

Sensitive analyte detection by magnetic beads-gold nanoparticles-immuno-PCR (MB-GNP-IPCR) with photocleavable barcodes

Technical Field

5 The present invention belongs to a medical field, especially, a medical diagnosis field, and relates to sensitive analyte detection by magnetic beads-gold nanoparticles-immuno-PCR (MB-GNP-IPCR) with photocleavable barcodes. In particular, the present invention relates to a method for sensitive detection of one or more target analytes, e.g., proteins in a sample, and in some cases, determining a measure of the concentration of the
10 target analytes in the sample.

Background

 Analyte detection is of utmost importance in research and medical applications. Several methods for detection of different types of analytes have been developed and
15 employed in many areas such as academic research, medical diagnosis and environmental assessment. In particular, there is an increasing need for hypersensitive detection of multiple analytes in clinical applications in which samples are limited or rare, and analyte quantities are low.

20 Most methods for sensitive detection of low amounts of analytes in a sample utilize amplification techniques to increase the amount of reporter molecules in order to produce measurable signal. Examples of these methods include enzyme-linked immunosorbent assays (ELISA) that enable signal amplification of antibody-based assays and polymerase chain reaction (PCR) that amplifies target DNA in nucleic acid-based assays. Immuno-PCR
25 (IPCR) refers to a technique combining the aforementioned two methods (see Sano, T.; Smith, C. L.; Cantor, C. R. Science 1992, 258, 120-122.) by replacing the enzyme linked to the detection antibody with DNA such that the enzyme-catalyzed substrate readout is converted to PCR amplification instead. IPCR increases sensitivity of traditional ELISA by the exponential signal amplification of PCR. Further improvements to IPCR have been
30 developed over the years and the method described here incorporates the use of magnetic beads (MB), gold nanoparticles (GNP) and photocleavable (PC) DNA oligonucleotides to further improve the sensitivity and specificity of IPCR to enable hypersensitive, multiplexed detection of analytes with a molecular readout.

Summary of the Invention

The inventive gist of this application lies in a method that combines the use of both magnetic beads and gold nanoparticles to form sandwich complexes with target analytes,
5 and photocleavable oligonucleotides as reporter barcodes.

The invention aims for a combined use of MB, GNP, IPCR and photocleavable oligonucleotides for a hypersensitive detection with respect to limited or rare samples and low analyte quantities.

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Specifically, the present invention relates to a bifunctionalized gold nanoparticle (GNP) with both detection antibodies (Abs) and detection oligonucleotides (Oligos) via a non-covalent binding approach or a covalent conjugation with a crosslinker (e.g., succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate, SMCC).

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According to the present invention, the non-covalent binding approach may include adsorption, SA-protein A chimera, receptor-ligand binding system, and SA-biotin coupling system, preferably SA-biotin coupling system.

20 According to the present invention, GNP is coated with streptavidin (SA), the detection Abs are biotinylated Abs, and the detection Oligos are biotinylated Oligos, preferably Oligos labelled with photocleavage (PC) biotin.

According to the present invention, GNP is 10-100nm, preferably 20nm, in size.

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The present invention also relates to use of magnetic beads (MB), gold nanoparticles (GNP) and photocleavable (PC) oligonucleotides (Oligos) for improving the sensitivity and specificity of immuno-PCR (IPCR) to enable hypersensitive, multiplexed detection of analytes.

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Furthermore, the present invention relates to a combined use of MB, GNP, IPCR and PC Oligo for a hypersensitive detection with respect to limited or rare samples and/or low analyte quantities.

According to the present invention, MB is a functionalized MB, e.g., conjugated to a capture Ab, if the analyte or target of interest in a sample is an antigen, and vice versa (i.e., MB is a conjugated MB to an antigen, if the analyte or target of interest in a sample is an antibody), or a capture receptor, if the analyte or target of interest is a ligand, and vice versa. Preferably, MB is 1-5 μm in size.

According to the present invention, GNP is the bifunctionalized GNP as mentioned above.

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The present invention relates to a method for hypersensitive detection of an analyte in a sample, comprising steps of:

- 1) Isolating a target of interest from the sample by means of MB to form a MB-target complex;
- 15 2) Binding of GNP to the MB-target complex to form a MB-target-GNP sandwich;
- 3) Releasing the detection oligonucleotide from the GNP by cleavage; and
- 4) Amplifying the detection oligonucleotide by PCR.

According to the present invention, MB is 1-5 μm in size.

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According to the present invention, MB is a conjugated MB with a capture Ab when the target of interest is an antigen, and vice versa.

According to the present invention, GNP is the bifunctionalized GNP as mentioned above.

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According to the present invention, step 1) further comprises collecting MBs with a magnet.

According to the present invention, steps 2) and 3) each further comprises releasing and collecting MBs with a magnet.

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According to the present invention, the detection oligonucleotide contains a cleavable region to allow their release from the GNP.

According to the present invention, the cleavable region is a sequence or substrate
5 that is specifically cleaved by a cleaving agent or when an optimal reaction condition is met.

According to the present invention, the cleaving agent is an endonuclease or uracil-DNA glycosylase, and the optimal reaction condition is UV light at 365 nm.

According to the present invention, the cleavable region is a photocleavable region,
10 such as PC-biotin.

According to the present invention, the detection oligonucleotide is an oligonucleotide labelled with PC-biotin, preferably selected from SEQ ID NO: 1, 5 or 9.
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According to the present invention, the target of interest is selected from IL-6, IL-8 and/or IL-1 β .

According to the present invention, PCR is selected from qRT-PCR, Rolling Circle
20 Amplification (RCA) or Loop-mediated Isothermal Amplification (LAMP), preferably qRT-PCR.

Finally, the present invention relates to a method for hypersensitive detection of an analyte in a sample by magnetic beads-gold nanoparticles-immuno-PCR (MB-GNP-IPCR)
25 with photocleavable barcodes, in particularly, comprising steps of:

- 1) Providing the sample containing one or more targets of interest, adding MBs conjugated to capture antibodies (Abs) to the sample, to form MB-target complexes, and collecting MBs with a magnet, for isolation of target(s) by capture Abs of MBs;
- 2) Releasing MBs, adding streptavidin (SA)-coated bifunctionalized GNP with both
30 biotinylated detection Abs and biotinylated detection oligonucleotides via SA-biotin coupling system to MB-target complexes, to form MB-target-GNP sandwiches for binding of targets by detection Abs, and collecting MBs with a magnet;

- 3) Releasing MBs, cleaving detection oligonucleotides labelled with PC-biotin from the GNPs by UV irradiation (e.g., at 365 nm) for releasing detection oligonucleotides, collecting MBs by a magnet, and obtaining a supernatant containing the released detection oligonucleotides;
- 5 4) Adding the supernatant to qRT-PCR master mix for amplification and detection of the detection oligonucleotides by qRT-PCR.

Positive effects

Usage of MBs increases both specificity and sensitivity by allowing efficient washing and enabling the use of many capture Abs on the surfaces of MBs. Besides signal amplification by PCR, signal amplification also arises from the numerous detection oligonucleotides bound to each GNP, thus further increasing the sensitivity of the method. Additionally, the release of detection oligonucleotides via combination of UV irradiation and PC-biotin contributes to specificity of the method as well.

15 MB-GNP-IPCR of the present invention works and is at least 10-fold more sensitive than both basic IPCR and ELISA.

Brief Description of the Drawings

To clearly indicate the technical solution of the present invention, a brief introduction thereto is provided below in reference to the Figures. Apparently, these Figures are merely some embodiments recorded in this application. The present invention includes but is not limited to these Figures.

Fig.1 shows a general scheme of MB-GNP-IPCR with PC barcodes;

Fig.2 shows a graph of mean ΔC_t for each IL-6 protein concentration tested for MB-GNP-IPCR;

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Fig.3 shows a graph of mean ΔC_t for each IL-6 protein concentration tested for IPCR;

Fig.4 shows a graph of mean normalized absorbance for each IL-6 protein concentration tested for ELISA; and

Fig.5 shows qRT-PCR amplification curves for IL-6, IL-8 and IL-1 β .

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Detailed Description

Abbreviations

Antibodies, Abs;

Bovine Serum Albumin, BSA;
 DeoxyriboNucleic Acid, DNA;
 Enzyme-Linked Immunosorbent Assay, ELISA;
 Gold NanoParticles, GNP;
 5 Hour(s), hr(s);
 Immuno-PCR, IPCR;
 Loop-mediated Isothermal Amplification, LAMP;
 Magnetic Beads, MB;
 Magnetic Beads-Gold NanoParticles-Immuno-PCR, MB-GNP-IPCR;
 10 Minutes, min;
 Phosphate-Buffered Saline, PBS;
 PhotoCleavable, PC;
 Polymerase Chain Reaction, PCR;
 quantitative Real-Time PCR, qRT-PCR;
 15 Rolling Circle Amplification, RCA;
 Streptavidin, SA;
 Succinimidyl-4-(N-Maleimidomethyl)Cyclohexane-1-Carboxylate, SMCC;
 Seconds, sec;
 UltraViolet, UV.

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Definitions

“a” or “an” herein refers to one or more, and thus an antigen may include one or more antigen, and an analyte may include one or more analytes.

Similarly, “target of interest” in a sample may include a plurality of targets of interest,
 25 and also refers to “an analyte” in a sample, or sometimes refers to “an antigen” in a sample.

“detection oligonucleotide” refers to “detection oligo”, “oligo” or “oligo barcode”, and they may be thus interchangeably used herein, unless specially illustrated otherwise.

“functionalized with” refers to “conjugated to”, and they may be thus interchangeably used herein, unless specially illustrated otherwise.

30 “binding system” usually refers to “coupling system”, and “SA-Biotin binding system” and “SA-Biotin coupling system” may be thus interchangeably used herein, unless specifically illustrated otherwise.

“detection antibody” may refer to another antibody against an antigen, relative to a capture antibody. However, sometimes, “detection antibody” may also refer to a secondary antibody against a first antibody when an antigen is used for a capture substance, and its first antibody is an analyte or target of interest in a sample.

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Preparation of functionalized MB

1 – 5 μm MB can be used for Ab conjugation and antigen capture, and 2.8 μm is the preferred size. 100 μg of capture Ab (Human/Primate α -IL-6 Ab, Human IL-1 β Ab, Human IL-8 Ab) is conjugated to 5 mg of MB (Dynabeads M-280 Tosylactivated, Invitrogen) according to manufacturer’s instructions. All Abs were purchased from R&D Systems. The conjugated MBs are resuspended in PBS Buffer with 0.1% bovine serum albumin (BSA, Sigma-Aldrich) to a final concentration of 20 mg/mL.

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Preparation of functionalized GNP

For our scheme, the GNP has to be functionalized with both detection oligonucleotides and detection Ab, and several methods of GNP functionalization can be utilized. This includes non-covalent binding approaches such as adsorption, SA-protein A chimera, SA and biotin binding system, and covalent conjugation with crosslinkers such as SMCC (succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate). The SA and biotin coupling system is preferred for its ease of conjugation and high affinity. 10 – 100 nm GNP can be used but 20 nm GNP is preferred.

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For preparation of functionalized GNP, 20 nm SA-GNP, biotinylated detection Ab (Human/Primate IL-6 Biotinylated Ab, Human IL-1 β Biotinylated Ab, Human IL-8 Biotinylated Ab) and biotinylated detection oligonucleotide (Bio-T2, Bio-T3 or Bio-T4) were purchased from BBI Solutions, R&D Systems and Integrated DNA Technologies respectively. Firstly SA-GNP’s storage buffer is removed by adding 1 mL of PBST (0.02% Tween 20 (Sigma-Aldrich) in 1x PBS (1st BASE)) to 250 μL SA-GNP, and after centrifugation (10,000 rpm for 30 minutes (min)), the supernatant is discarded and the SA-GNP pellet is resuspended in 50 μL PBST. 3.2×10^{13} cp biotinylated detection oligonucleotide is then added to the SA-GNP and allowed to mix together on a rotator at room temperature for an hour (hr). The conjugated GNP is washed with PBST as follows: Addition of 1 mL PBST to GNP and mix well, centrifugation to pellet GNP and then

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supernatant removal. The GNP is resuspended with 50 μ L PBST and 1.6 μ g of biotinylated Ab is added. The GNP solution is mixed well before being incubated on a rotator at room temperature for an hr. After incubation, the conjugated GNP solution is washed thrice with PBST as described above. Lastly, the GNP is resuspended in 50 μ L PBST and stored in 4°C
5 fridge.

Design of multiplexed qRT-PCR

The detection oligonucleotides can be amplified and detected with a variety of DNA amplification methods, including qRT-PCR and isothermal methods such as RCA and
10 LAMP. For ease of multiplexity design, qRT-PCR is the preferred embodiment.

Several sets of detection oligonucleotides (with their respective primers and probes) were designed such that multiplexed qRT-PCR can be carried out with them. The detection oligonucleotides (with their respective primers and probes) used in the present invention are
15 shown in Table 1. The detection oligonucleotide includes unique primers and probe sequences for specific amplification and detection. It can be 50 – 250 bases, but preferred to be less than 90 bases in length and it should not be aligned to any human sequences and other common environmental contaminants (such as bacteria). Detection oligonucleotide sequences were selected from plant-related genes, and a BLAST against NCBI nucleotide
20 databases was performed to ensure that there are no similar sequences found in human and bacteria. In addition, the detection oligonucleotides should not fold into stable structures which will hinder the amplification process.

The detection oligonucleotides are also designed to contain a cleavable region to
25 allow their release from the GNP for qRT-PCR. The cleavable region can be sequences or substrates that are specifically cleaved by cleaving agents (such as endonucleases and uracil-DNA glycosylase) or when optimal reaction conditions (such as UV light of certain wavelength) are met. The preferred embodiment is a photocleavable region such as PC-biotin.

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Table 1: Oligonucleotide and Probe Sequences

Name	Sequence (5'→3')
Bio-T2	PC-Biotin- CACAAGTACCAACTCGTCCCGGACCAGTCCAAGGTGTGCGA GACCCGAAATCCACTGCATCTGGCATTAACTTGAACGACG AGCATCT (SEQ ID NO:1)
F2	CACAAGTACCAACTCGTCCC (SEQ ID NO:2)
R2	AGATGCTCGTCGTTCCAAGT (SEQ ID NO:3)
P2	Cy5-ACCAGTCCA/TAO/AGGTGTGCGAGACC-Iowa Black RQ (SEQ ID NO:4)
Bio-T3	PC-Biotin- CGCACATACACCCGAGTACATGTTCTCGTCGCTGAGGGCAT CGCCGAGCAGCCGTGCGATTACGTGAGGATTCGGATTGGCT GTCT (SEQ ID NO:5)
F3	CGCACATACACCCGAGTACA (SEQ ID NO:6)
R3	AGACAGCCAATCCGAATCCT (SEQ ID NO:7)
P3	6-FAM-CCGAGCAGC-ZEN-CGTGCGATTACG-Iowa Black FQ (SEQ ID NO:8)
Bio-T4	PC-Biotin- TCGTGCTGTCAAGAGATGCTACTCTATCTGGTCTACATTGGC AGTCACGAACCAGATGTGTCGTTGTACGAACCACTGTGTAAG CCC (SEQ ID NO:9)
F4	TCGTGCTGTCAAGAGATGCT (SEQ ID NO:10)
R4	GGGCTTACACAGTGGTTCGT (SEQ ID NO:11)
P4	6-ROX-TCTGGTCTA/ZEN/CATTGGCAGTCACGAACC-Iowa Black RQ (SEQ ID NO:12)

In brief, the present invention relates to a method for hypersensitive detection of an analyte in a sample by magnetic beads-gold nanoparticles-immuno-PCR (MB-GNP-IPCR) with photocleavable barcodes.

Specifically, the present invention provides methods for hypersensitive detection of multiple analytes in a sample. There are four main steps in MB-GNP-IPCR as outlined in Figure 1. The first step is the isolation of targets of interest from a sample by capture antibodies (Abs). Magnetic beads functionalized with capture Abs are added to the sample and the capture Abs will bind specifically to the targets of interest. Subsequent MB collection with a magnet and repeated washes will result in isolation of bound targets and removal of unbound targets. Next the MB-target complexes are released from the magnet and streptavidin-coated GNPs functionalized with both biotinylated detection Abs and PC-biotinylated detection oligonucleotides are added. The detection Abs on the GNPs will bind to the targets bound to the MBs to form a sandwich. This is followed by MB collection with a magnet and repeated washes to isolate the MB-target-GNP sandwiches and remove unbound GNPs. The third step is the release of the PC-biotinylated detection oligonucleotides from the GNPs by UV irradiation. The MB-target-GNP sandwiches are collected by the magnet and the supernatant containing the released detection oligonucleotides is then removed for the final step. Lastly, the supernatant is added to qRT-PCR master mix for amplification and detection of the detection oligonucleotides by qRT-PCR. Detection of multiple analytes is achieved by the use of different and specific detection oligonucleotides (barcodes) for each target analyte, which are differentiated by their respective fluorescent probes in the qRT-PCR reaction.

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Examples

To further understand the present invention, preferable solutions of the present invention are described in details below in reference to examples. However, these examples are merely used to illustrate the characteristics and merits of the present invention, but not to limit the protection scope of the present invention.

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Example 1

MB-GNP-IPCR was performed to detect human IL-6 recombinant protein (R&D Systems). Serially diluted IL-6 protein was prepared in PBS Buffer with 0.1% BSA, ranging from 0.01 pg/mL to 1000 pg/mL. 100 μ L of functionalized MB was added to each 50 μ L IL-6 protein dilution in a microcentrifuge tube and incubated at room temperature for an hr on a rotator (to prevent settling of MB). The MBs were then washed thrice with 1 mL PBST by retaining the MB with a magnetic separation rack and discarding the supernatant. After

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the final wash, the MBs were resuspended in 100 μ L PBS Buffer with 0.1% BSA. 5 μ L functionalized GNP was added to the MB and the mixture was incubated at room temperature for an hr on a rotator. MB-IL-6-GNP sandwiches should be formed during the incubation. The MBs were then washed thrice with 1 mL PBST with a magnetic separation rack as described above. This is followed by resuspension of the MB in 100 μ L PBST. Next the MBs were exposed to 5 min of UV treatment (365 nm) to release the detection oligonucleotides from the GNP. The MBs were then retained by the magnetic separation rack and 50 μ L supernatant (containing the released detection oligonucleotides) were collected for qRT-PCR. 5 μ L of supernatant was added to 15 μ L of qRT-PCR mastermix (1x PrimeTime Gene Expression Master Mix, 200 nM of each primer and probe) and RT-PCR was carried out with the following program on a RT-PCR instrument: 95°C for 3 min, followed by 40 cycles of 95°C for 15 seconds (sec) and 60°C for 1 min. qRT-PCR was performed in triplicates, and all primers, probe and qRT-PCR mastermix were purchased from Integrated DNA Technologies.

Figure 2 shows the graph of mean Δ Ct for each IL-6 protein concentration tested. Δ Ct values were obtained by subtracting mean Ct of each sample from that of the negative control (0 pg/mL IL-6 protein). The lowest detectable IL-6 concentration that was tested was 0.1 pg/mL. IPCR and ELISA for IL-6 recombinant protein spiked in PBS Buffer with 0.1% BSA were also performed according to manufacturers' instructions. IPCR and ELISA kits were purchased from RayBiotech Life and Sigma-Aldrich respectively. Figure 3 shows the graph of mean Δ Ct for each IL-6 protein concentration tested for IPCR and Figure 4 shows the graph of mean normalized absorbance for each IL-6 protein concentration tested for ELISA. The lowest detectable IL-6 concentration that was tested was 1.0 pg/mL and 10 pg/mL for IPCR and ELISA respectively. Thus MB-GNP-IPCR works and is at least 10-fold more sensitive than both basic IPCR and ELISA.

Example 2

Multiplexed MB-GNP-IPCR was performed to detect human IL-6, IL-8 and IL-1 β in PBS Buffer with 0.1% BSA. All recombinant proteins were purchased from R&D Systems. IL-6, IL-8 and IL-1 β -specific functionalized MB and GNP were prepared as described above. 50 μ L of each target-specific functionalized MB was added to 50 μ L sample solution (1 pg/mL of each protein) in a microcentrifuge tube and incubated at room

temperature for 2 hrs on a rotator. The MBs were then washed thrice with 1 mL PBST by retaining the MB with a magnetic separation rack and discarding the supernatant. After the final wash, the MBs were resuspended in 200 μ L PBS Buffer with 0.1% BSA. 10 μ L of each target-specific functionalized GNP was added to the MB and the mixture was
5 incubated at room temperature for 2 hrs on a rotator. The MBs were then washed thrice with 1 mL PBST with a magnetic separation rack as described above. This is followed by resuspension of the MB in 100 μ L PBST. Next the MBs were exposed to 5 min of UV treatment (365 nm) to release the detection oligonucleotides from the GNP. The MBs were then retained by the magnetic separation rack and 50 μ L supernatant (containing the released
10 detection oligonucleotides) were collected for qRT-PCR. 5 μ L of supernatant was added to 15 μ L of multiplex qRT-PCR mastermix (1x PrimeTime Gene Expression Master Mix, 200 nM of each primer and probe) and RT-PCR was carried out with the following program on a RT-PCR instrument: 95°C for 3 min, followed by 40 cycles of 95°C for 15 seconds (sec) and 60°C for 1 min. qRT-PCR was performed in triplicates, and all primers, probe and qRT-
15 PCR mastermix were purchased from Integrated DNA Technologies.

All three proteins in the sample were detected as shown by the amplification curves in Figure 5. Thus MB-GNP-IPCR can be multiplexed (with at least 3 targets) and this is unlike traditional ELISA and IPCR methods that cannot be used to detect multiple targets
20 simultaneously from the same sample. This would also enable multiplex testing in small and/or limited sample volume.

CLAIMS

1. A bifunctionalized gold nanoparticle (GNP) with both detection antibodies (Abs) and detection oligonucleotides (Oligos) via a non-covalent binding approach or a covalent
5 conjugation with a crosslinker (e.g., succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate, SMCC).
2. The bifunctionalized GNP of claim 1, wherein the non-covalent binding approach is selected from adsorption, SA-protein A chimera, receptor-ligand binding system, or SA-
10 biotin coupling system, preferably SA-biotin coupling system.
3. The bifunctionalized GNP of claim 1 or 2, wherein GNP is coated with streptavidin (SA), the detection Abs are biotinylated Abs, and the detection Oligos are biotinylated Oligos, preferably Oligos labelled with photocleavage (PC) biotin.
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4. The bifunctionalized GNP of any of claims 1-3, wherein the GNP is 10-100nm, preferably 20nm, in size.
5. Use of magnetic beads (MB), gold nanoparticles (GNP) and photocleavable (PC)
20 oligonucleotides (Oligos) for improving the sensitivity and specificity of immuno-PCR (IPCR) to enable hypersensitive, multiplexed detection of analytes.
6. A combined use of MB, GNP, IPCR and PC Oligo for a hypersensitive detection with respect to limited or rare samples and/or low analyte quantities.
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7. Use of claim 5 or 6, wherein MB is a functionalized MB, e.g., conjugated to a capture Ab, if the analyte in a sample is an antigen, and vice versa, or conjugated to a capture receptor, if the analyte is a ligand, and vice versa.
- 30 8. Use of claim 7, wherein MB is 1-5 μm in size.
9. Use of any of claims 5-8, wherein GNP is the bifunctionalized GNP of any of claims 1-4.

10. A method for hypersensitive detection of an analyte in a sample, comprising steps of:
- 1) Isolating a target of interest from the sample by means of MB to form a MB-target complex;
 - 5 2) Binding of GNP to the MB-target complex to form a MB-target-GNP sandwich;
 - 3) Releasing the detection oligonucleotide from the GNP by cleavage; and
 - 4) Amplifying the detection oligonucleotide by PCR.
11. The method of claim 10, wherein MB is 1-5 μm in size.
- 10 12. The method of claim 10, wherein MB is a conjugated MB with a capture Ab when the target of interest is an antigen, and vice versa.
13. The method of claim 10, wherein GNP is the bifunctionalized GNP of any of claims 1-
- 15 4.
14. The method of claim 10, wherein step 1) further comprises collecting MBs with a magnet.
- 20 15. The method of claim 10, wherein steps 2) and 3) each further comprises releasing and collecting MBs with a magnet.
16. The method of claim 10, wherein the detection oligonucleotide contains a cleavable region to allow its release from the GNP.
- 25 17. The method of claim 16, wherein the cleavable region is a sequence or substrate that is specifically cleaved by a cleaving agent or when an optimal reaction condition is met.
18. The method of claim 17, wherein the cleaving agent is an endonuclease or uracil-DNA
- 30 glycosylase, and the optimal reaction condition is UV light at 365 nm.
19. The method of claim 16, wherein the cleavable region is a photocleavable region, such as PC-biotin.

20. The method of claim 16, wherein the detection oligonucleotide is an oligonucleotide labelled with PC-biotin, preferably selected from SEQ ID NOs: 1, 5 and 9.
- 5 21. The method of claim 10, wherein the target of interest is selected from IL-6, IL-8 and IL-1 β .
22. The method of claim 10, wherein PCR is selected from qRT-PCR, Rolling Circle Amplification (RCA) and Loop-mediated Isothermal Amplification (LAMP), preferably
10 qRT-PCR.
23. A method for hypersensitive detection of an analyte in a sample by magnetic beads-gold nanoparticles-immuno-PCR (MB-GNP-IPCR) with photocleavable barcodes.
- 15 24. A method of hypersensitive detection of an analyte in a sample, comprising steps of:
- 1) Providing the sample containing one or more targets of interest, adding MBs conjugated to capture antibodies (Abs) to the sample, to form MB-target complexes, and collecting MBs with a magnet, for isolation of target(s) by capture Abs of MBs;
 - 20 2) Releasing MBs, adding streptavidin (SA)-coated bifunctionalized GNP with both biotinylated detection Abs and biotinylated detection oligonucleotides via SA-biotin coupling system to MB-target complexes, to form MB-target-GNP sandwiches for binding of targets by detection Abs, and collecting MBs with a magnet;
 - 25 3) Releasing MBs, cleaving detection oligonucleotides labelled with PC-biotin from the GNPs by UV irradiation (e.g., at 365 nm) for releasing detection oligonucleotides, collecting MBs by a magnet, and obtaining a supernatant containing the released detection oligonucleotides;
 - 30 4) Adding the supernatant to qRT-PCR master mix for amplification and detection of the detection oligonucleotides by qRT-PCR.