

# Experimental report

15/09/2024

**Proposal:** 9-13-1047

**Council:** 10/2022

**Title:** Interaction of peptide-loaded photocatalytic nanoparticles with bacterial membrane components - lipopolysaccharides

**Research area:** Soft condensed matter

**This proposal is a new proposal**

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**Samples:** TiO<sub>2</sub> nanoparticles  
Smooth lipopolysaccharide from Escherichia Coli O111:B4  
LL-37 antimicrobial peptide  
Rough lipopolysaccharide from Escherichia Coli F583 (Rd mutant)

Instrument	Requested days	Allocated days	From	To
D17	4	4	04/04/2023	08/04/2023

## Abstract:

In the global fight against antimicrobial resistance, photocatalytic TiO<sub>2</sub> nanoparticles (TiO<sub>2</sub>NPs) represent potent non-conventional antibiotics, capable of inducing oxidative degradation of bacterial membranes under UV illumination. However, insufficient colloidal stability and poor selectivity between bacteria and human cells severely limit their clinical translation. Recently, we demonstrated that loading TiO<sub>2</sub>NPs with antimicrobial peptides (AMPs), specifically the AMP LL-37, improves colloidal stability and selectivity between bacteria-like and eukaryotic-mimicking bilayers. Here, we will extend this investigation to other key components of bacterial cell walls, i.e. bacterial lipopolysaccharides (LPS), composing the outer cell membrane of Gram-negative bacteria. We will use Neutron Reflectometry to investigate the structural modification induced by LL-37-TiO<sub>2</sub>NPs on either  $\zeta$ smooth $\zeta$  or  $\zeta$ rough $\zeta$  LPS surfaces (mimicking the two main phenotypes of bacterial LPS), under UV illumination. The results will provide mechanistic insights on the oxidative degradation of bacterial LPS by AMPs-loaded TiO<sub>2</sub>NPs and on how its susceptibility depends on the structure and composition of LPS layers.

**Interaction of peptide-loaded photocatalytic nanoparticles with bacterial membrane components – lipopolysaccharides**

**Abstract**

In the global fight against antimicrobial resistance, photocatalytic TiO<sub>2</sub> nanoparticles (TiO<sub>2</sub>NPs) represent potent non-conventional antibiotics, capable of inducing oxidative degradation of bacterial membranes under UV illumination. However, insufficient colloidal stability and poor selectivity between bacteria and human cells severely limit their clinical translation. Recently, we demonstrated that loading TiO<sub>2</sub>NPs with antimicrobial peptides (AMPs), specifically the AMP LL-37, improves colloidal stability and selectivity between bacteria-like and eukaryotic-mimicking bilayers. On this experiment, we extended this investigation to other key components of bacterial cell walls, i.e. bacterial lipopolysaccharides (LPS), composing the outer cell membrane of Gram-negative bacteria. We used Neutron Reflectometry to investigate the structural modification induced by LL-37-TiO<sub>2</sub>NPs on either “smooth” or “rough” LPS surfaces (mimicking the two main phenotypes of bacterial LPS), under UV illumination. The results provided mechanistic insights on the oxidative degradation of bacterial LPS by AMPs-loaded TiO<sub>2</sub>NPs and on how its susceptibility depends on the structure and composition of LPS layers. The findings obtained through this experiment are part of a paper currently published on ChemRxiv (1) and submitted on a peer-reviewed journal.

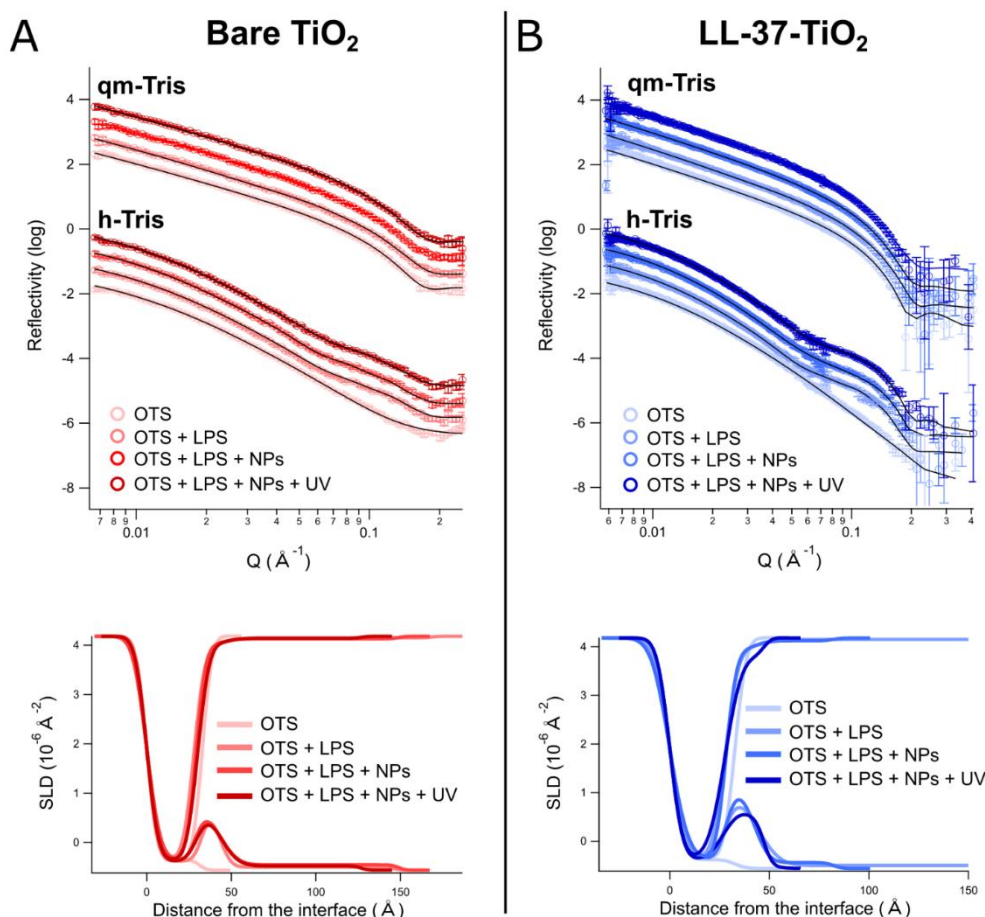
**Methodology**

The structure of supported LPS layers were characterized by NR, employing the D17 reflectometer (Institut Laue-Langevin). The Q-region of interest ( $\sim 0.01$  to  $0.3 \text{ \AA}^{-1}$ ) was covered using incident angles and  $0.8^\circ$  and  $3.0^\circ$ . Flow cells, the top plate of which having a circular 30 mm opening, were used in combination with UV-transparent quartz blocks (RMS  $< 4.5 \text{ \AA}$ , PI-KEM Ltd.) for in situ UV irradiation. The blocks were cleaned as described previously (2). After that, they were dried at  $100^\circ \text{C}$  and transferred to 1 mM OTS in toluene, followed by incubation (1 h) in a glovebox under N<sub>2</sub> flow. This procedure allowed to form a hydrophobic OTS monolayer onto the block surface, characterized by  $\sim 100^\circ$  contact water contact angle. HPLC tubing, PEEK troughs, and O-rings were cleaned by 2% Hellmanex (Hellma Analytics) (2).

OTS-coated surfaces were characterized in three contrasts, i.e., Tris, 150 mM NaCl in MQ (h-Tris), D<sub>2</sub>O (d-Tris), and 68.6/31.4 % v/v D<sub>2</sub>O/MQ to match the scattering length density (SLD) of the quartz substrate (qm-Tris). The cells were then rinsed, after which smooth LPS (1,000 ppm in h-Tris) was manually injected as described previously. The layers thus formed were characterized in h-, qm-, and d-Tris. After that, 15 mL of bare or LL-37-coated TiO<sub>2</sub> NPs (100 ppm in h-Tris) were injected manually, followed by incubation (10 min) and flushing by h-Tris to remove excess NPs, after which samples were characterized in h-, qm-, and d-Tris. The systems were then subjected to in situ UV irradiation (Spectroline lamp ENF-260C, 254 nm, 3 mW/cm<sup>2</sup>) for 2 h. Immediately after UV exposure, samples were rinsed with 20 mL of d-Tris (1 mL/min) and measured in the three contrasts. Experimental NR profiles were fitted by Motofit within IGOR Pro. The best fits were converted to SLD profiles perpendicular to the surface. To minimize fitting uncertainty, data was re-fitted 200 times by Monte Carlo error analysis.

## Results

Smooth LPS was then manually injected into the measurement chamber (after having formed an OTS onto the quartz block) and the reflectivity changes following the LPS layer formation were monitored. NR profiles for smooth LPS interacting with either bare or peptide-coated NPs are shown in Figure 1, together with best curve fits and corresponding SLDs. Key structural data determined through fitting are summarized in (1). Prior to UV illumination, the incubation with bare TiO<sub>2</sub> NPs did not induce significant structural modification in any of the LPS domains. In contrast, the addition of LL-37-TiO<sub>2</sub> NPs provoked a substantial thickness decrease for the outer O-antigen chains layer, from  $124 \pm 24$  Å to  $40 \pm 7$  Å. UV illumination induced a further decrease (to  $9 \pm 1$  Å) and hydration (from  $94 \pm 1$  % to  $59 \pm 8$  %), indicating an essentially complete removal of the O-antigen moiety. Contrasting this, only a relatively minor thickness reduction of the O-antigen layer was observed on UV illumination for bare TiO<sub>2</sub> NPs, from  $106 \pm 12$  Å to  $84 \pm 17$  Å, with negligible hydration changes. For both TiO<sub>2</sub> and LL-37-TiO<sub>2</sub> NPs, the OTS/lipid A and core oligosaccharides layers were essentially unaffected by UV illumination.



**Figure S9.** Neutron reflectivity curves with best model fits (upper) and corresponding SLD profiles (lower) for smooth LPS layers before and after incubation with bare TiO<sub>2</sub> NPs (A) and LL-37-TiO<sub>2</sub> NPs (B). Shown are also reflectivity curves with best model fits and SLD profiles for the corresponding systems after 2 h of *in situ* UV exposure. All experiments were performed in 10 mM

Tris buffer, pH 7.4, at a nanoparticle concentration of 100 ppm. Curves are shown for two different buffer contrasts, h-tris and qm-Tris, and data for the latter are offset by  $3 \cdot 10^{-1}$  for clarity. The grey box in the SLD profiles indicates the position of the silicon block and reflecting interface, consisting of bulk Si and a SiO<sub>2</sub> layer.

## References

1. Caselli L, Du G, Micciulla S, Traini T, Sebastiani F, Guldsmed Diedrichsen R, et al. Photocatalytic degradation of bacterial lipopolysaccharides by peptide-coated TiO<sub>2</sub> nanoparticles. ChemRxiv. 2024; doi:10.26434/chemrxiv-2024-0zz31. This content is a preprint and has not been peer-reviewed
2. Caselli, L., Traini, T., Micciulla, S., Sebastiani, F., Köhler, S., Nielsen, E.M., et al. Antimicrobial peptide coating of TiO<sub>2</sub> nanoparticles for boosted antimicrobial effects. Adv. Funct. Mater. 2024, 2405047.