Supplementary Information

Magnetic AuNPs@TiO_2@NF Heterojunction for Solar-Light Degradation of Antibiotic and Mitigation of Bacterial Resistance Risk

Joanna Breczko,[a] Anna Basa,[a] Katarzyna Niemirowicz-Laskowska,[b] Beata Skonieczna,[b] Raúl López-Martín,[c,d] Jose A. De Toro,[c,d] Halina Car,[b] and Elzbieta Regulska [*a,c,e]

[a] Faculty of Chemistry, University of Bialystok, Ciolkowskiego 1K, 15-245 Bialystok, Poland.

[b] Department of Experimental Pharmacology, Medical University of Bialystok, Szpitalna 37, 15-295 Bialystok, Poland.

[c] Instituto Regional de Investigación Científica Aplicada (IRICA), University of Castilla-La Mancha, Avenida Camilo José Cela 1, 13071, Ciudad Real, Spain.

[d] Departamento de Física Aplicada, Universidad de Castilla-La Mancha, 13071 Ciudad Real, Spain.

[e] Faculty of Pharmacy, University of Castilla-La Mancha, Almansa 14, 02008 Albacete, Spain.
*Corresponding author: elzbieta.regulska@uclm.es

1. Chemicals

Tigecycline (≥99.9% purity) was sourced from USP (China). Merck supplied the following reagents: ammonium oxalate monohydrate (98%), gold(III) chloride monohydrate (≥99.9%), iron(III) nitrate nonahydrate (≥98%), 1,4-benzoquinone (≥98.5%), dimethyl sulfoxide (≥99.9%), isopropanol (99.9%), titanium(IV) tert-butoxide (TBOT, ≥99.99%), tetraethyl orthosilicate (TEOS, 98%), titanium dioxide (anatase, ≥99%), and trisodium citrate (≥99%). Aqueous ammonia (≥99.9%) and sodium hydroxide (≥99.9%) were acquired from POCh (Gliwice, Poland). All chemicals were analytical grade and used as received. Heat-inactivated fetal bovine serum (certified) was obtained from Eurx (Gdańsk, Poland). Additional reagents included 2-mercaptoethanol (99%), penicillin (99%), and streptomycin (99%) from Gibco, Thermo Fisher Scientific (Waltham, MA, USA). For LC-MS applications, ammonium formate (≥99%), acetonitrile (≥99.9%), and methanol (≥99.9%) were provided by Fluka, while formic acid (≥99%) came from Merck. High-purity water (≥99.9%) was supplied by Honeywell.

2. Biological studies

2.1. Cell Lines Used and Cell Viability Assay

Normal skin fibroblast CCD-1079Sk (American Type Culture Collection–2097), Human hepatic cell line HepG2 (American Type Culture Collection HB-8065) and human monocytic cells THP-1 (American Type Culture Collection TIB-202) were used as control cell lines for assessment of the toxicological profile of tigecycline (TGC) and its photocatalytic derivatives. CCD-1079Sk cells and HepG2 were grown in 96-well plates at 5–7 × 10^3 cells per well to full confluence in EMEM medium supplemented with 10% with addition 10% fetal bovine serum (FBS), 50 U/mL penicillin, 50 mg/mL streptomycin under physiological conditions, at 37 °C with 5% CO_2. The cells were incubated in a 5% CO_2 atmosphere at 37 °C. THP-1 cells was cultured in Roswell Park Memorial Institute medium (RPMI-1640, ATCC) supplemented with 10% heat-inactivated fetal bovine serum (Eurx, Gdańsk, Poland), 1% penicillin/streptomycin mixture (Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA), and 2-mercaptoethanol (0.05 mM) (Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA).

The Neutral red uptake test[1] was used to assess the viability of CCD-1079Sk and HepG2 cells in vitro. Cells were seeded in 96-well plates, and after overnight incubation, the cells were treated with increasing content 10 and 100 uL of TGC and its photocatalytic products to 24 hours. Thereafter, the absorbance was recorded with a microplate reader (Varioscan lux Thermofisher) at 540 nm and normalized to control. Viability of non- adherent THP-1 cells after treatment by aforementioned samples added at volume 10 and 100 µL for 24h, was determined by the MTS assay.[2] This test evaluates the reduction of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) to formazan by enzymatic activity (mitochondrial dehydrogenases) of viable cells. THP-1 cells were seeded and differentiated as described above. After 24 h exposure, the absorbance of samples was measured using a microplate reader(Varioscan lux Thermofisher) at 570 nm and normalized to control cells without tested agents.

In other set of experiment, metabolic activity of all examined cells has been performed. For this purpose, the resazurin based assay was used.[2] The cells (CCD-1079Sk and HepG2 cells) were incubated with the presence of tested compounds (10 and 100 µL). After 24 h exposure, the fluorescence was measured at excitation 520 nm and emission 590 nm using a microplate reader (Varioscan lux Thermofisher) and normalized to control.

2.2. Oxidative stress pathway assays

2.2.1. Measurement of ROS formation

The ROS-Glo™ H_2O_2 Assay (Promega) was used to assess the level of reactive oxygen species (ROS) in human fibroblast CCD-1079Sk cells exposed to tigecycline (TG) and samples of the decomposed antibiotic (10 µL) in the presence photocatalysts. ROS-Glo™ H_2O_2 substrate was added to cell cultures at the end of the treatment and reacted with the H_2O_2 present in our samples. The reaction product, a luciferin precursor, was converted to luciferin by adding ROS-Glo™ Detection Solution. The bioluminescence was measured with the use of a microplate reader (Varioscan lux Thermofisher). The light signal was proportional to the amount of H_2O_2 in cultured cells [1]. The data were normalized to the bioluminescent signal intensity of non-treated cells and normalized to the control.

2.2.2. NAD/NADH assay

Human fibroblast CCD-1079Sk cells were plated in a white-walled culture plate, and treated with the tested compounds (10 µL) and incubated for 24 h. After that , the plate was removed from the incubator and equilibrated at room temperature for 5 -10 minutes. Then 50µL of NAD/NADH-Glo™ detection reagent was added to each well and the plate was carefully and briefly shaken to mix and lyse cells and incubated for 30–60 minutes at room temperature. In the final step the bioluminescence was recorded with the use of a microplate reader (Varioscan lux Thermofisher). The light signal produced is proportional to the amount of NAD+ and NADH in the sample. The data were normalized to the bioluminescent signal intensity of non-treated cells and normalized to the control.

2.2.3. Cytokine response assessment

To examine IL-6, IL-10 and TNFα levels, a homogeneous, non-wash bioluminescent cytokine assay was performed. For this purpose monocytic THP-1 cells were plated in a 96-well white-walled culture plate and differentiated into macrophages. Macrophages were divided into two groups with or without 24 hours of LPS-stimulation (0.5 µg/ml) - before antibiotic and their metabolites (20 µL) were added. After 24h exposition, samples were incubated with the antibody mixture for 1 hour. After the incubation is complete, the assay plate was equilibrated to room temperature for 15 minutes, and the Lumit™ Detection Reagent B (Promega GMBH) was added. In the next step, after 5 min incubation, the luminescence signal has been recorded by microplate reader

2.2.4. Cytochrome P450 activity

To examine the influence of the tested agents on the activity of the enzyme metabolizing xenobiotics, a luminescence analysis of cytochrome P450 CYP3A4 activity was performed. TGC and its metabolites were added to the HepG2 cells in 10 µL volume and incubated for 24 hours. The medium was then replaced, and the same volume (50 µl) of fresh medium containing CYP3A4 substrate (3 µM) was added. After 1 hour of incubation, 25 µL of culture medium from each well was transferred at room temperature to a 96-well opaque white luminometric plate, and 25 µL of luciferin detection reagent was added. After 20 minutes, the luminescence was read. Results were normalized to the untreated control.

2.3. Statistical analysis

Data were analyzed statistically by one-way analysis of variance (ANOVA). Bonferroni's post-test was used to determine differences between groups. Statistical evaluation of results was performed using the GraphPadPrism software package.

3. Results

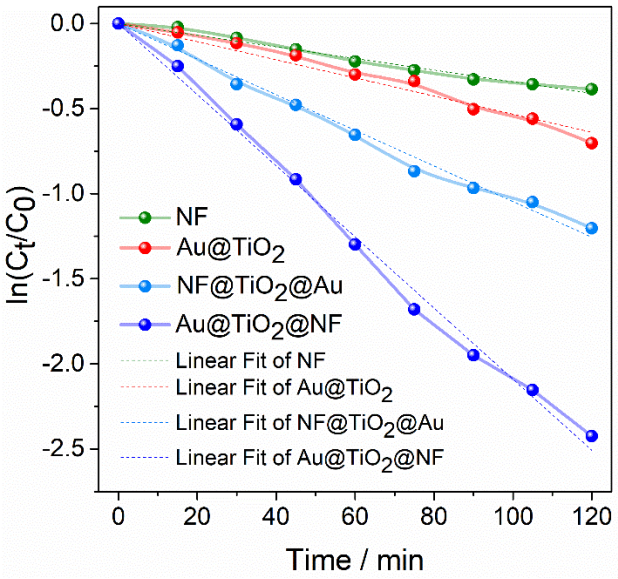


Fig. S1. Logarithmic plots of C_t/C_0 versus time for NF, Au@TiO_2, NF@TiO_2@Au, and Au@TiO_2@NF.

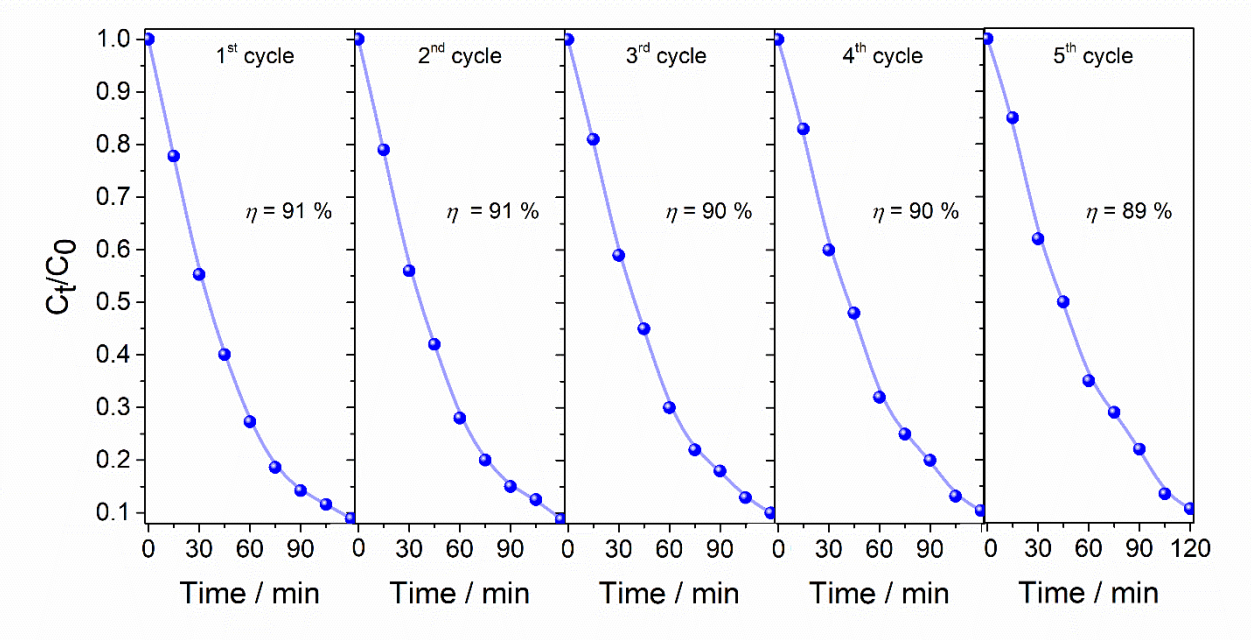


Fig. S2. Recyclability of Au@TiO_2@NF upon 5 consecutive cycles. Photodegradation efficiency is depicted as η.

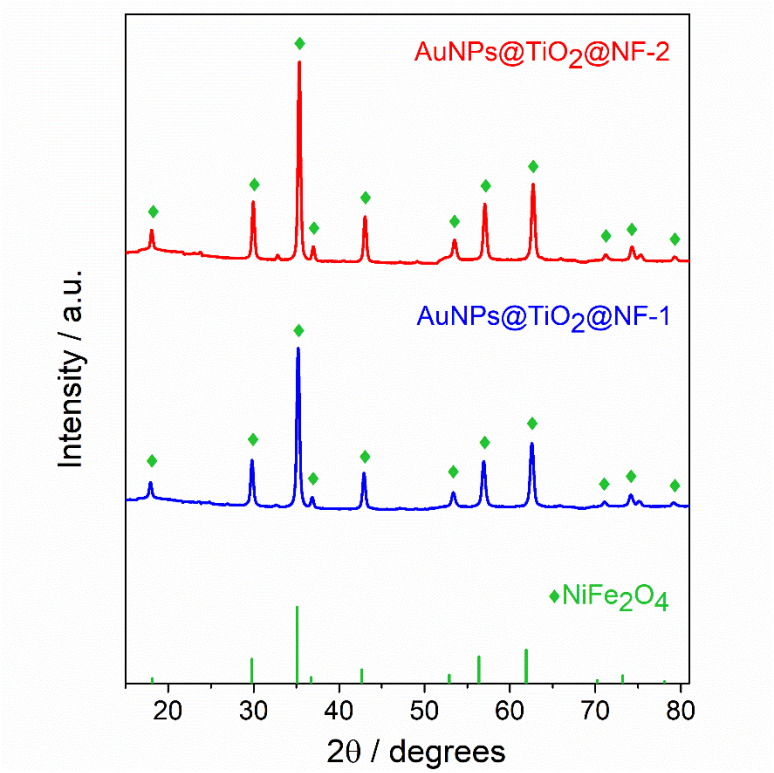


Fig. S3. PXRD patterns of Au@TiO_2@NF before (Au@TiO_2@NF-1) and after (Au@TiO_2@NF-2) photocatalytic cycle.

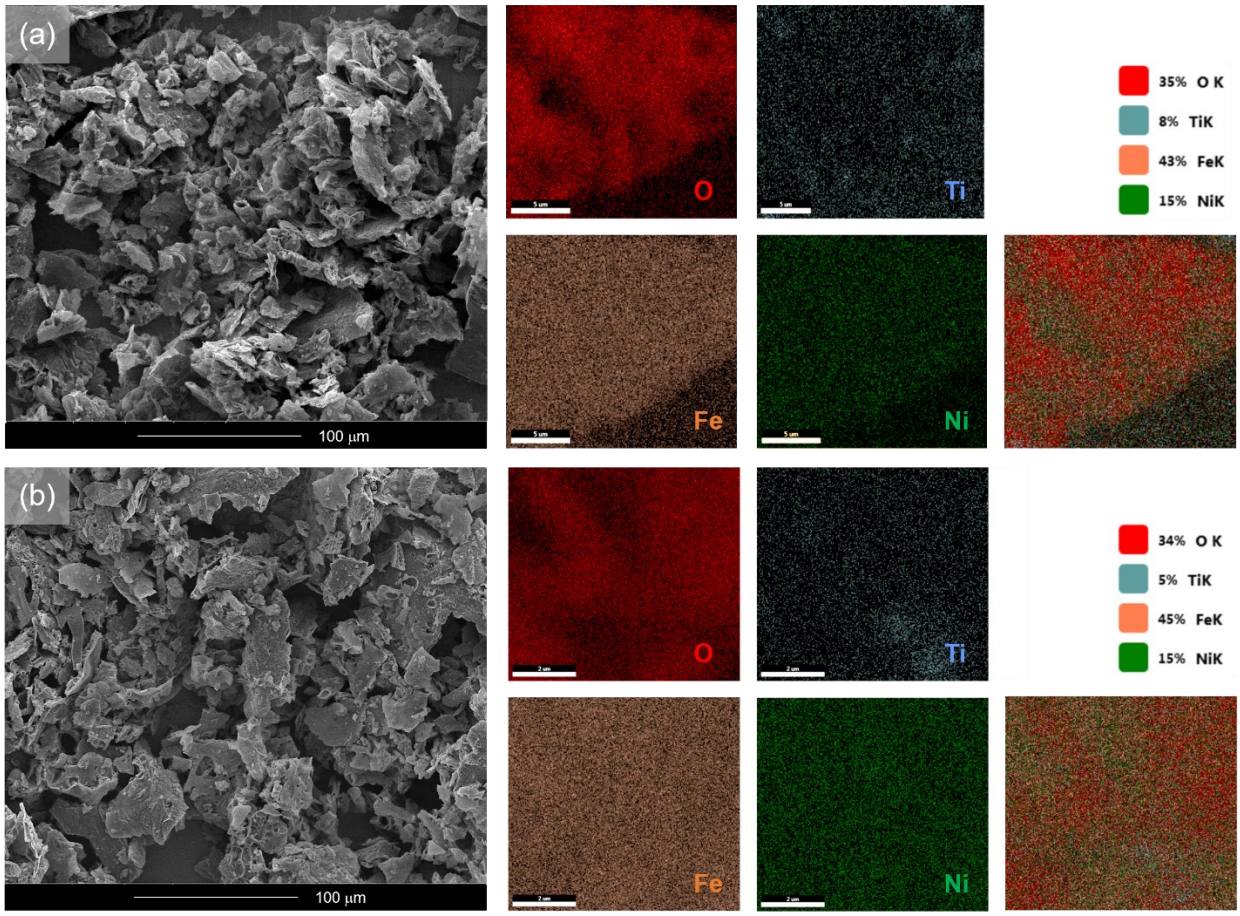


Fig. S4. SEM images and corresponding EDX mapping analysis of Au@TiO_2@NF (a) before and (b) after photocatalytic cycle.

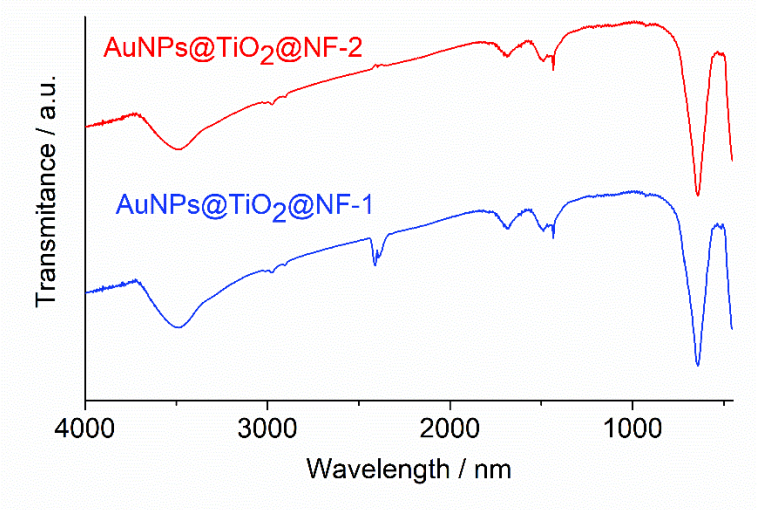
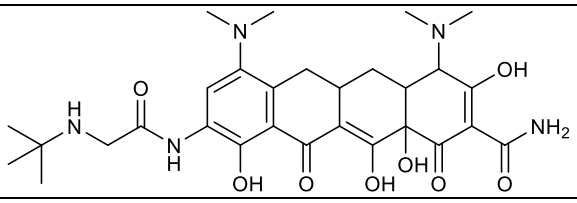
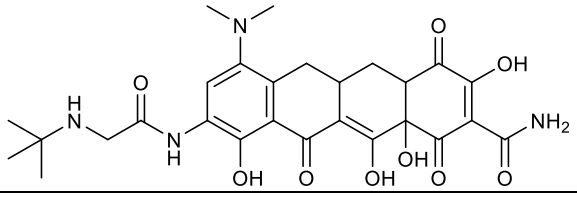
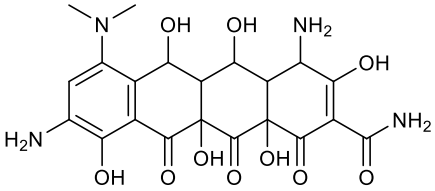
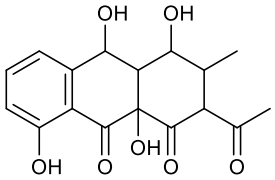
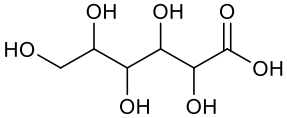
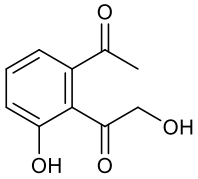
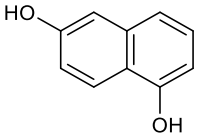
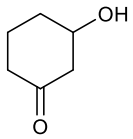
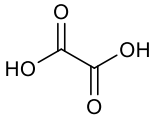


Fig. S5. IR spectra of Au@TiO_2@NF before (Au@TiO_2@NF-1) and after (Au@TiO_2@NF-2) photocatalytic cycle.

Table S1. List of compounds identified after the photocatalytic degradation of TG.

	Chemical structure	Chemical formula	*m/z* of $[M-H]^-$
TG		$C_{29}H_{39}N_5O_8$	584.27
7		$C_{27}H_{32}N_4O_9$	555.21
12		$C_{21}H_{24}N_4O_{10}$	491.14
14		$C_{17}H_{18}O_7$	333.10
16		$C_6H_{12}O_7$	195.05
17		$C_{10}H_{10}O_4$	193.05
18		$C_{10}H_8O_2$	159.04
20		$C_6H_{10}O_2$	113.06
21		$C_2H_2O_4$	88.99