Off-On Switchable Fluorescent Probe for Rapid Bacteria Detection

Supporting Information

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1. Graphic concept of indirect detection and effect of the probe

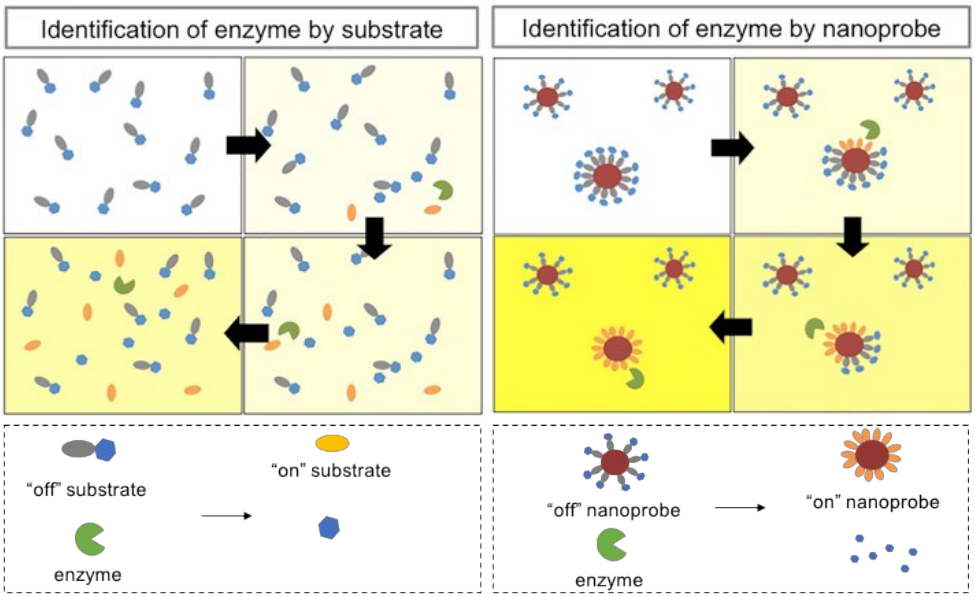

Figure S1: Schematic representation of how the sensitivity of the detection method can be enhanced by functionalising the nanoparticle surface with the substrate rather than use the free substrate.

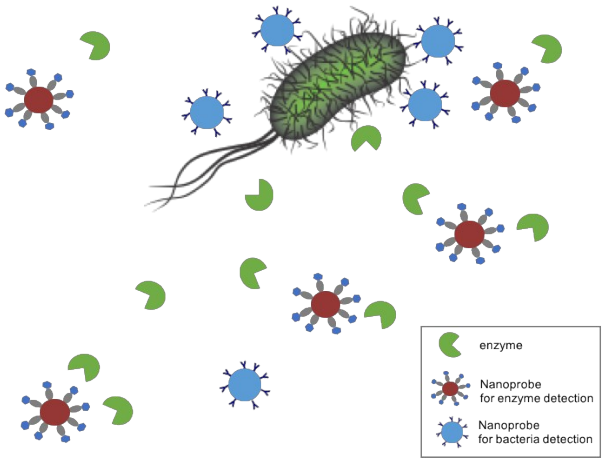

Figure S2: Schematic rappresentation of the different sensitivity between the antibody-mediated detection and the indirect enzymatic-mediated method. A single bacterium produces many enzymes that can be detected by the probe improving the LOD of the method.

2. Material and Methods

7-hydroxycoumarin-4-acetic acid (97%), ammonia, (ca. 7N solution in methanol, Acroseal®), lithium aluminium hy-dride (1M solution in THF, Acroseal®) and 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (98.5% stabilised) were purchased from Acros Organics (distributors: Fisher Scien-tific, Loughborough, UK). EDC (> 98.0%) and D-biotin (>98.0%) were purchased

from Tokyo Chemical Industries (TCI) (Oxford, UK). Streptavidin magnetic particles 0.7-0.9 μm were purchased from Kisker Biotech GmbH & Co. KG. The following reagents were purchased from Sigma Aldrich (Gillingham, Dorset, UK): barium chloride (99.9%), metha-nol (puriss. ≥99.8%), sodium sulfate, sodium methoxide reagent grade (95%), N,N-dimethylformimide (DMF-99.8% extra dry over molecular sieves Acroseal®), dimethyl sulfox-ide (anhydrous 99.9%), trimethylamine (≥99%), sodium hydroxide (≥98%), sulfuric acid ACS reagent (95-98%), so-dium hydride (NaH), β-glucosidase (from almonds ≥2 units/mg solid), LB broth with agar (Miller), LB broth, α-glucosidase (from Saccharomyces cerevisiae ≥100 units/mg protein), 4-methylumbelliferyl α-D-glucopyranoside (α-4-MUD) biotin (5-fluorescien) conjugate and Glucose Assay reagent kit. Absolute ethanol, phosphate buffer saline tablets (one tablet dissolved in 200 mL DI water yields 0.01 M phosphate buffer, pH 7.4) were purchased from Fisher Sci-entific. Hereafter, the use of 'PBS' refers to 0.01 M PBS, pH 7.4. Deionised water was filtered through 200 nm nylon membrane filters from Millipore. Nunclon Delta sterile flat bottom 96-well plate and Micro BCA™ were purchased from Thermo Fisher Scientific (Loughborough, UK). Dime-thyl sulfoxide-d6 (99.9%) for NMR analysis was purchased from Goss Scientific Instruments Ltd (Cheshire, UK). MERCK silica gel 60, 230-400 mesh ASTM was used for column chromatography. Thin-layer chromatography (TLC) was performed on aluminium-backed plates MERCK silica gel 60 or on aluminium-backed aluminium oxide F254 plates (Fluka Analytics). Glacial acetic acid, chloroform, dichloro-methane, absolute ethanol, ethyl acetate, sodium acetate, 2-isopropanol and n-hexane were purchased from Fisher Sci-entific. Bacteria were isolated from midstream sample urines (MSU) given by women with OAB (over-active bladder) attending an outpatient clinic for urinary problems at Med-way Maritime Hospital, Gillingham, Kent.

3. Instrumentation

Incubators: Bacteria samples were grown in Petri dishes on solid LB agar in a Genlab incubator at 37°C overnight. Bacteria were harvested in liquid LB broth under agitation (166 rpm) at 37°C in a New Brunswick Scientific incubator.

Autoclave: All tools used for the bacterial culturing and experiments were sterilised using an INUCLAV 18/23 class B autoclave purchased form UniEquip. Solid and liquid materials were autoclaved at 134°C.

Dynamic Light Scattering (DLS): DLS experiments were performed using a Malvern Zetasizer and the measurements were evaluated using the Zetasizer software. Data are reported as the average of three measurements (n = 3) ± SD. Particle diameter and PDI were used to characterise the particles after synthesis and to assess their evolution after the various surface functionalisation steps. Unless otherwise mentioned, particles were analysed at a concentration of 20 μg/mL in water at 25°C in DTS1070 disposable cuvettes. Using water as dispersant at 25°C, 0.8872 was used as parameter for solvent density during the measurements. RI 1.59, abs 0.01 were used as parameters for the analysis of streptavidin-coated magnetic particles.

UV-Vis analysis: Tecan Infinite 200 PRO was used for the UV-Vis analysis and data were evaluated by Tecan I-Control software. λ_{ex}/ λ_{em} 327/460 nm were used for both enzymatic and bacterial assays. Absorbance at 690 nm was used for the quantification of bacteria in the sample. 340 nm was the wavelength used for the glucose quantification assay.

4. Synthesis of C-MPs

After the first step of functionalisation all particles were intensively washes with repeated cycles of centrifugation and re-dispersion in water until no fluorescence signal was measured in the supernatant indicating that all the non-bonded fluorophore was removed. The amount of fluorophore washed away was quantified in each supernatant using the calibration curve for compound C.

5. Synthesis of Glu-C-MPs

The quantification of the glucose in the supernatants after the glycosylation step was accomplished by measuring the absorbance at 340 nm after treatment with a solution of 1.5 mM NAD, 1.0 mM ATP, 1 unit/mL of hexokinase and 1.0 unit/ml of glucose-6-phosphate dehydrogenase (Glucose Assay Reagent Kit). This mixture allows the production of NADH, which is quantified by measuring the absorption at 340 nm. In particular, the enzyme hexokinase transfers a phosphate group from ATP to the glucose. In presence of NAD and glucose-6-phosphate, a red-ox reaction is induced by glucose-6-phosphate dehydrogenase with formation of NADH. Since the stoichiometry of the reaction is 1:1 in each step, the amount of NADH is equivalent to the amount of glucose in first place.[1] The amount of α-D-Glucose (HO-Glu-Br) washed away was quantified in each supernatant using the calibration curve obtained with D-glucose solution at known concentration.

6. Probe characterisation

Table S1: The table shows the diameter (Ø) and fluorescence properties (Fluo) measured after each step of synthesis at concentration of 20 µg/mL of particles in water.

	Fluo (A.U.)	Ø (µm)
MPs	1869 ± 45	1.02 ± 0.02
C-MPs	6484 ± 237	3.17 ± 0.35
Glu-C-MPs	4378 ± 50	>10

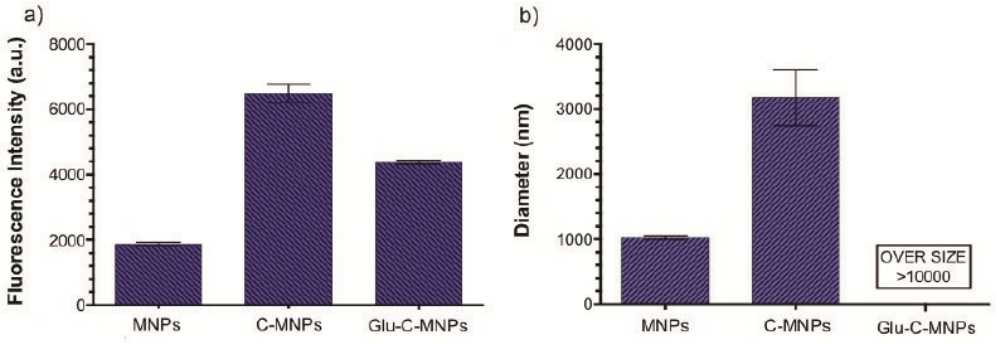

Figure S3: Characterisation of the probe after each step of synthesis. The fluorescence property of the probe was measured (a) and the size of the particles was determined by DLS (b).

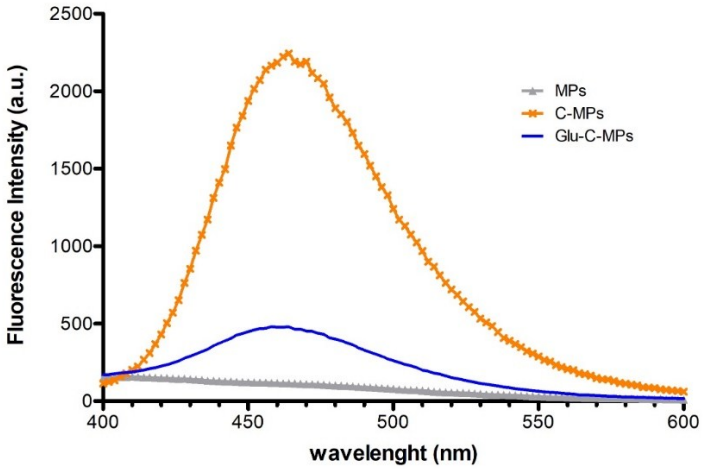

Figure S4: Fluorescence spectrum of particles at eact functionalisation step.

The quantum yield (Φ) has been calculated for C-MPs and Glu-C-MPs using 4-MU as reference (0.74, measured in water).[2] The Φ calculated are 0.21 and 0.11 respectively for C-MPs and Glu-C-MPs.

7. Functionalisation quantification

Quantification of substrate on the probe surface following *Method 1* and *Method 2* are shown in *Table S2.* The values are expressed both as mM of substrate per mg of probe. As expected, appears that *Method 1* overestimates the values while *Method 2* underestimates the amount of substrate present on the surface. As such, the average of the values obtained with the two methods have been considered to determine the concentration used in the enzymatic and bacterial experiments.

Table S2: Quantification of the corresponding fluorophores on the surface of each particles by method1 (green section) and method2 (purple section). The average value determined by means of the two method was calculated to tbe 0.965 mM per mg of probe (yellow section).

Method 1	C-MPs
Fluorescence (A.U.)	25936
mM of dye/0.1 mg of MPs	0.127
mM of dye/1 mg of MPs	1.27
Method 2	Glu-C-MPs
Max. fluorescence (A.U)	13377
mM of dye/0.1 mg of MPs	0.065
mM of dye/1 mg of MPs	0.65
Average mM/mg	0.965

8. Evaluation of the suitability of MNPs for the enzymatic detection of bacteria

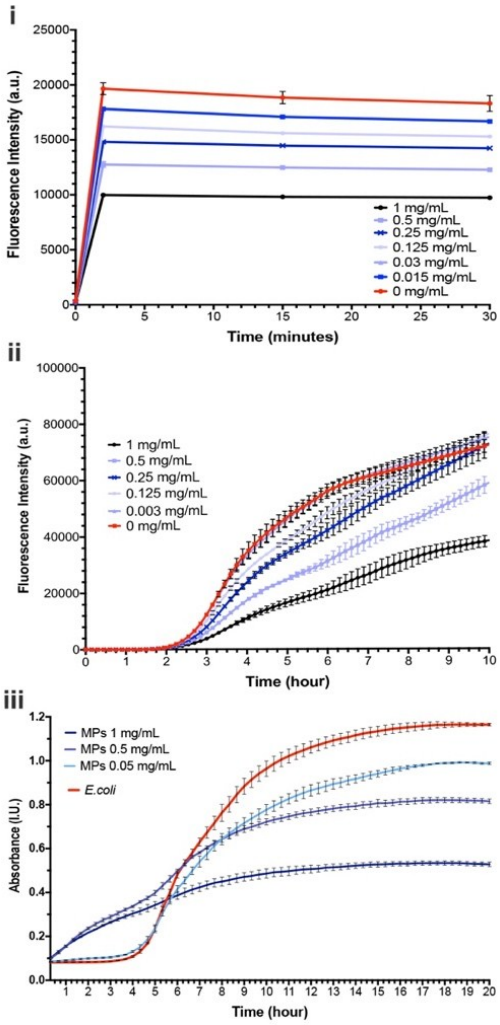

Figure S5: Evaluation of inhibition effect on the enzymatic activity (i and ii) and toxicity on bacteria (iii) of MPs. The inhibitory effects of MPs on the enzyme-substrate recognition event was evaluated by monitoring the recognition of the enzyme α-glucosidase (100 μg/mL) by the substrate α-4-MUD (50 mM) when particles are absent (red line) and in presence of MPs (i). Similarly, the activity of α-4-MUD (250 μM/mL) in the recognition of 25 CFU/mL of *E.coli* in absence of particles (red line) or in presence of MPs at different concentrations was compared (ii). MPs at highest concentration tested- black line; remaining concentrations-graduated blue. iii) Toxicity induced by MPs at different concentrations (blue shade lines) was evaluated comparing the growth of bacteria in absence of MPs (red line).

9. Evaluation of probe efficiency in the enzymatic detection

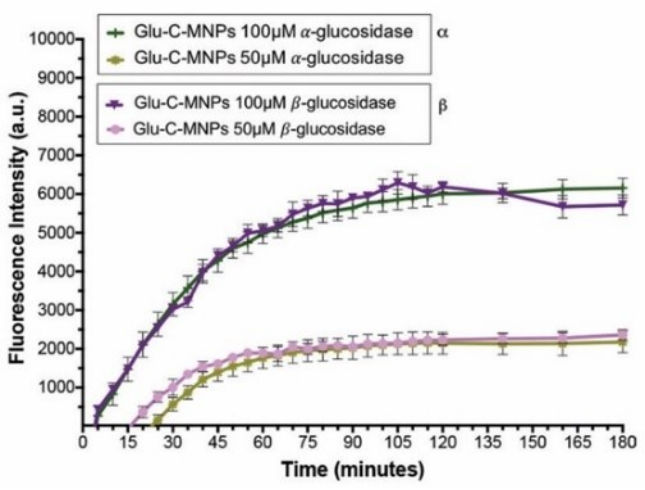

Figure S6: The increase of fluorescence was measured for 100 and 50 μM Glu-C-MNP treated with 100 μg/mL of α- (green lines) and β-glucosidase (purple lines). The curves were normalised to account for auto-hydrolysis.

10. Dose-response analysis

As observed in our previous work, intensity of the fluorescent signal is not dependent to the concentration of the bacteria but solely to the concentration of the substrate/probe used.[2] However, lower is the bacterial concentration and more time is required to detect an increase of fluorescent signal.

The time required for the detection has been exploited to analyse data from a "dose-response" point of view. As shown in Figure S7, higher is the concentration of bacteria and sooner the fluorescence increases. This is particularly evident in the case of *E.coli*: fluorescence increased after 1 hour when the concentration was 75 CFU while 2.5 hours were required to measure the signal for 1 CFU sample. The delay between sample 75 and 1 CFU was instead 1 hour in the case of *K.pneumoniae* and *S.aureus*. No relevant correlation between concentration and detection time was found for *Enterococcus* while in the case of *P.aeruginosa* in 1 hour, an increase in fluorescence was measured for sample 75 CFU and only 20 minutes extra were required for the detection of sample 1 CFU.

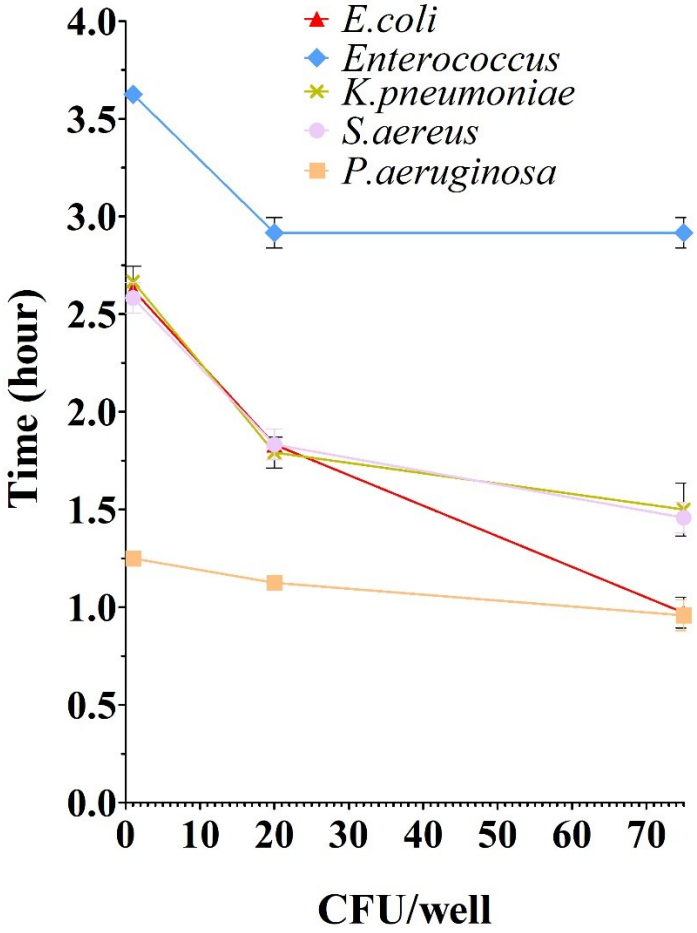

Figure S7: Dose-response analysis proposed to evaluate the correlation between detection and bacteria concentration. A constant amount of Glu-C-MPs (50 µM) was used to detect bacteria at different concentrations (1, 20 and 75 CFU/well). Considering that the fluorescence increment follows a sigmoidal growth, the time at which half of the maximum fluorescence was measured has been considered for the analysis. Data are reported as average values of three independent experiments ± SD.

1 L. Kern, J. Spreckels, A. Nist, T. Stiewe, C. Skevaki, B. Greene, M. Mernberger and H. Elsässer, Cell Tissue Res., 2016, 366, 651-665

2 G. Giovannini, A. J. Hall, V. Gubala. Talanta, 2018, 188, 448-453