Supporting Information:

Peroxidase-mimicking Activity of Biogenic Gold Nanoparticles Produced by Prunus nepalensis Fruit Extract: Characterizations and Application for the Detection of Mycobacterium bovis

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1. Additional Experimental Details

1.1. Optimization study of biogenic synthesis process of Au nanoparticles

Four parameters were monitored for optimizing the process of Au nanoparticle synthesis: temperature, reaction pH, reductant dilution factor and concentration of gold salt. The process was first optimized for the reaction pH and temperature. Four different reaction pH: 4.0, 7.0, 10.0, and 4.3 (the natural pH of the reductant) were taken and synthesis was observed at five different reaction temperatures: room temperature (RT), 4 °C, 50 °C, 70 °C and 90 °C. Four different concentrations of gold salt solution: 0.5 mM, 1 mM, 2 mM, 3 mM, and 4 mM were used for the optimization study and, similarly, four different dilutions of the reductant were prepared in aqueous solution: 1 (undiluted), 1:10, 1:100 and 1:1000. Spectroscopic results were used to analyze each pH and temperature condition and select the optimal parameters for biogenic synthesis of Au NPs.

1.2. Chemical analysis of Prunus nepalensis extract

1.2.1. Free radical scavenging activity and antioxidant potential of P. nepalensis fruit extract

To understand the chemical characteristics of the P. nepalensis fruit extract, and estimate the antioxidant potential and reducing ability, different assays were performed. Likewise, total phenolic content and flavonoid content responsible for the antioxidant and reduction potency were also assessed.

1.2.2. Reducing power assay

Reducing power assay was performed by adapting the protocol described by Yildirim et al.\textsuperscript{1}. *P. nepalensis* fruit extract (20 µL) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) followed by 2.5 mL of potassium ferricyanide as described by Yang et al.\textsuperscript{2}. After 30 min incubation at 50 °C, 2.5 mL of trichloroacetic acid was added and centrifuged at 1008 g force for 10 min.\textsuperscript{3} The supernatant (2.5 mL) was mixed properly by pipetting with 2.5 mL of distilled water followed by 0.5 mL of ferric chloride. Finally, the absorbance was measured at 700 nm using a UV-Visible spectrophotometer where the reducing power is directly proportional to the absorbance value.\textsuperscript{4,5}
1.2.3. DPPH (2,2-diphenyl-1-picrylhydrazyl-hydrate) scavenging ability

DPPH scavenging ability was determined according to the method reported by Blois (1958) with slight modifications. Two hundred microliters of *P. nepalensis* fruit extract was mixed with 2 mL of 0.1 mM DPPH and 50 mM Tris-HCl buffer (800 µL, pH 7.4). After 30 min incubation at RT, the reduction of DPPH was measured at 517 nm. Tubes without the samples were taken as control, whereas an ascorbic acid (1 mg/mL) solution was taken as the standard. This is represented as % DPPH radical scavenging activity and is calculated using SEq. (1).

\[
\text{DPPH scavenging activity (\%)} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100
\]

(SEq.1)

1.2.4. Determination of total phenolic content

Total phenolic content was calculated with a spectrophotometer by using Folin-Ciocalteu reagent. *P. nepalensis* fruit extract (2 mL) was mixed with 10 mL of Folin-Ciocalteu diluted reagent (1/10 with double-distilled deionized water). After 2 min incubation, 8 mL of sodium carbonate was added and the reaction solutions were incubated in dark conditions for 2 h at room temperature. The absorbance was measured at 765 nm. Gallic acid standard curve was prepared with the concentration range from 50 to 500 µg/mL. Results are articulated in micrograms (µg) of gallic acid equivalent (GAE) per mL of *P. nepalensis* fruit extract.

1.2.5. Total flavonoid content

Total flavonoid content was determined by the aluminium chloride colorimetric method. One mL of *P. nepalensis* fruit extract was mixed with 4 mL of distilled water, followed by the addition of 0.3% (v/v) NaNO₂ solution. After incubation (5 min), 0.3% AlCl₃ was added and the solution incubated for a further 6 min. Two mL of 1 M NaOH solution was added and the final volume of the reaction mixture was prepared to 10 mL by adding distilled water. After incubation (15 min), the absorbance was read at 570 nm. The total flavonoid content present in the solution was calculated from the calibration curve, and the result expressed as mg catechin equivalent per g dry weight.

1.3. Characterization of peroxidase mimicking activity of biogenic Au NPs

Peroxidase like activity of biogenic Au NPs was determined using TMB as a chromogenic substrate. Experiments were carried out using 100 pM of biogenic synthesized Au NPs in a
final reaction volume of 1 mL, with 1 mM of TMB and 6% (v/v) H$_2$O$_2$. The reaction mixture was incubated at RT for 10 minutes, and full-spectrum analysis was carried out using UV-Visible spectrophotometer. Time-dependent UV-Visible full spectral analysis of the catalyzed reaction using the same conditions was done in 1 mL reaction volume for 90 min $^{10}$.

1.3.1. Understanding the reaction mechanism of TMB oxidation catalyzed by peroxidase mimicking biogenic Au NPs

To understand the reaction mechanism of the TMB oxidation using biogenic Au NPs, 1 mM TMB was catalyzed using the same conditions, mentioned earlier (section 1.3). The reaction kinetics were monitored at three different wavelengths: 370 nm, 450 nm and 650 nm, using a UV-Visible spectrophotometer for 20 min and samples readings were recorded at one-minute intervals $^{11}$.

1.3.2. Determining the role of P. nepalensis fruit extract for the catalysis of TMB oxidation

To understand the role of P. nepalensis fruit extract for the oxidation of chromogenic substrate TMB in the presence of H$_2$O$_2$ a simple experiment was carried out by using 10 µL of fruit extract, 1 mM of TMB, 6 % H$_2$O$_2$ in a reaction volume of 1 mL. For a comparative study, 100 pM of biogenic Au NPs was taken as a positive control. For blank, only TMB along with H$_2$O$_2$ was taken without any NPs or fruit extract $^{12}$.

2. Additional Results and Discussions

2.1. Optimization study

The UV-visible readings for the effect of different parameters in the biogenic synthesis of Au nanoparticles can be seen in Figure S1. Figure S1A represents the optimization study of Au nanoparticles synthesis using different pH levels where natural pH was measured as 4.3 (a mixture of gold salt and prunus extract). From this figure, it is clearly visible that at natural pH rapid synthesis of Au nanoparticles took place with a SPR maximum at 520 nm. Additionally, an increase in the yield of the NPs is also noticed as compared to pH 4. A further increase in the pH to 7.0 and pH 10 has resulted in a rapid damping in the intensity of the SPR band. This suggests that the formation of Au NPs is favoured under acidic conditions (2 to 7). An increase in the pH to basic condition either has decreased the formation of initial metal nuclei or may have resulted in the agglomeration of the NPs. Figure S1B shows the effect of reaction temperature for the synthesis of Au NPs where increase in the temperature from 4°C to 90°C...
has shown a slight variation in the SP band. At RT (room temperature) yield of the synthesized nanoparticles has been considered as highest with an SP maximum at 522 nm. A decrease in the reaction temperature has resulted in the low-intensity SP band, which is possibly due to the formation of less number of nuclei during the reduction process. Figure S1C shows the effect of concentration of the gold salt solution on the synthesis of Au NPs. Reaction with 1 mM gold salt has shown a notable peak with a SPR maximum at 519 nm. It suggests that with an increase in the concentration from 2 mM to 4 mM, SP peak has shown redshift along with a significant amount of damping. The presence of a higher concentration of gold salt may have led to rapid nucleation and simultaneous growth (heterogeneous process), which might have resulted in the formation of particles with different morphology and size. Figure S1D shows the effect of the rate of dilution of prunus extract on the synthesis of Au NPs. Low dilution has resulted in aqueous NP dispersion with a broad SP peak, which centers at 550 nm. Increasing the dilution factor to 1:10 has produced particles with a sharp SP peak at 510 nm. But, further increasing the dilution factor, 1:100 and 1:1000, has not produced any Au NPs; this is probably due to the insufficient amount of reducing agents required for the synthesis of Au NPs.

2.2. Field Emission Scanning Electron Microscope (FESEM) analysis

Morphological characterization of biogenic Au nanoparticles was done by using field emission scanning electron microscope (FESEM). Agglomerated Au nanoparticles with a particles size range from 20-35 nm can be observed in Figure S2. Particles are present in polydisperse form due to the agglomeration (probably occurred during the sample preparation). Uniformity in the size of Au NPs can be observed. This observation was in accordance with the data obtained from UV-visible spectrum of Au nanoparticles.

2.3. Characterization of peroxidase mimicking activity of biogenic Au NPs

To understand the full reaction mechanism of the TMB oxidation process catalyzed by biogenic Au NPs, a kinetic analysis of the TMB oxidation was performed with 1 mM TMB, 6% H$_2$O$_2$, and 100 pM biogenic Au NPs at set conditions for 20 min. Figure S4A demonstrates the reaction mechanism of the TMB oxidation process where TMB, parent diamine (TMB$^0$) gets oxidized by one-electron oxidation to form cation radical (TMB$^{+1}$) ($\lambda_{\text{max}}$ 370 nm) giving vivid blue color, which is in equilibrium with a meriquinoid complex or charge transfer complex (CTC) ($\lambda_{\text{max}}$ 650 nm) displaying green color. The reaction is further catalyzed by peroxidase mimicking biogenic Au NPs in the presence of H$_2$O$_2$ to form diamine oxidation product (TMB$^{+2}$) or quinodiimine of yellow color ($\lambda_{\text{max}}$ 450 nm) $^{13}$. Figure S4 B-D, demonstrate the
UV-Visible spectral analysis of TMB oxidation at different stages of the TMB oxidation process at three different wavelengths such as 370, 650, and 450 nm. From previous studies, it has been observed that the peak absorbance at 650 nm has been utilized widely as a diagnostic peak to investigate the peroxidase mimicking activity of Au NPs. However, interference of physicochemical reaction parameters plays an imperative role in inducing aggregation of Au NPs in the high electrolyte reaction medium, attributing a red-shift of the plasmon peak around 600-700 nm. Thus, the absorption peak around 650 nm, representing the formation of oxidized TMB products is potentially less acceptable due to the potential overlapping of peaks generated from the aggregated nanoparticles. Additionally, Figure S4B shows an increase in absorbance at 370 nm in a time-dependent manner in comparison with 450 nm and 650 nm, (Figure S4C, D) while keeping all the reaction parameters similar for every three reactions. Based on this, we proposed to utilize the peak at 370 nm for characterizing the peroxidase mimicking activity of biogenic Au NPs in kinetic analysis. This result offers significant insight into the peroxidase mimicking activity of the biogenic Au NPs obtained by P. nepalensis extract.

Kinetic analysis of the TMB oxidation using biogenic Au NPs was performed by mixing various concentrations of TMB/ H$_2$O$_2$ with a fixed concentration of Au NPs and monitoring the oxidation process by absorption analysis at 370 nm. Figure S5A-G demonstrate the UV-Visible absorption spectra of TMB oxidation reaction kinetics in presence of different concentrations of H$_2$O$_2$. The results indicate the absorbance values of TMB oxidation at 370 nm increased with the increasing concentrations of TMB and H$_2$O$_2$ at fixed concentration of biogenic Au NPs (100 pM). At a lower concentration of H$_2$O$_2$ (0.0075 %) the TMB oxidation (for TMB concentration 0-1 mM) catalyzed by biogenic Au NPs occurred at a slower rate generating less amount of oxidized TMB product with the increasing time. However, when the H$_2$O$_2$ concentration was increased above 4 % (v/v), the rate of TMB oxidation catalytic reaction increased significantly with respect to time at a fixed concentration of Au NPs (Figure S5D-G). This findings attributed to the availability of the excess amount of the H$_2$O$_2$ for accepting the lone pair of electron from the Au NPs surface following with the oxidation of TMB.

Biogenic Au NPs showed excellent intrinsic peroxidase mimicking activity in presence of H$_2$O$_2$ indicating the ability to oxidize TMB with higher efficiency. For biological synthesis process, P. nepalensis fruit extract acted as a reducing as well as a capping agent for the synthesis of stable Au NPs, indicating the probable presence of biological components from
the fruit extract over the surface of the nanoparticles. Hence, the role for fruit extracts in possessing intrinsic peroxidase-like activity of Au NPs remains unknown. As a preliminary test of the ability of fruit extract to oxidize TMB, a simple reaction was carried out with the similar protocol outlined above. Figure S6 shows the bar diagram of the absorbance values at 370 nm for the comparative TMB oxidation efficiency of fruit extract and biogenic Au NPs at different time intervals. The result indicates that within 5 minutes of the reaction, the biogenic Au NPs showed the highest absorbance value at 370 nm indicating the oxidation of TMB, which generated blue color (Figure S6 inset (a&b (iii)) whereas no change in the absorbance value was observed in case of fruit extract. Furthermore, there was no changes in the color of the reaction solution for fruit extract in presence of 0.1 mM TMB and 6 % H₂O₂ attributed to the incapability of fruit extract alone to oxidize TMB (Figure S6 inset (a&b (ii)).
3. Additional Figures

Figure S1. UV-visible spectra of optimization study of Au NPs synthesis using *Prunus nepalensis* extract. (a) Effect of different pH, (b) effect of varying temperature, (c) effect of varying salt concentration, (d) effect of different dilution rates of *Prunus nepalensis* extract.
**Figure S2.** Field emission electron microscopy (FESEM) image of Au NPs synthesized by *P. nepalensis* fruit extract. Arrows indicating the biogenic Au NPs.

**Figure S3.** Zeta potential analysis of the biogenic Au NPs synthesized using *Prunus nepalensis* extract.
**Figure S4.** (a) Reaction mechanism of TMB oxidation process. Kinetic analysis of the catalyzed reaction of 1 mM TMB /6% H₂O₂ in presence of 100 pM Au NPs at different wavelengths 370nm (b), 650 nm (c) and 450nm (d) to demonstrate the reaction mechanism of TMB oxidation process (Figure 4. B,C,D Insite: (i) TMB, (ii) TMB+ H₂O₂, (iii) Au NPs, (iv) TMB+ H₂O₂+Au NPs)
Figure S5. UV-Visible absorption spectra (370 nm) of reaction kinetics of TMB oxidation process varying the concentration of H$_2$O$_2$ (a) 0.0075%, (b) 1%, (c) 2%, (d) 4%, (e) 6%, (f) 8%, (g) 10%. The concentration of biogenic Au NPs was kept constant (100 pM) while
different concentrations of TMB (0-1 mM) were utilized to analyse the reaction kinetics up to 90 min of reaction time.

**Figure S6.** Role of *P. nepalensis* fruit extract in TMB oxidation process. Absorbance values of TMB oxidation process in presence of 1 mM TMB, 6% H₂O₂ with *P. nepalensis* fruit extract (green), biogenic Au NPs (red) and blank (without biogenic Au NPs and fruit extract). (Figure inset visualization of the TMB oxidation reaction at different time intervals 0 min (a), after 10 min (b), with (i) Blank, (ii) fruit extract, (iii) biogenic Au NPs). Samples were analysed in triplicates (n=3) and standard deviation deduced form these data.
Figure S7. GC-MS analysis of the *P. nepalensis* fruit extract.
Figure S8. UV-Visible absorption spectra (370 nm) of reaction kinetics of TMB oxidation process using biogenic Au NPs and chemically synthesized Au NPs. The concentration of biogenic Au NPs and chemically synthesized Au NPs was kept constant (100 pM) while 0.1 mM TMB and 6% H$_2$O$_2$ was utilized to analyse the reaction kinetics up to 30 min of reaction time.

4. Additional Tables

Table S1. Michaelis-Menten kinetic parameters demonstrating the peroxidase-mimicking activities of HRP enzyme, biogenic Au NPs at different concentrations of H$_2$O$_2$. $K_{cat}$ was demonstrated as the ratio of $V_{max}$ to molar concentration of enzyme. The concentration of biogenic Au NPs was calculated according to the previously published article$^1$. The concentration of biogenic Au NPs was determined to be 100 pM.
| H$_2$O$_2$ concentration | Catalyst            | $V_{\text{max}}$ (M min$^{-1}$) | $K_M$ (mM) | $K_{\text{cat}}$ (min$^{-1}$) | $K_{\text{cat}}/K_M$ (mM$^{-1}$ min$^{-1}$) |
|-------------------------|---------------------|---------------------------------|------------|------------------------------|-----------------------------------------------|
| 0.0075%                 | HRP                 | $5.75 \times 10^{-6}$           | $1.1 \times 10^{-1}$ | $5.75 \times 10^{4}$          | $5.19 \times 10^{4}$                          |
|                         | Biogenic Au NPs     | $1.68 \times 10^{-7}$           | $1.17 \times 10^{-2}$ | $1.68 \times 10^{3}$          | $9.69 \times 10^{4}$                          |
| 1.0%                    | HRP                 | $9.86 \times 10^{-7}$           | $1.5 \times 10^{-1}$ | $9.86 \times 10^{3}$          | $6.44 \times 10^{4}$                          |
|                         | Biogenic Au NPs     | $1.13 \times 10^{-6}$           | $1.12 \times 10^{-2}$ | $1.14 \times 10^{4}$          | $4.16 \times 10^{3}$                          |
| 2.0%                    | HRP                 | $5.086 \times 10^{-7}$          | $7.8 \times 10^{-2}$ | $5.09 \times 10^{3}$          | $6.47 \times 10^{4}$                          |
|                         | Biogenic Au NPs     | $1.84 \times 10^{-6}$           | $3.5 \times 10^{-2}$ | $1.84 \times 10^{4}$          | $5.19 \times 10^{3}$                          |
| 4.0%                    | HRP                 | $4.13 \times 10^{-7}$           | $6.7 \times 10^{-2}$ | $4.41 \times 10^{3}$          | $6.17 \times 10^{4}$                          |
|                         | Biogenic Au NPs     | $2.9 \times 10^{-6}$            | $5.7 \times 10^{-2}$ | $3.0 \times 10^{4}$           | $5.17 \times 10^{3}$                          |
| 6.0%                    | HRP                 | $4.16 \times 10^{-7}$           | $7.9 \times 10^{-2}$ | $4.17 \times 10^{3}$          | $5.24 \times 10^{4}$                          |
|                         | Biogenic Au NPs     | $4.016 \times 10^{-6}$          | $6.9 \times 10^{-2}$ | $4.02 \times 10^{4}$          | $5.80 \times 10^{3}$                          |
| 8.0%                    | HRP                 | $7.78 \times 10^{-7}$           | $8.4 \times 10^{-2}$ | $7.79 \times 10^{3}$          | $9.21 \times 10^{4}$                          |
|                         | Biogenic Au NPs     | $5.11 \times 10^{-6}$           | $1.06 \times 10^{-1}$ | $5.12 \times 10^{4}$          | $4.82 \times 10^{3}$                          |
| 10.0%                   | HRP                 | $1.07 \times 10^{-6}$           | $1.00 \times 10^{-1}$ | $1.08 \times 10^{4}$          | $1.08 \times 10^{5}$                          |
|                         | Biogenic Au NPs     | $5.34 \times 10^{-6}$           | $1.02 \times 10^{-1}$ | $5.35 \times 10^{4}$          | $5.24 \times 10^{5}$                          |
Table S2. GC MS analysis of *P. nepalensis* extract.

| Retention Time (mins) | Compound Name | Match Factor | Best Hit | Formula | Base peak | Base Peak Height | Base Peak MZ |
|-----------------------|---------------|--------------|----------|---------|-----------|-----------------|-------------|
| 5.0657                | D(-)-Lactic acid, 2TMS derivative | 88.5 | True | C_{12}H_{20}O_{11}Si_{5} | 31832558.7 | 23075921.5 | 146.8 |
| 5.0658                | Lactic Acid, 2TMS derivative | 85.4 | True | C_{12}H_{20}O_{11}Si_{5} | 30486708.9 | 22447359.1 | 147.0 |
| 5.4558                | L-Alanine, 2TMS derivative | 96.5 | True | C_{2}H_{4}NO_{5}Si_{4} | 37169626.6 | 39654803.8 | 115.9 |
| 6.6430                | Benzoic Acid, TMS derivative | 92.7 | True | C_{12}H_{4}O_{11}Si_{5} | 5686425.7 | 6980443.0 | 179.0 |
| 6.8742                | Silanol, trimethyl-, phosphate (3:1) | 92.6 | True | C_{12}H_{2}O_{11}PSi_{3} | 151859382.8 | 111219864.8 | 299.0 |
| 6.9946                | Niacin, TBDMS derivative | 92.5 | True | C_{12}H_{2}NO_{11}Si_{5} | 912420.4 | 882556.4 | 179.8 |
| 7.1187                | Butanedioic acid, 2TMS derivative | 85.3 | True | C_{12}H_{4}O_{11}Si_{5} | 14771708.6 | 16226669.6 | 147.0 |
| 8.3407                | Malic acid, 3TMS derivative | 97.8 | True | C_{12}H_{4}O_{11}Si_{5} | 121527394.2 | 107029973.7 | 72.9 |
| 8.5486                | L-Aspartic acid, 3TMS derivative | 94.5 | True | C_{12}H_{2}NO_{11}Si_{5} | 15638448.1 | 18859400.3 | 232.0 |
| 8.5777                | L-5-Oxoproline, . 2TMS derivative | 92.4 | True | C_{12}H_{2}NO_{11}Si_{5} | 18332164.4 | 19450196.0 | 155.9 |
| 8.6238                | 4-Aminobutanoic acid, 3TMS derivative | 95.8 | True | C_{12}H_{2}NO_{11}Si_{5} | 58110907.6 | 59860974.8 | 173.9 |
| 9.4246                | D-Arabinose, tetrakis(trimethylsilyl) ether, ethyloxyxime (isomer 2) | 95.8 | True | C_{10}H_{18}NO_{11}Si_{4} | 29455211.9 | 31603140.3 | 72.9 |
| 9.4560                | Asparagine, 3TMS derivative | 97.0 | True | C_{12}H_{2}N_{12}O_{11}Si_{3} | 51602786.1 | 42892177.3 | 72.9 |
| 9.6669                | Isocitric acid lactone, 2TMS derivative | 89.2 | True | C_{12}H_{2}O_{11}Si_{5} | 8127325.9 | 9204626.4 | 72.9 |
| 9.7338                | D-Ribose, 4TMS derivative | 89.3 | True | C_{12}H_{2}O_{11}Si_{5} | 17240072.0 | 10547564.4 | 72.9 |
| 9.8496                | Aconitic acid, (Z)-, 3TMS derivative | 85.1 | True | C_{12}H_{2}O_{11}Si_{5} | 6264977.2 | 6639073.9 | 146.8 |
| 9.8769                | D(-)-Ribono-1,4-lactone (R,S,R), 3TMS derivative | 86.1 | True | C_{12}H_{2}O_{11}Si_{5} | 11466409.4 | 11196669.3 | 72.9 |
| 10.2717               | D(-)-Fructofuranose, pentakis(trimethylsilyl) ether (isomer 2) | 88.9 | True | C_{2}H_{2}O_{11}Si_{5} | 174777483.1 | 114665453.9 | 72.9 |
| 10.2792               | Protocatechoic acid, 3TMS derivative | 88.9 | True | C_{16}H_{30}O_{14}Si_{3} | 27104803.8 | 23922438.0 | 193.0 |
| 10.2950               | Citric acid, 4TMS derivative | 92.4 | True | C_{12}H_{2}O_{11}Si_{5} | 106504159.0 | 119468828.1 | 273.0 |
| 10.3241               | Citric acid, 4TMS derivative | 88.1 | True | C_{12}H_{2}O_{11}Si_{5} | 142817799.3 | 105891386.2 | 273.0 |
| 10.3842               | D-Fructose, 5TMS derivative | 91.0 | True | C_{2}H_{2}O_{11}Si_{5} | 210686565.7 | 115781313.5 | 204.0 |
| 10.4516               | D(-)-Talofuranose, pentakis(trimethylsilyl) ether (isomer 2) | 89.1 | True | C_{2}H_{2}O_{11}Si_{5} | 184561001.0 | 70009484.6 | 216.9 |
| 10.6292               | D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime | 88.3 | True | C_{2}H_{2}O_{11}Si_{5} | 128282065.2 | 107598635.1 | 307.0 |
| Identifier | Name | Formula | Molar Mass | Retention Time | Peak Area | Peak Height | Signal-to-Noise Ratio |
|------------|------|---------|------------|----------------|-----------|-------------|----------------------|
| 10.6549    | D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime | C_{22}H_{55}NO_{6}Si_{5} | 10159464.3 | 106586314.0 | 307.0 |
| 10.7737    | d-Glucose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime, (1Z)- | C_{22}H_{55}NO_{6}Si_{5} | 133610277.6 | 113105142.3 | 319.0 |
| 10.7975    | d-Glucose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime, (1Z)- | C_{22}H_{55}NO_{6}Si_{5} | 107547390.7 | 118127368.9 | 319.0 |
| 10.8936    | D-\(+\)-Talose, pentakis(trimethylsilyl) ether, methyloxime (syn) | C_{22}H_{55}NO_{6}Si_{5} | 181009781.2 | 112067712.3 | 319.0 |
| 10.9307    | Methyl galactoside, 4TMS derivative | C_{19}H_{46}O_{6}Si_{4} | 116916945.0 | 85558000.0 | 203.9 |
| 10.9615    | D-Glucitol, 6TMS derivative | C_{24}H_{62}O_{6}Si_{6} | 12496763.7 | 13930667.5 | 319.0 |
| 11.0103    | D-Glucitol, 6TMS derivative | C_{24}H_{62}O_{6}Si_{6} | 104028762.2 | 102536266.7 | 204.9 |
| 11.0106    | D-Sorbitol, 6TMS derivative | C_{24}H_{62}O_{6}Si_{6} | 101109025.1 | 100877240.3 | 205.0 |
| 11.0430    | D-\(+\)-Turanose, octakis(trimethylsilyl) ether | C_{36}H_{86}O_{11}Si_{8} | 51830744.4 | 44762341.2 | 73.0 |
| 11.0430    | Maltose, octakis(trimethylsilyl) ether, methyloxime (isomer 2) | C_{37}H_{89}NO_{11}Si_{8} | 77766977.5 | 52530507.1 | 216.9 |
| 11.1210    | \beta.-D-Allopyranose, 5TMS derivative | C_{21}H_{52}O_{6}Si_{5} | 166050185.2 | 147030437.3 | 203.9 |
| 11.1411    | \beta.-D-Allopyranose, 5TMS derivative | C_{21}H_{52}O_{6}Si_{5} | 149575034.7 | 147215141.3 | 203.9 |
| 11.3433    | Palmitic Acid, TMS derivative | C_{19}H_{40}O_{2}Si | 14475458.2 | 13786928.9 | 116.9 |
| 11.7465    | Myo-Inositol, 6TMS derivative | C_{24}H_{50}O_{6}Si | 146536676.7 | 94795723.3 | 72.9 |
| 13.6066    | 3-alpha.-Mannobiose, octakis(trimethylsilyl) ether (isomer 1) | C_{36}H_{86}O_{11}Si_{8} | 77102174.6 | 53186414.4 | 216.9 |
| 14.1113    | Sucrose, 8TMS derivative | C_{36}H_{86}O_{11}Si_{8} | 179173810.6 | 121187820.6 | 361.0 |
| 14.1397    | D-\(+\)-Trehalose, octakis(trimethylsilyl) ether | C_{36}H_{86}O_{11}Si_{8} | 409741617.4 | 119836756.6 | 361.0 |
Table. S3. Comparison between different biosensing assays for the detection of bacteria

| Biosensing strategy                          | Target analyte | Linear range CFU mL$^{-1}$ | LOD (CFU mL$^{-1}$) | Advantages                                      | Disadvantages                                      | Ref. |
|----------------------------------------------|----------------|---------------------------|---------------------|------------------------------------------------|--------------------------------------------------|------|
| Immuno magnetic separation (IMS)-PCR         | *M. bovis*     | $10^0 - 10^5$            | 57.7                | High specificity                                | Complex procedure                                  | 18   |
| IMS-LFD (lateral flow device)                | *M. bovis*     | $10^{4.5}$               | $1.7 \times 10^4$  | novel, rapid immunochromatographic lateral flow device (LFD) as a non-invasive test to detect *M. bovis* cells in badger faeces | Low sensitivity                                   | 19   |
| RT-PCR assay                                 | *M. bovis*     | -                         | $10^4$              | High specify                                    | DNA amplification based assay, which is time consuming and involves loads of instrumentation. Not suitable for point of care applications | 20   |
| Biogenic-Au nanozyme-based colorimetric biosensing | *M. bovis* | $10^0 - 10^2$ | $\sim 53$ in washing buffer and $\sim 71$ in spiked sample | Low cost, effective, sensitive and specific biosensing assay | Interference of different bio matrixes in real sample analysis | This paper |
| Double-tagging PCR and electrochemical genosensing | *M. bovis* | -                        | -                   | Synergistic approach resulting in higher specificity | Complicated steps involved and qualitative assay | 21   |
| Pd@PtNPs-based colorimetric biosensor        | *Escherichia coli O157:H7* | -                        | $9 \times 10^2$ | Cost effective and rapid biosensing strategy | Low sensitivity                                    | 22   |
| Fe$_3$O$_4$ NPC                              | *Listeria monocytogenes* | $5.4 \times 10^2$- $10^8$ | $5.4 \times 10^3$ | Low cost and highly specific biosensing strategy | Lower sensitivity                                  | 23   |
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