BENOÎT&CÔTÉ

Your Reference: 2,692,882
Our Reference: P3024CA00

February 13, 2015

RESPONSE

The Commissioner of Patents
The Canadian Intellectual Property Office
50 Victoria Street
Phase I, Place du Portage
Gatineau, Quebec
K1A 0C9

Dear Commissioner:

Re	:	**Canadian Patent Application No. 2,692,882 Filed on July 11, 2008**
Title	:	**ULTRASENSITIVE DETECTION OF TARGET USING TARGET-READY PARTICLES**
Applicant	:	**NATIONAL RESEARCH COUNCIL OF CANADA**
Classification		**C12Q 1/68**
Examiner		**Mary Murphy**

This is responsive to the Office Action ("Action") dated September 15, 2014, a Response to which is due by March 15, 2015. Accordingly, this Response is timely filed.

Please note that our reference number for this case has been updated to P3024CA00. Please update your records to refer to this number going forward. Thank you for your attention to this matter.

Please note that an Appointment of Agent in favor of the undersigned is being concurrently filed herewith. (A copy of our submission is enclosed with the present)

Please amend the above-mentioned application as follows:

IN THE DESCRIPTION

Kindly substitute the enclosed pages 18a, 25 and 27 for the corresponding pages presently on file.

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IN THE CLAIMS

Please replace claims 1-57 currently of record with claims 1-56 submitted herewith.

REMARKS

Description Amendments

The description has been amended on page 27 (paragraph [00144]) to remove certain statements to comply with subsection 27(3) of the *Patent Act*. Moreover, page 27 (paragraph [00144]) has been amended to recite the following statement which is believed to be acceptable under Canadian practice: "*The scope of the claims should not be limited by the preferred embodiments and examples, but should be given the broadest interpretation consistent with the description as a whole*".

The description has been amended on page 27 to remove certain statements to comply with subsection 81(1) of the *Patent Rules*.

The description has been amended on page 27 to comply with subsection 81(3) of the *Patent Rules*.

The description has been amended on pages 18a and 25 to provide proper identification of trademarks to comply with Section 76 of the *Patent Rules*.

Applicant submits that no new matter has been added to the application as a result of these amendments and their entry is respectfully requested.

Claim Amendments

Claims 1 to 56 are now in the application.

The claims have been amended to a form which Applicant desires to present for further examination. A marked-up copy showing the claim amendments and claim deletions has been annexed to the present response for the Examiner's convenience. In summary:

- Claims 1-31, 35-43, 45, 48-52 and 54-57 have been amended; and
- Claim 29 has been deleted; and

- The claims and the dependencies of the dependent claims have been renumbered in accordance with the above-amendments.

Non-limiting support for the claim amendments can be found throughout the specification as originally filed (see e.g. paragraphs [0027], [0033], [0035], [0036], [0071], [00133]-[00138] and [00140]-[00141]).

The claim amendments have been made without prejudice and without acquiescing to any of the Action's objections. Applicant submits that no new matter has been added to the claims as a result of these amendments and that the amended claims submitted herewith are fully supported by the application as originally filed. Entry of the claim amendments is thus respectfully requested.

The Office Action dated September 16, 2014 has been carefully considered. It is believed that the following comments represent a complete response to the Action's rejections and place the present application in condition for allowance. Reconsideration is respectfully requested.

REQUISITION UNDER SUBSECTION 30(2) OF THE PATENT RULES

Rejections based on Section 28.2 of the Patent Act

1. In the Office Action, claims 1-6, 10-18, 20-31, 33-44 and 46-57 have been rejected pursuant to Section 28.2 of the *Patent Act*. The Action alleges that the analysis presented in the International Preliminary Report on Patentability (IPRP) regarding novelty is applicable to the above-referenced Canadian application. Applicant submits that Najari, A. *et al.* (Reagentless Ultrasensitive Specific DNA Array Detection Based on Responsive Polymeric Biochips, Analytical Chemistry, Vol. 78, 22, 7896-7899) (**D3**) was cited in the IPRP with respect to novelty.

As an initial matter, Applicant submits that amended claim 1 relates to a nano- or micro-sized particle comprising pre-assembled aggregates formed by the association of complexes comprising a nucleic acid probe and a polymer of formula A, wherein the aggregates are grafted to the surface of the nano- or micro-sized particle.

Amended claim 28 relates to a composition comprising multiple nano- or micro-sized particle species, wherein each nano- or micro-sized particle species comprises pre-

assembled aggregates formed by the association of complexes comprising a distinct nucleic acid probe species and a polymer of formula A, wherein the aggregates are grafted to the surface of the nano- or micro-sized particle species.

Amended claim 31 (renumbered as claim 30) relates to a method for detecting the presence or absence of a target in a sample comprising or suspected of comprising the target, the method comprising:

contacting the sample with a nano- or micro-sized particle comprising a pre-assembled aggregate formed by the association of a complex comprising a nucleic acid probe and a polymer of formula A;

allowing a sufficient period of time for the target to bind the nucleic acid probe; and

measuring or identifying a signal emitted upon binding of the target and the nucleic acid probe, and wherein the aggregate is grafted to the surface of the nano- or micro-sized particle.

Amended claim 41 (renumbered as claim 40) relates to a method for the simultaneous detection of multiple target species from a sample, the method comprising:

contacting the sample with a composition comprising multiple nano- or micro-sized particle species, wherein each nano- or micro-sized particle species comprises pre-assembled aggregates formed by the association of complexes comprising a distinct nucleic acid probe species and a polymer of formula A;

allowing a sufficient period of time for the target species to bind the nucleic acid probe species and;

measuring or identifying a signal emitted upon binding of the target species and the nucleic acid probe species; and

wherein each nano- or micro-sized particle species further comprises a distinct and selectable tag allowing its distinction among the multiple nano- or micro-sized particle species and wherein the aggregates are grafted to the surface of each of the nano- or micro-sized particle species.

Applicant submits that for a publication to be found anticipatory one must be able to look at the publication and find in it all the information which, for practical purposes, is needed to produce the claimed invention without the exercise of any inventive skill. The

prior publication must contain so clear a direction that a skilled person reading and following it would in every case and without the possibility of error be led to the claimed invention.[1] The test for anticipation by publication in *Beloit,* was cited with approval by the Supreme Court of Canada in *Free World Trust* v. *Électro Santé Inc.*[2]

Applicant further submits that the prior publication must, for the purposes of practical utility, be equal to that given by the patent in question. It cannot be merely a foreshadowing of the invention or the nucleus of the idea behind the invention. It must clearly show the whole of the invention with all the directions necessary to instruct the public how to put it to use (*i.e.* give clear and unmistakable directions). The claimed invention must necessarily always be obtained.

Applicant respectfully submits that **D3** does not teach nor suggest a nano- or micro-sized particle such as currently recited by the amended set of claims as submitted by way of the present response. More specifically, **D3** is silent regarding a nano- or micro-sized particle comprising pre-assembled aggregates formed by the association of complexes comprising a nucleic acid probe and a polymer of formula A, wherein the aggregates are grafted to the surface of the nano- or micro-sized particle.

Applicant respectfully submits that at least for these reasons, the amended set of claims as submitted with the present response is novel over **D3**, and requests that the rejection under Section 28.2 of the *Patent Act* be withdrawn.

Rejections based on Section 28.3 of the Patent Act

1. In the Office Action, claims 1-6, 10-31, 33-44 and 46-57 have been rejected pursuant to Section 28.3 of the *Patent Act.* The Action alleges that the analysis presented in the International Preliminary Report on Patentability (IPRP) regarding inventive step (obviousness) is applicable to the above-referenced Canadian application. Applicant submits that Dubuis, S. *et al.* (PCR-Free DNA Detection Using a Magnetic Bead-Supported Polymeric Transducer and Microelectromagnetic Traps; Analytical Chemistry, Vol. 78, 13, 4457-4464) (**D1**); US 7,083,928 (**D2**); and Aberim, M. B. *et al.* (Protein Detecting Array Based on Cationic Polythiophene-DNA-Aptamer

[1] *Beloit Canada Ltd. v. Valmet OY* (1986), 8 C.P.R. (3d) 289 (F.C.A.) at pp. 298-299.
[2] *Free World Trust v. Électro Santé Inc., 2000 SCC 66, [2000] 2 S.C.R. 1024 at para. 26.*

Complexes, Advanced Materials, Vol. 18, 20, 2703-2707) (**D4**) were cited in the IPRP with respect to inventive step.

As discussed hereinabove with respect to Section 28.2 of the *Patent Act*, Applicant respectfully submits that the teachings of **D3** cannot be relied upon as teaching a nano- or micro-sized particle such as currently recited by the amended set of claims as submitted by way of the present response. Applicant further submits that the deficiencies of **D3** are not complemented by any one of **D1**, **D2** or **D4**, taken alone or in combination.

Applicant thus respectfully submits that in view of the above remarks none of the references applied by the Examiner, taken alone or in combination, teach a nano- or micro-sized particle such as currently recited by the amended set of claims and submits that at least for this reasons, the set of claims as submitted by way of the present response is inventive over the applied art and requests that the rejection under Section 28.3 of the *Patent Act* be withdrawn.

Finally, Applicant respectfully points out that **D1**-**D4** were cited during the prosecution of the corresponding U.S. application which has matured into U.S. Patent 8,765,369 issued on July 1, 2014 and which contains substantially similar claims to those submitted by way of the present response.

Rejections based on Subsection 27(4) of the Patent Act

1. In the Office Action, claims 1, 28, 31 and 41 have been rejected pursuant to subsection 27(4) of the *Patent Act* as being indefinite in view of the phrase "m is an integer ranging for 2 to 3". [Emphasis added] Applicant has amended claims 1, 28, 31 and 41 such that this phrase now reads "m is an integer ranging from 2 to 3". [Emphasis added] Claims 1, 28, 31 and 41 have been further rejected pursuant to subsection 27(4) of the *Patent Act* as being indefinite in view of the phrase "in association with a surface of the particle". Applicant has amended claims 1, 28, 31 and 41 such that this phrase now reads "grafted to the surface of the nano- or micro-sized particle".

In view of the above, Applicant respectfully requests that the rejection to claims 1, 28, 31 and 41 (renumbered as claims 1, 28, 30 and 40) pursuant to subsection 27(4) of the *Patent Act* be withdrawn.

2. In the Office Action, claim 10 has been rejected pursuant to subsection 27(4) of the *Patent Act* as being indefinite in view of the phrase "The particle any one of claims". Applicant has amended claim 10 such that this phrase now reads "The nano- or micro-sized particle of any one of claims". [Emphasis added]
In view of the above, Applicant respectfully requests that the rejection to claim 10 pursuant to subsection 27(4) of the *Patent Act* be withdrawn.

3. In the Office Action, claim 21 has been rejected pursuant to subsection 27(4) of the *Patent Act* as being indefinite in view of the phrase "every nucleic acid probes". [Emphasis added] Applicant has amended claim 21 such that this phrase now reads "every nucleic acid probe".
In view of the above, Applicant respectfully requests that the rejection to claim 21 pursuant to subsection 27(4) of the *Patent Act* be withdrawn.

4. In the Office Action, claim 26 has been rejected pursuant to subsection 27(4) of the *Patent Act* as being indefinite in view of the phrase "wherein particle is". Applicant has amended claim 26 such that this phrase now reads "wherein the particle is". [Emphasis added]
In view of the above, Applicant respectfully requests that the rejection to claim 26 pursuant to subsection 27(4) of the *Patent Act* be withdrawn.

5. In the Office Action, claim 28 has been rejected pursuant to subsection 27(4) of the *Patent Act* as being indefinite in view of the phrase "by the association complexes". Applicant has amended claim 28 such that this phrase now reads "by the association of complexes". [Emphasis added] Claim 28 has been further rejected pursuant to subsection 27(4) of the *Patent Act* as being indefinite in view of the term "the aggregate". Applicant has amended claim 28 such that this term now reads "the aggregates". [Emphasis added]

In view of the above, Applicant respectfully requests that the rejection to claim 28 pursuant to subsection 27(4) of the *Patent Act* be withdrawn.

6. In the Office Action, claim 29 has been rejected pursuant to subsection 27(4) of the *Patent Act* as being indefinite.

Applicant has deleted claim 29 from the application thus rendering this objection moot.

7. In the Office Action, claim 31 has been rejected pursuant to subsection 27(4) of the *Patent Act* as being indefinite in view of the phrase "the association of complex". Applicant has amended claim 31 such that this phrase now reads "the association of a complex". [Emphasis added]

In view of the above, Applicant respectfully requests that the rejection to claim 31 (renumbered as claim 30) pursuant to subsection 27(4) of the *Patent Act* be withdrawn.

8. In the Office Action, claims 36, 39 and 40 have been rejected pursuant to subsection 27(4) of the *Patent Act* as being indefinite in view of the terms "the aggregates" (claim 36) and "the particles" (claims 39 and 40). Applicant has amended claim 36 such that the term "aggregates" now reads "aggregate". Applicant has amended claims 39 and 40 such that the term "particles" now reads "particle".

In view of the above, Applicant respectfully requests that the rejection to claims 36, 39 and 40 (renumbered as claims 35, 38 and 39) pursuant to subsection 27(4) of the *Patent Act* be withdrawn.

9. In the Office Action, claims 41 and 45 have been rejected pursuant to subsection 27(4) of the *Patent Act* as being indefinite. Applicant has amended claim 41 by reciting that the aggregates are grafted to the surface of each of the nano- or micro-sized particle species. [Emphasis added] Applicant has amended claim 45 by reciting that each of the nano- or micro-sized particle species is capable of detecting a target at a concentration as low as 10^{-19} mole/L. [Emphasis added]

In view of the above, Applicant respectfully requests that the rejection to claims 41 and 45 (renumbered as claims 40 and 44) pursuant to subsection 27(4) of the *Patent Act* be withdrawn.

10. In the Office Action, claims 50 and 52 have been rejected pursuant to subsection 27(4) of the *Patent Act* as being indefinite in view of the term "the particles species". Applicant has amended claims 50 and 52 such that the term "the particles species" now reads "the nano- or micro-sized particle species". [Emphasis added]
In view of the above, Applicant respectfully requests that the rejection to claims 50 and 52 (renumbered as claims 49 and 51) pursuant to subsection 27(4) of the *Patent Act* be withdrawn.

11. In the Office Action, claim 54 has been rejected pursuant to subsection 27(4) of the *Patent Act* as being indefinite in view of the phrases "under condition allowing" and "each manufacturing step are performed". Applicant has amended claim 54 such that these phrases now read "under conditions allowing" and "each manufacturing step is performed". [Emphasis added]
In view of the above, Applicant respectfully requests that the rejection to claim 54 (renumbered as claim 53) pursuant to subsection 27(4) of the *Patent Act* be withdrawn.

12. In the Office Action, claim 55 has been rejected pursuant to subsection 27(4) of the *Patent Act* as being indefinite in view of the term "the particle". As an initial matter, Applicant believes that the Examiner intended to object to the term "the particles" for lacking proper antecedent basis. [Emphasis added] Applicant has amended claim 55 such that the term "the particles" now reads "the nano- or micro-sized particle". [Emphasis added] Applicant respectfully submits that the use of the single tense for the term "particle" is properly supported in claim 54 (renumbered as claim 53).
In view of the above, Applicant respectfully requests that the rejection to claim 55 (renumbered as claim 54) pursuant to subsection 27(4) of the *Patent Act* be withdrawn.

13. In the Office Action, claim 57 has been rejected pursuant to subsection 27(4) of the *Patent Act* as being indefinite. The Action alleges that since these claims relate to a kit, it is implied that at least two components be recited. Applicant has amended claim 57 by further reciting that the kit comprises water.
In view of the above, Applicant respectfully requests that the rejection to claim 57 (renumbered as claim 56) pursuant to subsection 27(4) of the *Patent Act* be withdrawn.

Rejections based on subsection 27(3) of the Patent Act

1. In the Office Action the description has been rejected pursuant to subsection 27(3) of the *Patent Act* as containing statements indicating that the claims are to be viewed as broader than the teachings of the description. Such a statement has been deleted from page 27 (paragraph [00144]). Moreover, paragraph [00144] has been further amended to recite the following statement which is believed to be acceptable under Canadian practice: "*The scope of the claims should not be limited by the preferred embodiments and examples, but should be given the broadest interpretation consistent with the description as a whole*".

In view of the above, Applicant respectfully requests that the rejection to the description pursuant to subsection 27(3) of the *Patent Act* be withdrawn.

Rejections based on subsection 81(1) of the Patent Rules

1. In the Office Action, the description has been rejected pursuant to subsection 81(1) of the *Patent Rules*. The statement incorporating by reference has been deleted from page 27.

In view of the above, Applicant respectfully requests that the rejection to the description pursuant to subsection 81(1) of the *Patent Rules* be withdrawn.

Rejections based on subsection 81(3) of the Patent Rules

1. In the Office Action, the description has been rejected pursuant to subsection 81(3) of the *Patent Rules*. The document referred to on page 27 (line 12) has been identified by author and complete source.

In view of the above, Applicant respectfully requests that the rejection to the description pursuant to *Section 81(3) of the Patent Rules* be withdrawn.

Rejections based on Section 76 of the Patent Rules

1. In the Office Action the description has been rejected pursuant to Section 76 of the *Patent Rules* as containing trademarks that are not identified as such. The terms "*Ficoll*" (page 18a) and "*Tween*" (page 25) have been identified as trademarks.

In view of the above, Applicant respectfully requests that the rejection to the description pursuant to *Section 76 of the Patent Rules* be withdrawn.

In view of the foregoing, Applicant respectfully submits that the application is in order for allowance and an early indication of that effect is respectfully requested. Should the Examiner deem it beneficial to discuss the application in greater detail, the Examiner is kindly requested to contact Erwin Schultz by telephone at (514) 658-4844 (Ext. 205) at the Examiner's convenience.

Respectfully submitted,

Benoit & Côté

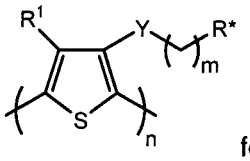
BENOIT & COTE INC.

/am

Enclosure Amended set of claims [Claims 1-57];
Marked-up copy of the claims illustrating amendments;
Amended description pages 18a, 25 and 27; and
Appointment of Agent.

CLAIMS

1. A nano- or micro-sized particle comprising pre-assembled aggregates formed by the association of complexes comprising a nucleic acid probe and a polymer of formula A:



formula A

wherein:

m is an integer ranging from 2 to 3;

n is an integer ranging from 3 to 100;

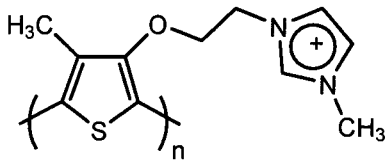
R* is a quaternary ammonium;

Y is an oxygen atom or a methylene; and

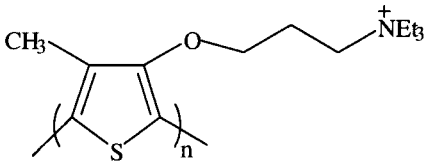
R^1 is a methyl group or a hydrogen atom; and

wherein the aggregates are grafted to the surface of the nano- or micro-sized particle.

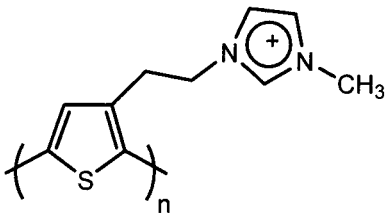
2. The nano- or micro-sized particle of claim **1**, wherein the polymer comprises a formula selected from the group consisting of:



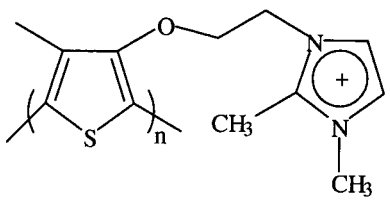
formula I



formula II

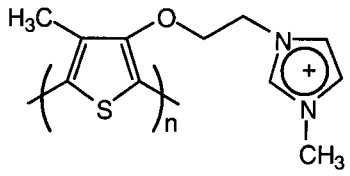


formula III and



formula IV.

3. The nano- or micro-sized particle of claim **2**, wherein the polymer comprises a formula Ia



Ia

wherein n is an integer ranging from 6 to 100.

4. The nano- or micro-sized particle of any one of claims **1** to **3**, wherein the nucleic acid probe is single-stranded.

5. The nano- or micro-sized particle of any one of claims **1** to **4**, wherein the nucleic acid probe comprises a label.

6. The nano- or micro-sized particle of any one of claims **1** to **5**, wherein the particle is capable of detecting a target at a concentration as low as 10^{-16} mole/L.

7. The nano- or micro-sized particle of claim **6**, wherein the particle is capable of detecting a target at a concentration as low as 10^{-17} mole/L.

8. The nano- or micro-sized particle of claim **7**, wherein the particle is capable of detecting a target at a concentration as low as 10^{-18} mole/L.

9. The nano- or micro-sized particle of claim **8**, wherein the particle is capable of detecting a target at a concentration as low as 10^{-19} mole/L.

10. The nano- or micro-sized particle of any one of claims **1** to **9**, wherein the nucleic acid probe comprises a portion/section for specific recognition of a target.

11. The nano- or micro-sized particle of any one of claims **1** to **10**, wherein the particle is in an aqueous solution.

12. The nano- or micro-sized particle of any one of claims **1** to **11**, wherein the probe is RNA or DNA.

13. The nano- or micro-sized particle of any one of claims **1** to **12**, wherein the probe is from 8 to 50 bases long.

14. The nano- or micro-sized particle of any one of claims **6** to **13**, wherein the target has affinity for nucleic acids.

15. The nano- or micro-sized particle of claim **14**, wherein the target comprises a nucleic acid.

16. The nano- or micro-sized particle of claim **15**, wherein the nucleic acid is single-stranded or double stranded.

17. The nano- or micro-sized particle of claim **15** or **16**, wherein the nucleic acid is DNA, RNA or a DNA/RNA chimera.

18. The nano- or micro-sized particle of claim **17**, wherein the DNA is a PCR amplicon, a genomic DNA or a restriction fragment.

19. The nano- or micro-sized particle of claim **14**, wherein the target comprises a protein or a peptide.

20. The nano- or micro-sized particle of any one of claims **10** to **19** wherein the target is unlabeled.

21. The nano- or micro-sized particle of any one of claims **1** to **20**, wherein every nucleic acid probe of the aggregates is identical.

22. The nano- or micro-sized particle of any one of claims **1** to **21**, wherein the aggregates provide for resonance energy transfer.

23. The nano- or micro-sized particle of claim **5**, wherein the label comprises a fluorophore.

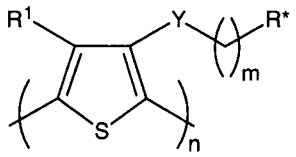
24. The nano- or micro-sized particle of claim **5**, wherein the label comprises a chromophore.

25. The nano- or micro-sized particle of any one of claims **1** to **24**, wherein the nucleic acid probe and the polymer are in stoichiometric amounts.

26. The nano- or micro-sized particle of any of claims **1** to **25**, wherein the particle is a mobility-controllable particle.

27. The nano- or micro-sized particle of any one of claims **1** to **26**, wherein the particle comprises a tag allowing identification of at least one of the nucleic acid probe and target associated with the particle.

28. A composition comprising multiple nano- or micro-sized particle species, wherein each nano- or micro-sized particle species comprises pre-assembled aggregates formed by the association of complexes comprising a distinct nucleic acid probe species and a polymer of formula A:



formula A

wherein:

m is an integer ranging from 2 to 3;

n is an integer ranging from 3 to 100;

R* is a quaternary ammonium;

Y is an oxygen atom or a methylene; and

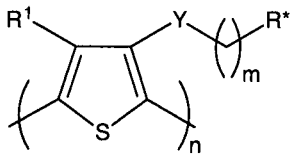
R^1 is a methyl group or a hydrogen atom; and

wherein the aggregates are grafted to the surface of the nano- or micro-sized particle species.

29. The use of the nano- or micro-sized particle of any of claims **1** to **27** for determining the presence or absence of a target in a sample or for isolating the target from the sample.

30. A method for detecting the presence or absence of a target in a sample comprising or suspected of comprising the target, the method comprising:

contacting the sample with a nano- or micro-sized particle comprising a pre-assembled aggregate formed by the association of a complex comprising a nucleic acid probe and a polymer of formula A:



formula A

wherein:

m is an integer ranging from 2 to 3;

n is an integer ranging from 3 to 100;

R* is a quaternary ammonium;

Y is an oxygen atom or a methylene; and

R^1 is a methyl group or a hydrogen atom;

allowing a sufficient period of time for the target to bind the nucleic acid probe; and

measuring or identifying a signal emitted upon binding of the target and the nucleic acid probe, and wherein the aggregate is grafted to the surface of the nano- or micro-sized particle.

31. The method of claim **30**, wherein the target is at a concentration as low as 10^{-19} mole/L in the sample.

32. The method of claim **30**, wherein the nucleic acid probe comprises a label.

33. The method of any one of claims **30** to **32**, wherein the detection is performed in aqueous conditions.

34. The method of claim **32** or **33**, wherein the label is a fluorescent acceptor molecule.

35. The method of any one of claims **30** to **34**, wherein the aggregate provides for resonance energy transfer.

36. The method of any of claims **30** to **35**, wherein the nano- or micro-sized particle is a mobility-controllable particle.

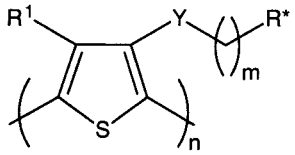
37. The method of any one of claims **30** to **36**, wherein the nano- or micro-sized particle comprises a tag allowing identification of the nucleic acid probe associated with the nano- or micro-sized particle.

38. The method of any one of claims **30** to **37**, wherein the nano- or micro-sized particle is concentrated to a smaller volume than the original volume of the contacting step and is submitted to a flow of clean media before measuring or identifying the signal.

39. The method of any one of claims **30** to **38**, wherein the nano- or micro-sized particle is mixed with the sample so as to enable capture of substantially all targets from the sample.

40. A method for the simultaneous detection of multiple target species from a sample, the method comprising:

contacting the sample with a composition comprising multiple nano- or micro-sized particle species, wherein each nano- or micro-sized particle species comprises pre-assembled aggregates formed by the association of complexes comprising a distinct nucleic acid probe species and a polymer of formula A:



formula A

wherein:

m is an integer ranging from 2 to 3;

n is an integer ranging from 3 to 100;

R* is a quaternary ammonium;

Y is an oxygen atom or a methylene; and

R^1 is a methyl group or a hydrogen atom;

allowing a sufficient period of time for the target species to bind the nucleic acid probe species and;

measuring or identifying a signal emitted upon binding of the target species and the nucleic acid probe species; and

wherein each nano- or micro-sized particle species further comprises a distinct and selectable tag allowing its distinction among the multiple nano- or micro-sized particle

species and wherein the aggregates are grafted to the surface of each of the nano- or micro-sized particle species.

41. The method of claim **40**, further comprising a step of isolating each nano- or micro-sized particle species based on the identity of the tag.

42. The method of claim **40** or **41**, wherein each nucleic acid probe species comprises a distinct nucleic acid sequence.

43. The method of any one of claims **40** to **42**, wherein each of the nucleic acid probe species comprises a label.

44. The method of any one of claims **40** to **43**, wherein each of the nano- or micro-sized particle species is capable of detecting a target at a concentration as low as 10^{-19} mole/L.

45. The method of claim **44**, wherein the label is a fluorescent acceptor molecule.

46. The method of any one of claims **40** to **45**, wherein the detection is performed in an aqueous solution.

47. The method of any one of claims **40** to **46**, wherein each aggregate of the nano- or micro-sized particle species is independently providing for resonance energy transfer.

48. The method of any of claims **40** to **47**, wherein the nano- or micro-sized particle species are mobility-controllable.

49. The method of any one of claims **40** to **48**, wherein the nano- or micro-sized particle species are concentrated to a smaller volume than the original volume of the contacting step.

50. The method of any one of claims **40** to **49**, wherein the nano- or micro-sized particle species are confined in a delimited space and are submitted to a flow of clean media before the measuring or identifying step.

51. The method of any one of claims **40** to **50**, wherein the nano- or micro-sized particle species are mixed with the sample so as to enable capture of substantially all target species from the sample.

52. The method of any one of claims **40** to **51**, wherein the method is used for determining whether the target species is an optimal target or a suboptimal target, wherein the method further comprises comparing a signal emitted upon binding of the target species to the nucleic acid probe species to a reference signal obtained for an optimal target, whereby a signal equal or higher than the reference signal is indicative of the presence of an optimal target in the sample and whereby a signal lower than the reference signal is indicative of the presence of a sub-optimal target in the sample.

53. A method of manufacturing the nano- or micro-sized particle of any one of claims **1** to **27**, the method comprising assembling aggregates by mixing a nucleic acid capture probe comprising an attaching means and the polymer of formula A, formula I, formula Ia, formula II, formula III or formula IV under conditions allowing for their electrostatic interaction, and grafting the aggregates onto a surface of a receptive nano- or micro-sized particle and wherein each manufacturing step is performed in an aqueous solution.

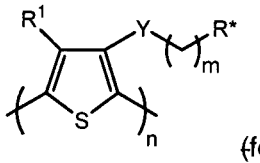
54. The method of claim **53**, wherein the aggregates are grafted to the nano- or micro-sized particle in a native form obtained in solution and wherein the aggregates retain photonic properties upon grafting to the nano- or micro-sized particle.

55. The method of claim **53** or **54**, wherein the nano- or micro-sized particles are dispersed in liquid media.

56. A kit comprising the nano- or micro-sized particles of any one of claims **1** to **27** and water.

CLAIMS

1. A nano- or micro-sized particle comprising pre-assembled aggregates formed by the association of complexes comprising a nucleic acid probe and a polymer of formula A:



(formula A)

wherein:

m is an integer ranging from for 2 to 3;

n is an integer ranging from 3 to 100;

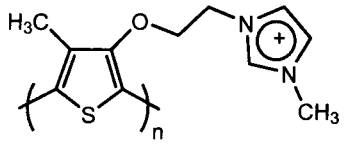
R* is a quaternary ammonium;

Y is an oxygen atom or a methylene; and

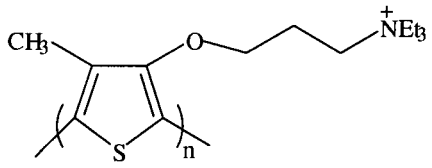
R^1 is a methyl group or a hydrogen atom; and

wherein the aggregates are grafted in association with a to the surface of the nano- or micro-sized particle[.]

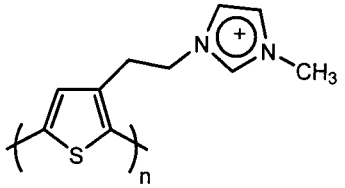
2. The nano- or micro-sized particle of claim **1**, wherein the polymer comprises a formula selected from the group consisting of:



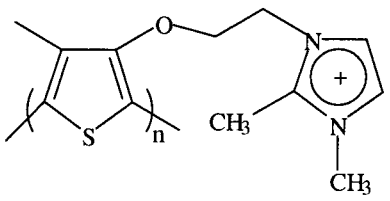
(formula I)



(formula II)

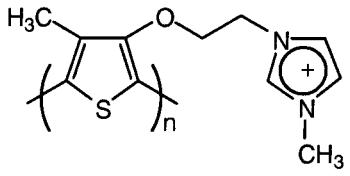


(formula III) and



{formula IV}[.]

3. The nano- or micro-sized particle of claim **2**, wherein the polymer comprises a formula Ia



Ia

wherein n is an integer ranging from 6 to 100.

4. The nano- or micro-sized particle of any one of claims **1** to **3**, wherein the nucleic acid probe is single-stranded.

5. The nano- or micro-sized particle of any one of claims **1** to **4**, wherein the nucleic acid probe comprises a label.

6. The nano- or micro-sized particle of any one of claims **1** to **5**, wherein the particle is capable of detecting a target at a concentration as low as 10^{-16} mole/L.

7. The nano- or micro-sized particle of claim **6**, wherein the particle is capable of detecting a target at a concentration as low as 10^{-17} mole/L.

8. The nano- or micro-sized particle of claim **7**, wherein the particle is capable of detecting a target at a concentration as low as 10^{-18} mole/L.

9. The nano- or micro-sized particle of claim **8**, wherein the particle is capable of detecting a target at a concentration as low as 10^{-19} mole/L.

10. The nano- or micro-sized particle of any one of claims **1** to **9**, wherein the nucleic acid probe comprises a portion/section for specific recognition of a target.

11. The nano- or micro-sized particle of any one of claims **1** to **10**, wherein the particle is in an aqueous solution.

12. The nano- or micro-sized particle of any one of claims **1** to **11**, wherein the probe is RNA or DNA.

13. The nano- or micro-sized particle of any one of claims **1** to **12**, wherein the probe is from 8 to 50 bases long.

14. The nano- or micro-sized particle of any one of claims **6** to **13**, wherein the target has affinity for nucleic acids.

15. The nano- or micro-sized particle of claim **14**, wherein the target comprises a nucleic acid.

16. The nano- or micro-sized particle of claim **15**, wherein the nucleic acid is single-stranded or double stranded.

17. The nano- or micro-sized particle of ~~any one of~~ claim[s] **15** ~~to~~ or **16**, wherein the nucleic acid is DNA, RNA or a DNA/RNA chimera.

18. The nano- or micro-sized particle of claim **17**, wherein the DNA is a PCR amplicon, a genomic DNA or a restriction fragment.

19. The nano- or micro-sized particle of claim **14**, wherein the target comprises a protein or a peptide.

20. The nano- or micro-sized particle[~~s~~] of any one of claims **10** to **19** wherein the target is unlabeled.

21. The nano- or micro-sized particle of any one of claims **1** to **20**, wherein every nucleic acid probe[~~s~~] of the aggregates ~~are~~ is identical.

22. The nano- or micro-sized particle of any one of claims **1** to **21**, wherein the aggregates ~~are capable of~~ provide for resonance energy transfer.

23. The nano- or micro-sized particle of claim **5**, wherein the label comprises a fluorophore.

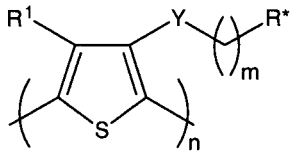
24. The nano- or micro-sized particle of claim **5**, wherein the label comprises a chromophore.

25. The nano- or micro-sized particle of any one of claims **1** to **24**, wherein the nucleic acid probe and the polymer are in stoichiometric amount[s].

26. The nano- or micro-sized particle of any of claims **1** to **25**, wherein the particle is a mobility-controllable particle.

27. The nano- or micro-sized particle of any one of claims **1** to **26**, wherein the particle comprises a tag allowing identification of at least one of the nucleic acid probe and~~/or~~ target associated with the particle.

28. A composition comprising multiple nano- or micro-sized particle species, wherein each nano- or micro-sized particle species comprises pre-assembled aggregates formed by the association of complexes comprising a distinct nucleic acid probe species and a polymer of formula A:



~~(~~formula A~~)~~

wherein:

m is an integer ranging from ~~for~~ 2 to 3;

n is an integer ranging from 3 to 100;

R* is a quaternary ammonium;

Y is an oxygen atom or a methylene; and

R^1 is a methyl group or a hydrogen atom; and

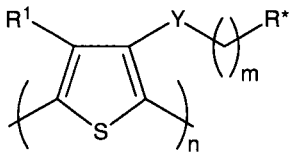
wherein the aggregate[s] are grafted ~~is in association with a~~ to the surface of the nano- or micro-sized particle species.

~~**29.** A method for *in vitro* detecting the presence or absence of a target using the particle of any one of claims **1** to **27**.~~

~~30~~**29.** The use of the nano- or micro-sized particle of any of claims **1** to **27** for determining the presence or absence of a target in a sample or for isolating the target from the sample.

~~31~~30. A method for detecting the presence or absence of a target in a sample comprising or suspected of comprising the target, the method comprising:

contacting the sample with a nano- or micro-sized particle comprising a pre-assembled aggregate formed by the association of a complex comprising a nucleic acid probe and ~~and~~ a polymer of formula A:



~~(~~formula A~~)~~

wherein:

m is an integer ranging from ~~for~~ 2 to 3;

n is an integer ranging from 3 to 100;

R* is a quaternary ammonium;

Y is an oxygen atom or a methylene; and

R^1 is a methyl group or a hydrogen atom;

allowing a sufficient period of time for the target to bind the nucleic acid probe; and

measuring or identifying a signal emitted upon binding of the target and the nucleic acid probe, and wherein the aggregate is grafted to the ~~in association with a~~ surface of the nano- or micro-sized particle[.]

~~32~~31. The method of claim ~~31~~30, wherein the target is at a concentration as low as 10^{-19} mole/L in the sample.

~~33~~32. The method of claim ~~31~~30, wherein the nucleic acid probe comprises a label.

~~34~~33. The method of any one of claims ~~31~~30 to ~~33~~32, wherein the detection is performed in aqueous conditions.

~~35~~34. The method of ~~any one of~~ claim[~~s~~] ~~33~~32 or ~~34~~33, wherein the label is a fluorescent acceptor molecule.

~~36~~35. The method of any one of claims ~~31~~30 to ~~35~~34, wherein the aggregate[~~s~~] ~~are capable of~~ provides for resonance energy transfer.

~~37~~36. The method of any of claims ~~31~~30 to ~~36~~35, wherein the nano- or micro-sized particle is a mobility-controllable particle.

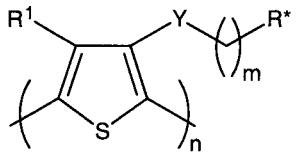
~~38~~37. The method of any one of claims ~~31~~30 to ~~37~~36, wherein the nano- or micro-sized particle comprises a tag allowing identification of the nucleic acid probe associated with the nano- or micro-sized particle.

~~39~~38. The method of any one of claims ~~31~~30 to ~~38~~37, wherein the nano- or micro-sized particle[~~s~~] is ~~are~~ concentrated to a smaller volume than the original volume of the contacting step and is ~~are~~ submitted to a flow of clean media before measuring or identifying the signal.

~~40~~39. The method of any one of claims ~~31~~30 to ~~39~~38, wherein the nano- or micro-sized particle[~~s~~] is ~~are~~ mixed with the sample so as to enable capture of substantially all targets from the sample.

~~41~~40. A method for the simultaneous detection of multiple target species from a sample, the method comprising:

contacting the sample with a composition comprising multiple nano- or micro-sized particle species, wherein each nano- or micro-sized particle species comprises pre-assembled aggregates formed by the association of complexes comprising a distinct nucleic acid probe species and a polymer of formula A:



~~(~~formula A~~)~~

wherein:

m is an integer ranging from ~~for~~ 2 to 3;

n is an integer ranging from 3 to 100;

R* is a quaternary ammonium;

Y is an oxygen atom or a methylene; and

R^1 is a methyl group or a hydrogen atom;

allowing [a] sufficient period of time for the target species to bind the nucleic acid probe species and;

measuring or identifying a signal emitted upon binding of the target species and the nucleic acid probe species; and

wherein each nano- or micro-sized particle species further comprises a distinct and selectable tag allowing its distinction among the multiple nano- or micro-sized particle species and wherein the aggregates are grafted to the ~~in association with a~~ surface of each of the nano- or micro-sized particle species.

~~42~~41. The method of claim ~~41~~40, further comprising a step of isolating each nano- or micro-sized particle species based on the identity of the tag.

~~43~~42. The method of ~~any one of~~ claim[~~s~~] ~~41~~40 or ~~42~~41, wherein each nucleic acid probe species comprises a distinct nucleic acid sequence.

~~44~~43. The method of any one of claims ~~41~~40 to ~~43~~42, wherein each of the nucleic acid probe species comprises a label.

~~45~~44. The method of any one of claims ~~41~~40 to ~~44~~43, wherein each of the nano- or micro-sized particle species is capable of detecting a target at a concentration as low as 10^{-19} mole/L.

~~46~~45. The method of claim ~~45~~44, wherein the label is a fluorescent acceptor molecule.

~~47~~46. The method of any one of claims ~~41~~40 to ~~46~~45, wherein the detection is performed in an aqueous solution.

~~48~~47. The method of any one of claims ~~41~~40 to ~~47~~46, wherein each aggregate of the nano- or micro-sized particle species is ~~are~~ independently providing for ~~capable of~~ resonance energy transfer.

~~49~~48. The method of any of claims ~~41~~40 to ~~48~~47, wherein the nano- or micro-sized particle species are mobility-controllable.

~~50~~49. The method of any one of claims ~~41~~40 to ~~49~~48, wherein the nano- or micro-sized particle[~~s~~] species are concentrated to a smaller volume than the original volume of the contacting step.

~~51~~50. The method of any one of claims ~~41~~40 to ~~50~~49, wherein the nano- or micro-sized particle[~~s~~] species are confined in a delimited space and are submitted to a flow of clean media before the measuring or identifying step.

~~52~~51. The method of any one of claims ~~41~~40 to ~~51~~50, wherein the nano- or micro-sized particle[~~s~~] species are mixed with the sample so as to enable capture of substantially all target species from the sample.

~~53~~52. The method of any one of claims ~~41~~40 to ~~52~~51, wherein the method is used for determining whether the target species is an optimal target or a suboptimal target, wherein the method further comprise[s] comparing a signal emitted upon binding of the target species to the nucleic acid probe species to a reference signal obtained for an optimal target, whereby a signal equal or higher than the reference signal is indicative of the presence of an optimal target in the sample and whereby a signal lower than the reference signal is indicative of the presence of a sub-optimal target in the sample.

~~54~~53. A method of manufacturing the nano- or micro-sized particle of any one of claims **1** to **27**, the method comprising assembling aggregates by mixing a nucleic acid capture probe comprising an attaching means and the polymer of formula A, formula I, formula Ia, formula II, formula III or formula IV under condition[s] allowing for their electrostatic interaction, and grafting ~~associating~~ the aggregates onto a surface of a receptive nano- or micro-sized particle and wherein each manufacturing step ~~are~~ is performed in an aqueous solution.

~~55~~54. The method of claim ~~54~~53, wherein the aggregates are ~~associated~~ grafted to the nano- or micro-sized particle[~~s~~] in a native form obtained in solution and wherein the aggregates retain photonic properties upon ~~association with~~ grafting to the nano- or micro-sized particle.

~~56~~55. The method of ~~manufacturing of~~ claim **53** or **54** ~~56 or 57~~, wherein the nano- or micro-sized particles are dispersed in liquid media.

~~57~~56. A kit comprising the nano- or micro-sized particles of any one of claims **1** to **27** and water.

In order to carry the methods of the present invention, the nucleic acid probe (probe species) and the polymer may be in stoichiometric amount.

Also in accordance with the present invention, each nucleic acid probe species may comprise a predetermined nucleic acid sequence.

In accordance with the present invention, the particles species may be concentrated to a smaller volume than the original volume of the contacting step. The particles species may be confined in a delimited space before the measuring or identifying step. Further in accordance with the present invention, the particles species may be mixed with the sample so as to enable capture of substantially all target species from the sample.

[0090] In accordance with the present invention, the target may be isolated. The target may be purified or substantially purified using the method described herein.

[0091] Hybridization may be performed under various stringency conditions in order to control the interaction between the probes and the targets. Higher stringency minimizes unspecific binding between capture probes and target molecules.

[0092] "Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization also depends on the ability of denatured DNA target to reanneal with complementary strands when present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel et al., Current Protocols in Molecular Biology, Wiley Interscience Publishers, (1995).

[0093] Exemplary embodiment of "stringent conditions" or "high stringency conditions", as defined herein, may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C.; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1 % bovine serum albumin/0.1 % Ficoll®/0.1% polyvinylpyrrolidone/50

and the resulting complex (dubbed duplex) was grafted to the surface of the particles as described below. Such labelled anionic capture probes and cationic polymer associate (preferably stoichiometrically) through electrostatic interactions and thus form nano-aggregates which may then be transferred onto the surface of controllable-mobility particles.

[00134] Target-ready particles production

[00135] AF546-labeled probes were diluted into pure, autoclaved water to a final concentration of $2x10^{-5}$ M of oligonucleotide strands (final volume of 10 μL) and mixed stoicheiometrically (on a repeat unit basis) with the cationic water-soluble polythiophene (6.1 μL of $3.3x10^{-5}$ M) in order to form the duplex. The mixture was then gently shaken during 10 minutes at 30°C. Target-ready particles were prepared by mixing the resulting duplex solution with magnetic particle (typically 10^6 beads) in Tween®20/LiCL/Tris buffer (30 μL) and stirring for 10 minutes at room temperature. Aggregate-grafted particles were then rinsed twice with Tween20 solution (0.5% v/v) and suspended in water until use. This protocol represents a reproducible method for aggregates formation and for the conservation of the detection properties (sensitivity and specificity) during transfer onto particles. Optimized aggregates functionalization protocol can be established with different particles surface such carboxylic, epoxyde or aldehyde functionality and probe with terminal reactive group (amine, sulphide...), using activator or not.

[00136] For the examples described herein, target hybridization was performed in pure water at 65°C.

[00137] Figure 2 shows typical results for the detection of targets in a highly diluted suspension, i.e. 200 particles suspended in a total volume of 3 mL (using a 3 mL fluorescence cuvette). The concentrations of the solutions used to generate these response curves varied from 0 to ~6500 copies of ssDNA targets diluted in the 3 mL volume (0 to $3.6x10^{-18}$ mole/L). For each measurement, the total time required for hybridization and optical detection was less than five minutes. The detection limit (defined as 3 times the standard error on the signal measured from blank beads, i.e. aggregate-grafted particles without any target) calculated from these measurements was 15 target DNA molecules in the 150-μL effective probed volume ($2x10^{-19}$ mole/L). Interestingly, this detection limit is closer to that measured previously for FCR aggregates free in homogenous solution using the same fluorometer, i.e. 3×10^{-21} mole/L (JACS 2005) than to that reported for glass slide based FCR (5×10^{-16} mole/L), which tends to demonstrate that the one-pot procedure used to attach the aggregates

example were purified and fractionated genomic DNA targets (typical fragment length 500-2000 base pairs) initially dispersed in a 4uL aqueous sample. The controllable mobility of FCR-grafted particles grants them the primordial advantage of changing their local chemical environment at will, to exploit, as shown in this example, the lower binding equilibrium constant of non complementary material with the FCR aggregates and hence dynamically maximizing the discrimination between perfectly matched and non matched targets.

[00142] Fluorescence Measurement

[00143] Although other apparatus and devices may be used, fluorescence measurements were performed with two custom fluorescence readers. Experiments with highly diluted particle concentrations were performed with a custom-made portable fluorometer tailored for the polythiophene sensor (Doré et al. J. Am Chem Soc. 126, 4240-4244 (2004)). Fluorescence detection of particles magnetically confined in μ-electro-magnetic traps and particles physically confined on a weir within a microfluidic device was performed with a custom-made fluorescence detection system dedicated to the collection of the optical signal coming from a solid support surface. For each apparatus, the excitation wavelength and the narrow bandpass of the interference emission filter (centered at 575nm) overlapped well with the absorption of the polymer transducer and emission of AlexaFluor 546 spectral profiles, respectively.

[00144] Although the present invention has been described herein by way of exemplary embodiments, the scope of the claims should not be limited by the preferred embodiments and examples, but should be given the broadest interpretation consistent with the description as a whole.

BENOÎT&CÔTÉ

February 13, 2015

Canadian Intellectual Property Office (CIPO)
Place du Portage I
50, Victoria Street
Gatineau (Québec)
K1A 0C9

Re :	REVOCATION OF APPOINTMENT OF AN AGENT AND APPOINTMENT OF ANOTHER AGENT Canadian Patent Application No. 2,692,882 Filed on July 11, 2008
Applicant :	NATIONAL RESEARCH COUNCIL OF CANADA
Title :	ULTRASENSITIVE DETECTION OF TARGET USING TARGET-READY PARTICLES
Our Ref :	P3024CA00

Sir:

You will find enclosed the "Revocation of Appointment of Agent and Appointment of Another Agent".

RESPECTFULLY SUBMITTED.

Benoît & Côté

Benoît & Côté Inc.

/AM

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REVOCATION OF APPOINTMENT OF AGENT AND APPOINTMENT OF ANOTHER AGENT

The Applicant, **NATIONAL RESEARCH COUNCIL OF CANADA** whose full address is: 1200 Montreal Road, Building M55, Room 29, Ottawa, Ontario K1A 0R6, Canada hereby revokes any and all previous appointments of agent and representative for service and appoints **Benoît & Côté Inc.** whose full address is 1550, Metcalfe Street, suite 800, Montreal, QC, H3A 1X6 - CANADA as its agent in respect of the Patent Application Number **2,692,882** filed on July 11, 2008 and entitled ULTRASENSITIVE DETECTION OF TARGET USING TARGET-READY PARTICLES with full power to appoint an associate agent when required to do so and to revoke such appointment, to sign the petition and drawings, to amend the specification and drawings, to prosecute the application, and to receive the patent granted thereon, and ratifies any act done by the last named appointee in respect of the foregoing application (Agent ref.: P3024CA00).

Signed at Ottawa (City or Town) Canada (Country),

this 30th day of January, 2015.

By: _____,
Name: Dick Bourgeois-Doyle
Title: Secretary-General
National Research Council of Canada