Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information for

A Gold Nanoparticles Growth-based Immunoassay for Detection of Antibiotic Residues

Wenbo Yu, [*a] Maria Knauer, [a] Christoph Kunas, [a] Ulas Acaroz, [a, b] Richard Dietrich, [a] and Erwin Märtlbauer [a]

[a] Department of Veterinary Sciences, Faculty of Veterinary Medicine, Ludwig-Maximilians-Universität München, Schönleutnerstr. 8, 85764 Oberschleißheim, Germany

[b] Present address: Food Hygiene and Technology Department, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey

Corresponding Author

E-mail: Wenbo.Yu@mh.vetmed.uni-muenchen.de

EXPERIMENTAL SECTION

Materials and chemicals. Gold (III) chloride hydrate, cloxacillin, D-glucose, sodium borohydride, trisodium citrate dehydrate, glucose oxidase (GlcOx), and casein sodium salt were purchased from Sigma-Aldrich GmbH (Steinheim, Germany). Goat anti-mouse IgG labeled with GlcOx was purchased from Abcam (Cambridge, UK). GlcOx-cloxacillin conjugates and monoclonal antibodies against cloxacillin (mAb 1F7) were produced in our laboratory as described.[1,2] All the other reagents required for the experiments were of analytical grade and used as received.

Gold seed solution. Gold seed solution was produced using the previously published method.[3] Briefly, 23.0 µmol gold (III) chloride hydrate and 26.4 µmol trisodium citrate dehydrate were dissolved in 100 mL deionized water. Then, 20 mL of the mixture were transferred into a sterile centrifugation tube, 600 µL freshly made 0.01 M $NaBH_4$ were added and the tube was inverted 3 times. To allow the borohydride to react completely, the reaction mixture was left at room temperature for 4 hours and stored at 4 ^{o}C until use. The final concentration was 15 nM and the nanoparticles had a diameter of 7 nm.

Identification of GlcOx-mediated sensing. GlcOx was serially diluted with deionized water to reach a final concentration ranging from 200 to 0 mU mL^{-1} in cuvettes. Then, 200 µL glucose (10 mM in H_2O) were mixed with the GlcOx solutions at a 2:1 volume ratio and the mixtures were incubated at 37 ^{o}C for 1h. Subsequently, 150 µL $HAuCl_4$ (1.25 mM in H_2O) and 150 µL gold seed solution were added into each cuvette. This solution was incubated at ambient temperature for another 40 min for the AuNPs growth reaction. The photographs were taken by a digital camera (D7000, Nikon, Japan) on a light table (SlimLine S, Rex, Germany). The spectrum from 400 to 800 nm of the AuNPs solutions was recorded by UV-Vis spectroscopy (Specord 200 Plus, Analytik Jena, Germany).

Sample preparation. Bovine muscle samples obtained from local retail shops were used to demonstrate the applicability of the approach for cloxacillin determination. Exactly 2.00 g of

bovine muscle samples were homogenized in 10 mL PBS by using BMT-20-S tubes containing stainless steel balls and an Ultra-Turrax Tube driver (Ika, Germany) at 6000 rpm for 10 s. The homogenates were spiked with cloxacillin at concentrations of 150, 300, and 600 ng mL^{-1}. After that, the spiked homogenates were mixed for another 30 s at 6000 rpm, filtered by medium fast filter paper (Grade 597, Whatmann, UK), and directly analyzed by immunoassay without any further clean-up.

Plasmonic ELISA for food samples. For the determination of cloxacillin in bovine muscle, microtiter plates were coated with GlcOx-cloxacillin (2 µg mL^{-1} in PBS; 100 µL per well) at room temperature overnight. After washing for 3 times with wash solution (0.85% NaCl containing 0.025% Tween-20 in H_2O), the microtiter plate was blocked with 3% casein in PBS for 30 min. Subsequently, various concentrations of cloxacillin (diluted in PBS) or samples (50 µL) were added together with 50 µL mAb 1F7 (0.5 µg mL^{-1} in PBS) and incubated for 1 h. Not adsorbed antibody and antigen were then removed by washing 4 times with wash solution. Then, GlcOx labeled goat anti-mouse IgG from Abcam was employed as secondary antibody (1:125 diluted in PBS; 100 µL per well) and incubated for 1 h at room temperature. Subsequently, the plate was carefully washed 4 times with wash solution and another 4 times with deionized water. Then, 100 µL glucose (10 mM in H_2O) were added into each well and incubated at 37 ^{o}C for 1 h. For the colorimetric detection, 50 µL gold seed solution and 50 µL $HAuCl_4$ (1.25 mM in H_2O) were added into each well and kept to react at ambient temperature for 40 min. Quantitative measurements were obtained with an high-throughput ELISA Reader (Sunrise RC, Tecan, Germany). The calibration curve was analyzed with a four-parameter logistic equation using Prism 5 (GraphPad Software, La Jolla, USA).

SUPPLEMENTARY FIGURE AND TABLE

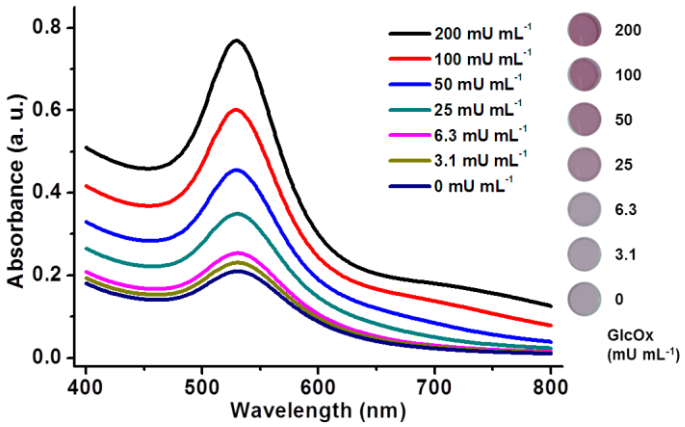

Figure S1 UV-vis spectra of AuNPs growth-based colorimetric nanosensor using varying GlcOx concentrations (0 – 200 mU mL^{-1}). Measurements were performed after incubating the enzyme in the presence of AuNPs and $HAuCl_4$ for 40 min. Photograph of different solutions was taken at 40 min.

Table S1 Comparison between the developed GlcOx/AuNPs/$HAuCl_4$-based signal generation mechanism to the most commonly used HRP/TMB-based mechanism

		HRP/TMB-based mechanism	**GlcOx/AuNPs/$HAuCl_4$-based mechanism**
Enzyme	Protein	Horseradish peroxidase	Glucose oxidase
	Price	175 Euro per 10 KU	47.8 Euro per 10 KU
	Secondary antibody conjugate	Commercial available	Commercial available
Substrate	Chemicals	3,3',5,5'-tetramethylbenzidine	AuNPs and $HAuCl_4$
	Price for 100 mL substrate solution	1.08 Euro	0.69 Euro
	Stability	Need stop solution	Stable signal
	Toxicity	Potentially toxic	Not toxic

Table S2 Comparison of the performances of various immunoassay variants for cloxacillin detection

Platform	**Signal readout**	**Antibody**	**LOD (ng mL^{-1})**	**Sample**	**Ref.**
Biosensor BIAcoreTM	Optical Response Units	Polyclonal Antibody	60	PBS	[4]
Automated microarray	Chemiluminescence	Monoclonal antibody	0.29	Milk	[5]
ELISA Plate	Colorimetric (3,3',5,5'-tetramethylbenzidine)	Monoclonal antibody	6.3	Milk	[6]
ELISA Plate	Colorimetric (3,3',5,5'-tetramethylbenzidine)	Polyclonal antibody	4	Milk	[7]
ELISA Plate	Colorimetric (AuNPs)	Monoclonal antibody	3.4	Bovine muscle	This work

Table S3 Recovery of cloxacillin from artificially contaminated bovine muscle samples at different concentration levels (n = 5)

Added	Found			
(ng mL^{-1})	Mean (ng mL^{-1})	±SD [a] (ng mL^{-1})	Recovery (%)	CV [b] (%)
150	158.8	16.0	105.9	10.1
300	294.2	36.5	98.1	12.4
600	562.7	80.5	93.8	14.3

[a]: standard deviation

[b]: coefficient of variation

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