probe targeted the hTR sequence of telomerase mRNA. Therefore, cancer cells should have high expression levels of telomerase mRNA while normal cells minimally express telomerase mRNA. Confocal imaging (see FIGURE 3) was performed every 2 hours after transfection until 14 hours and every 4 hours thereafter up to
10 38 hours post-transfection. The siRNA probe was excited using a 488 nm Argon laser which minimally excites the Cy5 fluorophore. Therefore, Cy5 fluorescence would be attributed to FRET. Microscopy settings were held constant during imaging to allow for direct visual comparison between normal and cancer cells.

FIGURE 3 demonstrates the ability to distinguish normal and cancer cells based on
15 siRNA probe targeted to telomerase mRNA. Interestingly, the 3400 MW siRNA probe discriminated differences between normal and cancer cells earlier than the 5000 MW siRNA probe. The ideal time for imaging the siRNA probe appeared to be 6-10 hours post-transfection and 10-14 hours post-transfection for the 3400 MW siRNA probe and 5000 MW siRNA probe, respectively. At 24 hours post-transfection, no difference could be observed
20 between normal and cancer cells (no FRET observed), presumably due to complete loss of the siRNA probe stem by degradation from intracellular exonucleases.

Example 4: Telomerase Activity of Transfected Cells

The modification of siRNA strands to form the siRNA-based probe was evaluated to determine if they were still able to mediate RNA interference. The siRNA-based probe
25 developed utilized a sequence previously shown to silence the expression of telomerase. Telomerase activity of the cells was assessed 44 hours post-transfection of the siRNA probe using a commercial fluorescence-based TRAPEZE^R XL telomerase detection kit (Intergen, Purchase, NY). This kit is a refined fluorometric version of the original TRAP (Telomeric Repeat Amplification Protocol) assay using quenched fluorophore primers to generate
30 fluorescently labeled TRAP products. It provides a highly sensitive and quantitative detection of telomerase activity *in vitro*. Lysates (1000 cell-equivalents) are mixed with TRAPEZE^R

XL reaction mix containing Amplifluor™ primers, and incubated at 30°C for 30 minutes. Samples undergo PCR amplification. Amplified telomerase products are quantitated with a SpectraMax M2 fluorescent plate reader. Telomerase activity is calculated by comparing the ratio of telomerase products to an internal standard for each lysate, as described by the
5 manufacturer.

Cells appeared healthy after transfection prior to telomerase assay. FIGURE 4 demonstrates effective gene silencing of telomerase by the siRNA-based probe. This finding is consistent with previous observations of telomerase silencing using this siRNA probe sequence. More importantly, the modifications to siRNA did not inhibit effects of gene
10 silencing in agreement with previous studies.

Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contain certain errors necessarily resulting from the standard deviation found in their
15 respective testing measurements.

Therefore, the present invention is well adapted to attain the ends and advantages mentioned as well as those that are inherent therein. While numerous changes may be made by those skilled in the art, such changes are encompassed within the spirit of this invention as illustrated, in part, by the appended claims.

What is claimed is:

1. An siRNA nanoprobe comprising a sense strand and an antisense strand coupled by a polymer linker, and a reporter-indicator pair formed from a reporter and an indicator, wherein the reporter and indicator are coupled to a different strand.
- 5 2. The siRNA nanoprobe of claim 1, wherein the sense strand and antisense strand is modified.
3. The siRNA nanoprobe of claim 1, wherein the reporter-indicator pair comprises Cy3 and Cy5.
4. The siRNA nanoprobe of claim 1, wherein the reporter-indicator pair
10 comprises a dye-quencher pair.
5. The siRNA nanoprobe of claim 1, wherein the reporter of the reporter-indicator pair comprises a quantum dot nanoparticle.
6. The siRNA nanoprobe of claim 1, wherein the polymer loop comprises a polyethylene glycol.
- 15 7. The siRNA nanoprobe of claim 1, wherein the antisense strand is capable of hybridizing under stringent conditions with a telomerase template sequence.
8. A method of detecting, treating, or both detecting and treating a disease comprising introducing into a cell a siRNA nanoprobe comprising a sense strand and an antisense strand coupled by a polymer linker, and a reporter-indicator pair formed from a
20 reporter and an indicator, wherein the reporter and indicator are coupled to a different strand.
9. The siRNA nanoprobe of claim 8, wherein the sense strand and antisense strand is modified.
10. The siRNA nanoprobe of claim 8, wherein the reporter-indicator pair comprises Cy3 and Cy5.
- 25 11. The siRNA nanoprobe of claim 8, wherein the reporter-indicator pair comprises a dye-quencher pair.
12. The siRNA nanoprobe of claim 8, wherein the reporter of the reporter-indicator pair comprises a quantum dot nanoparticle.
13. The siRNA nanoprobe of claim 8, wherein the polymer loop comprises a
30 polyethylene glycol.

14. The siRNA nanoprobe of claim 8, wherein the antisense strand is capable of hybridizing stringent conditions with a telomerase template sequence.

15. The method of claim 8 wherein the cell is a cancer cell.

16. The method of claim 8 wherein the siRNA nanoprobe is delivered to the
5 cell using a non-charged liposome.

17. A method of simultaneously detecting and treating cancer comprising introducing into a cell a siRNA nanoprobe comprising a modified sense strand and antisense strand coupled by a polymer linker, and a reporter-indicator pair formed from a reporter and an indicator, wherein the reporter and indicator are coupled to a different strand.

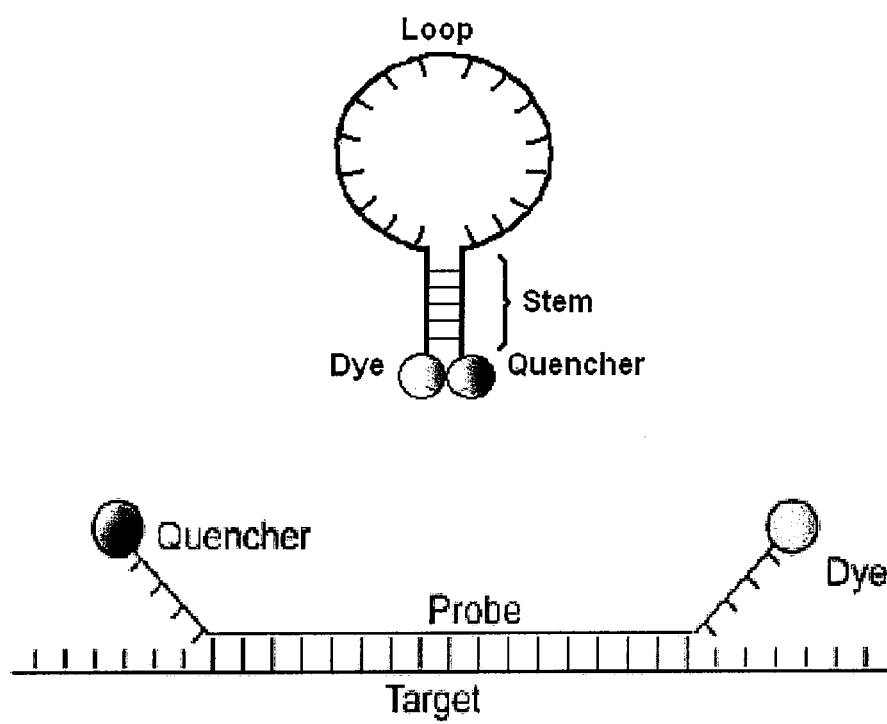
18. The siRNA nanoprobe of claim 17, wherein the polymer loop comprises a
10 polyethylene glycol.

19. The siRNA nanoprobe of claim 17, wherein the antisense strand is capable of hybridizing stringent conditions with a telomerase template sequence.

20. The method of claim 17 wherein the siRNA nanoprobe is delivered to the
15 cell using a non-charged liposome.

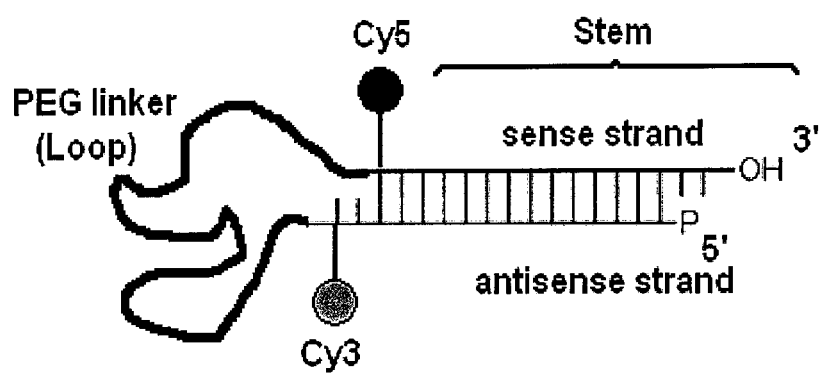
1/5

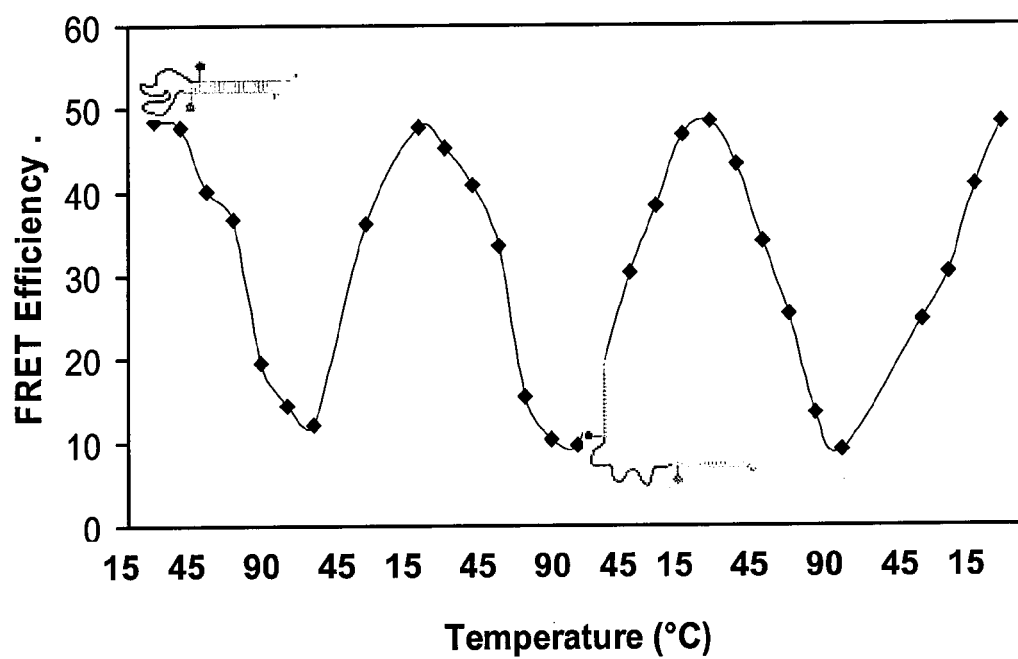
FIGURE 1A.



2/5

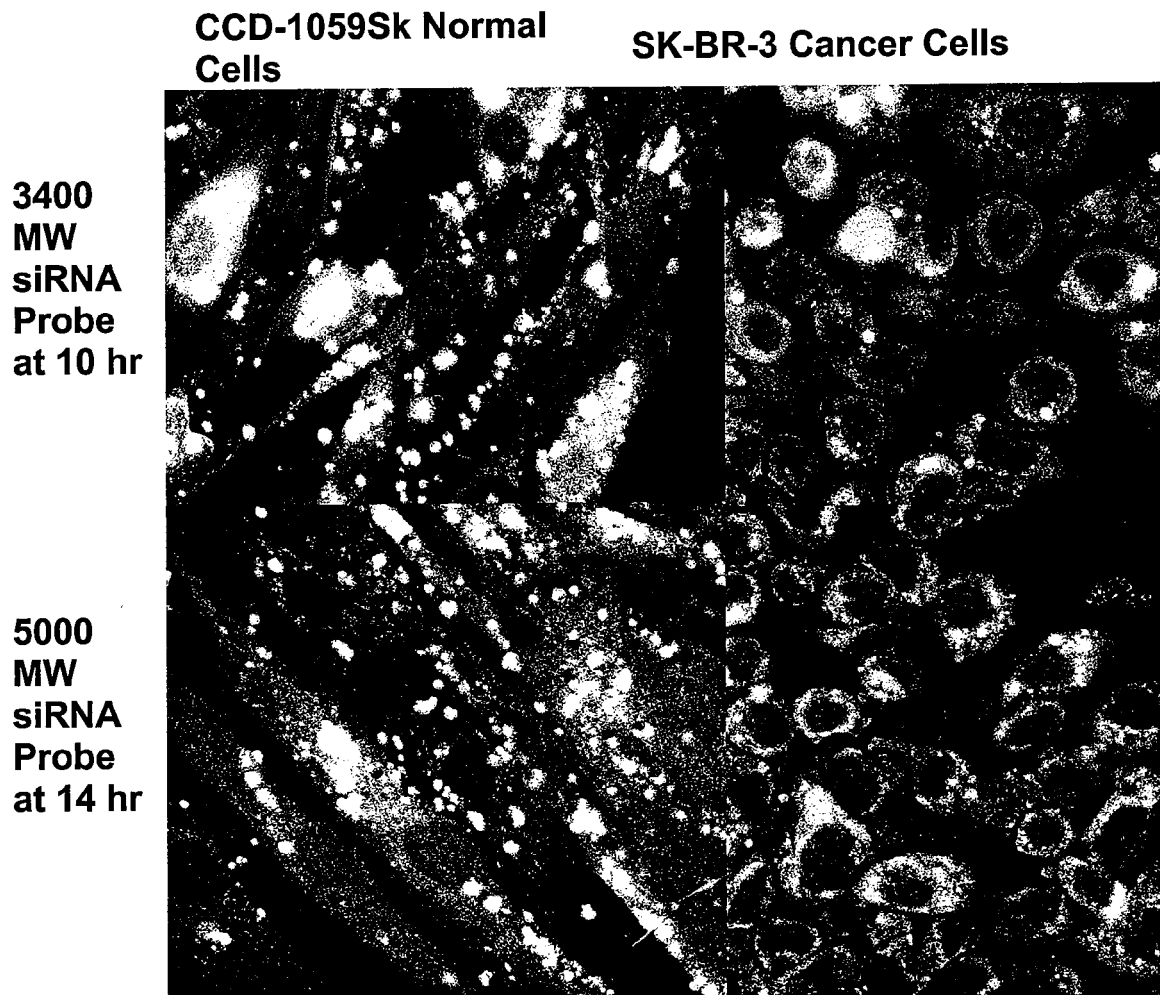
FIGURE 1B.





4/5

FIGURE 3.



5/5

FIGURE 4.

