Zirconyl Clindamycinphosphate Antibiotic Nanocarriers for Targeting Intracellular Persisting *Staphylococcus aureus*

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1. Analytical techniques for material characterization

Scanning electron microscopy (SEM) was carried out with a Zeiss Supra 40 VP (Zeiss, Germany), equipped with a field emission gun (acceleration voltage 2 kV, working distance 3 mm). Samples were prepared by placing a droplet of a diluted aqueous \([\text{ZrO}]^{2+}[\text{CLP}]^{2-}\) IOH-NP suspension on a silica wafer that was left for drying overnight.

Energy-dispersive X-ray spectroscopy (EDXS) was performed with an Ametek EDAX device (Ametek, U.S.A.) mounted on the above described Zeiss SEM Supra 40 VP scanning electron microscope. For this purpose, the \([\text{ZrO}]^{2+}[\text{CLP}]^{2-}\) IOH-NPs were pressed to dense pellets in order to guarantee for a smooth surfaces and a quasi-infinite layer thickness. These pellets were fixed with conductive carbon pads on aluminum sample holders.

Fourier-transformed infrared (FT-IR) spectra were recorded on a Bruker Vertex 70 FT-IR spectrometer (Bruker, Germany) in the range 4000-370 cm\(^{-1}\) with a resolution of 4 cm\(^{-1}\). To this concern, 1 mg of dried \([\text{ZrO}]^{2+}[\text{CLP}]^{2-}\) IOH-NPs was pestled with 300 mg of KBr and pressed to a pellet, which thereafter was measured in transmission.

Differential thermal analysis/thermogravimetry (DTA/TG) was performed with a STA409C device (Netzsch, Germany). The measurements were performed in air to guarantee for total organics combustion. The vacuum dried \([\text{ZrO}]^{2+}[\text{CLP}]^{2-}\) IOH-NPs (20 mg in corundum crucibles) were heated to 1000 °C with a rate of 5 K/min.

Elemental analysis (C/H/N/S analysis) was performed via thermal combustion with an Elementar Vario Microcube device (Elementar, Germany) at a temperature of 1100 °C.

2. Synthesis of \([\text{ZrO}]^{2+}[\text{CLP}]^{2-}\) and \([\text{ZrO}]^{2+}[(\text{CLP})_{0.995}(\text{DUT})_{0.005}]^{2-}\) IOH-NPs

a) Synthesis of \([\text{ZrO}]^{2+}[\text{CLP}]^{2-}\) IOH-NPs

Na\(_2\)(CLP) (25 mg, Aldrich, 95.7%) was dissolved in water (50 mL). The pH of this solution was adjusted to 7.0 upon addition of diluted NaOH (140 µL, 0.5 M). Thereafter, an aqueous solution (5 mL) of ZrOCl\(_2\)×8H\(_2\)O (4.25 mg, Aldrich, 99%) was injected. After 2 min of intense stirring, the nanoparticles were separated via centrifugation (25,000 rpm, 15 min). To remove all remaining salts, the colorless \([\text{ZrO}]^{2+}[\text{CLP}]^{2-}\) was resuspended in and centrifuged from H\(_2\)O three times. Subsequent to redispersion, highly stable colloidal suspension in water can be obtained (main paper: Figure 1b).
b) Synthesis of [ZrO]$^{2+}$[(CLP)$_{0.995}$(DUT)$_{0.005}$]$^{2-}$ IOH-NPs

[ZrO]$^{2+}$[(CLP)$_{0.995}$(DUT)$_{0.005}$]$^{2-}$ containing the antibiotic clindamycinphosphate ([CLP]$^{2-}$) as well as the near-infrared emitting dye-modified nucleoside triphosphate DY-647-dUTP ([DUT]$^{2-}$) was obtained similarly to [ZrO]$^{2+}$[CLP]$^{2-}$ described above. Thus, Na$_2$(CLP) (25 mg, Aldrich, 95.7%) and the dye-modified nucleoside triphosphate DY-647-dUTP (1.2 mg, DY-647-dUTP, Dyomics, Germany) were dissolved in water (50 mL). The pH of this solution was adjusted to 7.0 upon addition of diluted NaOH (140 µL, 0.5 M). Thereafter, an aqueous solution (5 mL) of ZrOCl$_2$×8H$_2$O (4.25 mg, Aldrich, 99%) was injected. After 2 min of intense stirring, the nanoparticles were separated via centrifugation (25,000 rpm, 15 min). To remove all remaining salts, the slightly bluish [ZrO]$^{2+}$[(CLP)$_{0.995}$(DUT)$_{0.005}$]$^{2-}$ was resuspended in and centrifuged from H$_2$O three times. Subsequent to redispersion, highly stable colloidal suspension in water can be obtained.

In view of the low amount of [DUT]$^{2-}$, the properties of [ZrO]$^{2+}$[(CLP)$_{0.995}$(DUT)$_{0.005}$]$^{2-}$ are similar to [ZrO]$^{2+}$[CLP]$^{2-}$. However, the presence of [DUT]$^{2-}$ in [ZrO]$^{2+}$[(CLP)$_{0.995}$(DUT)$_{0.005}$]$^{2-}$ is clearly validated by its slightly bluish color and its intense deep red to infrared fluorescence (Figure S1; main paper: Figure 3c,d). Furthermore, it is to be noted that pure [ZrO]$^{2+}$[(CLP)$_{0.995}$(DUT)$_{0.005}$]$^{2-}$ IOH-NPs were not studied due to the very high costs of [DUT]$^{2-}$ (i.e., 1 nM, 250 €). All relevant properties – e.g., particle diameter, cell uptake, etc. – of [ZrO]$^{2+}$[(CLP)$_{0.995}$(DUT)$_{0.005}$]$^{2-}$ due to the low amount of [DUT]$^{2-}$ are identical to [ZrO]$^{2+}$[CLP]$^{2-}$ within the significance of the experiment.

![Figure S1. Excitation and emission spectra of [ZrO]$^{2+}$[(CLP)$_{0.995}$(DUT)$_{0.005}$]$^{2-}$ IOH-NPs.](image-url)
3. Material characterization

a) Fluorescence of \([\text{ZrO}]^{2+}[(\text{CLP})_{0.995}(\text{DUT})_{0.005}]^{2-}\)

Excitation and emission spectra of fluorescence-marked \([\text{ZrO}]^{2+}[(\text{CLP})_{0.995}(\text{DUT})_{0.005}]^{2-}\) IOH-NPs are shown in Figure S1. Accordingly, the excitation can be performed at 500-700 nm with \(\lambda_{\text{max}} = 655\) nm. Emission occurs at 630-780 nm with \(\lambda_{\text{max}} = 675\) nm. The emission of the \([\text{ZrO}]^{2+}[(\text{CLP})_{0.995}(\text{DUT})_{0.005}]^{2-}\) IOH-NPs – as expected – is identical to pure Dyomics-647 uridine triphosphate.

b) Size distribution

In addition to statistical evaluation of particles on SEM images, particle diameter and size distribution of the as-prepared \([\text{ZrO}]^{2+}[(\text{CLP})]^{2-}\) IOH-NPs were also validated via DLS in water. As a result a mean hydrodynamic diameter of 73(14) nm was determined (Figure S2). Such difference between the mean diameter of nanoparticles obtained by statistical evaluation of particles on SEM images and the mean hydrodynamic diameter obtained via DLS in aqueous suspension is not surprising. SEM, on the one hand, shows the “real” particle diameter without any solvent shell and surface conditioning. The hydrodynamic diameter obtained via DLS, on the other hand, reflects the diameter in the presence of adsorbed solvent molecules. Due to the very high polarity of water, the rigid layer of adsorbed solvent molecules is often significantly expanded. Moreover, the nanoparticles are typically not agglomerated as such but the layers of adsorbed solvent molecules are merged so that the nanoparticles do not show independent diffusion. As a result, DLS shows larger diameters. Such effect is again strongly supported by the high polarity of water and extensive hydrogen bonding. These effects of water as a solvent can be easily verified by re-dispersing IOH-NPs in a less polar but more coordinating solvent such as diethylene glycol [S1]. After re-dispersion of IOH-NPs in diethylene glycol, the size distribution curve is only slightly shifted in comparison to the size distribution curve obtained from electron microscopy. Diethylene glycol, however, is not the relevant solvent for biomedical application. Finally, it must be noted that the as-prepared \([\text{ZrO}]^{2+}[(\text{CLP})]^{2-}\) IOH-NPs do not contain any specific stabilizer (e.g., dextran, protamine, polyethylene glycol). Nevertheless, the IOH-NPs are colloidally stable and do not show precipitation over several weeks.
c) Determination of chemical composition

Fourier-transform infrared spectroscopy (FT-IR) evidences the presence of [CLP]^{2-} in the [ZrO]^{2+}[CLP]^{2-} IOH-NPs (see main paper: Figure 1e). Thus, all characteristic vibrations of clindamycinphosphate are very comparable for the [ZrO]^{2+}[CLP]^{2-} IOH-NPs and Na_2(CLP) as the starting material (ν(O−H) at 3600-3200 cm\(^{-1}\), ν(C−H): 3000-2800 cm\(^{-1}\), ν(C=O): 1750-1500 cm\(^{-1}\), ν(PO\(_4\)): 1250-1000 cm\(^{-1}\), fingerprint area: 1500-1250 and 1000-500 cm\(^{-1}\)). The strong vibration ν(O−H), moreover, indicates the presence of H\(_2\)O.

Furthermore, the presence of zirconium and phosphorus was qualitatively proven by energy dispersive X-ray spectroscopy (EDXS).

To quantify the chemical composition of the [ZrO]^{2+}[CLP]^{2-} IOH-NPs, thermogravimetry (TG) was performed to verify the total organics content via thermal combustion (Figure S3). [ZrO]^{2+}[CLP]^{2-} shows more-or-less continuous decomposition in a temperature range of 100-1000 °C with a total weight loss of 66% (samples were dried at 100 °C) due to total combustion of the [CLP]^{2-} anion. This value matches very well with the calculated weight loss of 68%. The remnant of the thermal decomposition was identified via X-ray powder diffraction as a mixture of ZrO\(_2\) and Zr\(_3\)(PO\(_4\))\(_4\). In sum, the total organics decomposition of [ZrO]^{2+}[CLP]^{2-} can be rationalized based on the following reaction:

\[ 4[ZrO]^{2+}[C_{18}H_{33}PSClN_{2}O_{8}]^{2−} + 99O_2 \rightarrow ZrO_2 + Zr_3(PO_4)_4 + 72CO_2↑ + 4N_2↑ + 4SO_2↑ + 4HCl↑ + 64H_2O↑ \]

The composition of the [ZrO]^{2+}[CLP]^{2-} IOH-NPs was further confirmed by elemental analysis (EA) (see main paper).

Finally, it is to be noted that the fluorescent [ZrO]^{2+}[(CLP)\(_{0.995}(DUT)_{0.005}\)]^{2−} IOH-NPs show an identical chemical composition due to the low amount of [DUT]^{2−}. The presence of [DUT]^{2−} in
[ZrO]^{2+}[(CLP)_{0.995}(DUT)_{0.005}]^{2-}, however, is clearly validated by its slightly bluish color and its intense deep red to infrared fluorescence (see main paper: Figure 3c,d; Figure S1).

![Thermogravimetry graph](image)

**Figure S3.** Thermogravimetry of the as-prepared [ZrO]^{2+}[CLP]^{2-} IOH-NPs.

### 4. Biological studies

**a) Bacteria**

The bacterial strain used in this study was *S. aureus* strain SH1000. Staphylococci were grown to the mid-log phase at 37 °C with shaking (150 rpm) in brain heart infusion (BHI) medium. Bacteria were collected by centrifugation, washed with sterile PBS, and diluted to the required concentration. The number of viable bacteria was determined after serial diluting and plating on blood-agar.

**b) Generation of bone marrow derived macrophages**

BMDM were isolated from 4- to 6-week-old C57BL/6 mice (Harlan Laboratories United Kingdom). Briefly, mice were sacrificed by CO₂ asphyxiation. Femurs and tibias were removed and the bone marrow was harvested by repeated flushing with Iscove’s modified Dulbecco’s medium (DMEM). Bone marrow cells were incubated in DMEM supplemented with 10% FCS, 2 mM of L-glutamine, 1 mM of pyruvate, 100 U/mL of penicillin, 100 µg/mL of streptomycin, and 20 U/mL of recombinant murine M-CSF (BioLegend, San Diego, USA) for 6 days.

**c) In vitro infection of bone marrow derived macrophages**

BM-derived macrophages were infected with *S. aureus* at a multiplicity of infection (MOI) of 10:1 (10 bacteria per macrophage) and incubated for 1 h at 37 °C, 5% CO₂. Macrophages were washed 2-times with sterile PBS, and some cells were then disrupted with dH₂O to release intracellular bacteria, the
amount of viable intracellular bacteria was then calculated by plating serial dilutions on blood agar plates. This was considered time 0 h relative to drug treatment and the amount of intracellular bacteria was referred to as 100%. The remaining cells were then exposed to [ZrO]$^{2+}$[CLP]$^{2-}$ IOH-NPs and free, dissolved clindamycin (50 µg/mL Na$_2$(CLP)) at equal concentration in fresh cell culture medium and incubated further for the indicated time points at 37 °C, 5% CO$_2$. These cells were then also disrupted with dH$_2$O to release intracellular bacteria, and the amount of viable intracellular bacteria was calculated by plating on blood agar. This was considered time 4 h relative to drug treatment. For the generation of cell lysates, cells were treated as described above, washed twice with sterile PBS and lysed with 500 µL of sterile water.

d) Hep2 Cell culture

Cells were maintained in modified Eagle's medium (DMEM, high-glucose, L-Glutamine, Phenol red, HEPES, Thermo Fisher Scientific) supplemented with 10% sterile filtered (Sartorius Minisart Filters 0.2 µm, Sigma-Aldrich) fetal bovine serum (FBS, Thermo Fisher Scientific) and 1% Penicillin/Streptomycin [Penicillin-Streptomycin (10,000 U/mL), Thermo Fisher Scientific]. The cells were cultured in a humidified incubator with a 5% (v/v) CO$_2$ atmosphere at 37 °C in T-75 or T-150 cell culture flasks (Tissue Culture Flasks, TPP).

e) Transmission electron microscopy

Macrophages (10$^6$/mL) were grown in Permanox® 4 well chamber slides overnight at 37 °C. Subsequently, they were infected with S. aureus SH1000 (MOI 10:1). Accordingly, they were treated for 4, 6, and 12 hours with [ZrO]$^{2+}$[CLP]$^{2-}$ IOH-NPs, or they were untreated as a nanoparticle-free control. Samples were prepared for embedment and ultrathin sectioning as it has been described in [S2].

For electron energy-loss spectroscopy, 40 nm ultrathin sections were sectioned 'en face' and were picked up with neopren-coated 300 mesh Cu-grids. Unstained sections were analyzed with an integrated energy-filtered transmission electron microscope (EF-TEM; Zeiss Libra120plus; Zeiss, Oberkochen, Germany) and spectra (parallel electron energy-loss spectroscopy, PEELS) as well as elemental maps (electron spectroscopic imaging, ESI) were recorded by a bottom-mounted, cooled 2048×2048 CCD camera (Sharp.Eye; Tröndle, Moorenweis, Germany). PEELS were recorded within an energy range from 90 eV to 350 eV with a chanel width of 0.112 eV (Figure S4). ESI sequences have been recorded according to the 3-window method, with energy-windows set at 148, 172 and 211 eV with the energy-slit set to 10 eV energy width (main paper: Figure 3a,b). The magnification applied ranged from nominally ×8000 to ×20000 at 120 kV. The illumination aperture was set to 0.63 to 0.80 mrad, with an emission current set to 1 µA to 4 µA. Data acquisition and evaluation were performed with the iTEM-System software (version 5.2, OSIS, Münster, Germany).
Figure S4. EELS of [ZrO]$^{2+}$(CLP)$^{2-}$-treated BMDMs: a) Intracellular cluster of [ZrO]$^{2+}$(CLP)$^{2-}$ IOH-NPs (dashed red circle) for analysis in (b). b) PEEL spectra with Zr-M45 ionization edge of IOH-NP cluster in (a) (PEELS of ZrO$_2$ reference: green line).

PEELS and ESI clearly validate the presence of [ZrO]$^{2+}$(CLP)$^{2-}$ IOH-NPs within the primary lysosomes (Figure S4; main paper: Figure 3a,b). The analysis also allows discriminating [ZrO]$^{2+}$(CLP)$^{2-}$ IOH-NPs from other electron dense nanostructures such as ferritins or Fe$_3$O$_4$ particles that can be present in BMDMs. Within a measuring aperture of 1 µm in diameter (Figure S4a: dashed red circle) the corresponding PEELS was recorded, and the presence of Zr could be verified via the M45 ionization edge (187.0-211.0 eV) (Figure S4b: red line). PEELS of [ZrO]$^{2+}$(CLP)$^{2-}$ clusters shows rather similar features of the energy-loss near edge fine structure (ELNES; 190-270 eV) as ZrO$_2$ reference spectra (Figure S4b: green line). Similar results were obtained via Zr-M45 elemental maps as well as for S. aureus-infected macrophages (main paper: Figure 3a,b: net Zr-M45 in red) indicating the presence and high load of internalized [ZrO]$^{2+}$(CLP)$^{2-}$ IOH-NPs.

f) LDH assays

The level of LDH (released lactate dehydrogenase) was measured in the supernatant of treated macrophages by using the CytoTox 96 (Promega, USA) according to manufacturer’s instructions. Briefly, 50 µL of the supernatant from macrophages treated with different concentrations of the [ZrO]$^{2+}$(CLP)$^{2-}$ IOH-NPs were transferred to a 96-well enzymatic assay plate. Reconstituted substrate mix (50 µL) was then added to each well and plates were incubated for 30 min at room temperature. The absorbance was measured at 490 nm (Figure S5). The percentage of cytotoxicity was calculated as [(sample LDH release – spontaneous LDH release) / (maximum LDH release – spontaneous LDH release)] × 100. The spontaneous release was the amount of LDH released from the cytoplasm of uninfected macrophages,
whereas the maximum release was the amount released by total lysis of uninfected cells after treatment with 0.1% Triton X-100.

**Figure S5.** Determination of cytotoxicity induced in bone marrow derived macrophages after treatment with $[\text{ZrO}]^{2-}[\text{CLP}]^{2-}$ IOH-NPs and free, dissolved clindamycin (i.e. CLP: Na$_2$(CLP)) for 24 h by measuring the released levels of LDH over untreated control cells in the culture supernatant of treated cells. Cells lysed with water served as maximum LDH release. Summary of three independent experiments are shown.

**g) Sample preparation and analysis of macrophage cell lysates**

All cell lysate samples were analyzed via HPLC-MS/MS using an Agilent 1290 HPLC system equipped with a diode array UV detector and coupled to an AB Sciex QTrap 6500 mass spectrometer. First, a calibration curve was prepared by spiking different concentrations of clindamycinphosphate (CLP) or clindamycin hydrochloride (CL) into macrophage cell lysate. The lower limits of quantification are indicated in Table S1. Caffeine was used as an internal standard. In addition, quality control samples (QCs) were prepared for clindamycinphosphate and clindamycin hydrochloride in cell lysate. For clindamycinphosphate and clindamycin hydrochloride as well as for cell lysate samples the same extraction procedure was used: 10 µL of a sample (calibration samples, QCs or samples) was extracted with 20 µL of methanol containing 12.5 ng/mL of caffeine as internal standard for 10 min at 2000 rpm on an Eppendorf MixMate® vortex mixer. Then samples were spun down at 13,000 rpm for 5 min at 12 °C. Supernatants were transferred to standard HPLC-glass vials. HPLC conditions were as follows: column: Agilent Zorbax Eclipse Plus C18, 50×2.1 mm, 1.8 µm; temperature: 30 °C; injection volume: 1 µL; flow rate: 700 µL/min; solvent A: water +0.1% formic acid; solvent B: acetonitrile +0.1% formic acid; the same gradient was used for clindamycinphosphate and clindamycin hydrochloride: 99% A at 0 min, 99%-0% A from 0.1 min to 5.50 min, 0% A until 6.00 min, 0%-99% A from 6.00 min to 6.20 min, 99% A
until 8.00 min; UV detection: 190-400 nm. Mass spectrometric conditions were as follows: Scan type: MRM, positive mode; Q1 and Q3 masses for caffeine, clindamycinphosphate and clindamycin hydrochloride can be found in Table S2; peak areas of each sample and of the corresponding internal standard were analyzed using MultiQuant 3.0 software (AB Sciex). Peak areas of the respective sample containing clindamycin hydrochloride or phosphate were normalized to the internal standard peak area. For clindamycinphosphate \( m/z \) 505.259 → 126.100 was used for quantification and \( m/z \) 505.259 → 457.200 was used for qualification. For clindamycin hydrochloride \( m/z \) 425.193 → 125.900 was used for quantification and \( m/z \) 425.193 → 82.000 was used for qualification. For caffeine \( m/z \) 195.116 → 138.100 was used for quantification and \( m/z \) 195.116 → 110.000 was used for qualification. Peaks of samples were quantified using the calibration curve. The accuracy of the calibration curve was determined using QCs independently prepared on different days (Table S1).

| Sample                  | Limits of quantification [ng/mL] | Lower limit of qualification [ng/mL] | Accuracy [\%] |
|-------------------------|----------------------------------|--------------------------------------|---------------|
| Clindamycin hydrochloride | 25–4000                          | 2.5                                  | 85.67–114.31  |
| Clindamycinphosphate    | 2.5–4000                         | 1.0                                  | 87.29–113.85  |

| Sample                  | Q1 Mass [Da] | Q3 Mass [Da] | Time [msec] | CE [volts] | CXP [volts] |
|-------------------------|--------------|--------------|-------------|------------|-------------|
| Clindamycinphosphate    | 505.259      | 126.100      | 50          | 31         | 8           |
| Clindamycinphosphate    | 505.259      | 457.200      | 50          | 25         | 24          |
| Clindamycin hydrochloride | 425.193      | 125.900      | 50          | 25         | 4           |
| Clindamycin hydrochloride | 425.193      | 82.000       | 50          | 111        | 14          |
| Caffeine                | 195.116      | 138.100      | 50          | 27         | 10          |
| Caffeine                | 195.116      | 110.000      | 50          | 31         | 6           |

The superior bactericidal efficiency of clindamycin-loaded \([\text{ZrO}]^{2+}[\text{CLP}]^{2-}\) IOH-NPs over dissolved, free clindamycin is shown upon incubation with BMDMs in comparison to dissolved clindamycinphosphate (CLP, *main paper: Figure 5*) and dissolved clindamycin hydrochloride (CL, Figure S6). The amount of intracellular clindamycin was determined in BMDM lysates by HPLC-MS/MS to determine the intracellular clindamycin levels.
In comparison to both – solutions of CLP and CL – the internalized clindamycin concentration in macrophages turned out as 70 to 150-times higher after treatment with \([\text{ZrO}^{2+}][\text{CLP}]^{2–}\) suspensions after 1 h of incubation (Figure S6; main paper: Figure 5b). After 4 h, the internalized clindamycin concentration is even 150-times higher after treatment with \([\text{ZrO}^{2+}][\text{CLP}]^{2–}\) suspensions (Figure S6; main paper: Figure 5b).

**Figure S6.** Intracellular clindamycin levels within macrophages 1, 2 and 4 h after co-cultivation with \([\text{ZrO}^{2+}][\text{CLP}]^{2–}\) IOH-NPs and dissolved clindamycin hydrochloride (CL) at identical concentration (50 µg CL/mL): a) Illustration of uptake and cell treatment; b) Internalized clindamycin after treatment with dissolved CL (white bars), \([\text{ZrO}^{2+}][\text{CLP}]^{2–}\) (red bars), lysates of untreated cells (negative control, grey bars). Data measured by HPLC-MS/MS; mean ±SD of triplicate samples (**p<0.001).

**h) Statistics**

Data were analyzed by using Excel 2016 (Microsoft Office) or GraphPad Prism 5.0 (GraphPad software). All data are presented as mean ±SD. Comparison between groups was made by use of T-test. *P* values ≤0.05 were considered as significant.

**i) Determination of minimal inhibitory concentration (MIC)**

To obtain the minimal inhibitory concentration (MIC) of CLP of the applied *S. aureus* strain, the bacteria were grown in a volume of 300 µL in a 96-well plate (Sarstedt, Germany) for 24 h in the absence or the presence of the indicated concentrations of antibiotics. Bacterial growth was then measured as absorbance at OD\(_{600nm}\) by using a TECAN Sunrise® ELISA reader (Tecan Group, Switzerland) and transferred for better visualization to a heat map (Figure S7). Accordingly, a MIC of <0.625 µg per mL was obtained. In comparison, an intracellular CLP level of 1.027 µg per mL was reached upon incubation with IOH-NPs (in suspension) for 4 h. Using dissolved CLP only results in an intracellular concentration <0.02 µg per mL.
Figure S7. Determination of the minimal inhibitory concentration (MIC) of CLP of the applied S. aureus strain SH1000. Bacterial growth is indicated by high OD$_{600\text{nm}}$ values (red color), absence of bacterial growth by low OD$_{600\text{nm}}$ values (green color).

j) Fluorescence microscopy

For immunofluorescence microscopy (Figures S8,S9; main paper: Figure 3c,d), a total of $10^6$ BMDMs were seeded on coverslips and were either infected with a gfp expressing S. aureus strain at an MOI of 10 to 1 and/or treated with [ZrO]$^{2+}$[(CLP)$_{0.995}$(DUT)$_{0.005}$]$^{2-}$ IOH-NPs. After the indicated time of incubation, the coverslips were rinsed with sterile PBS and cells were fixed with 3.7% formaldehyde. The fluorescence images were obtained using a Zeiss Axiophot microscope with an attached Zeiss Axiocam HRc digital camera and Axiovision 4.8 software (Zeiss).

The [ZrO]$^{2+}$[(CLP)$_{0.995}$(DUT)$_{0.005}$]$^{2-}$ IOH-NPs show time depending uptake as indicated upon incubation with macrophages on different time scales (Figure S8). Thus, the fluorescence of [ZrO]$^{2+}$[(CLP)$_{0.995}$(DUT)$_{0.005}$]$^{2-}$ IOH-NPs (indicated by purple colour) clearly increases after incubation for 1 h (Figure S8a), 3 h (Figure S8b), and 6 h (Figure S8c). Cellular nuclei show blue emission due to DAPI staining.

The dose depending uptake of [ZrO]$^{2+}$[(CLP)$_{0.995}$(DUT)$_{0.005}$]$^{2-}$ IOH-NPs can be illustrated via fluorescence microscopy showing the fluorescence of the IOH-NPs (Figure S9). Whereas untreated bone marrow derived macrophages only show the blue emission of DAPI-stained cellular nuclei (Figure S9a), the fluorescence of [ZrO]$^{2+}$[(CLP)$_{0.995}$(DUT)$_{0.005}$]$^{2-}$ IOH-NPs (indicated by purple colour) is clearly visible after incubation for 1 h with 5 µg/mL of IOH-NPs (Figure S9b). This IOH-NP-related fluorescence was significantly increased upon incubation for 1 h with 50 µg/mL of IOH-NPs (Figure S9c).
Figure S8. Time-depending uptake of IOH-NPs according to fluorescence microscopy showing $[\text{ZrO}]^2^-[(\text{CLP})_{0.995}(\text{DUT})_{0.005}]^{2^-}$ IOH-NPs (purple), and the cellular nucleus stained with DAPI (blue). a-c) Bone marrow derived macrophages co-incubated with $[\text{ZrO}]^2^-[(\text{CLP})_{0.995}(\text{DUT})_{0.005}]^{2^-}$ IOH-NPs for 1 h (a), 3 h (b) and 6 h (c).

Figure S9. Dose-depending uptake of IOH-NPs according to fluorescence microscopy showing $[\text{ZrO}]^2^-[(\text{CLP})_{0.995}(\text{DUT})_{0.005}]^{2^-}$ (purple) and cellular nucleus (stained with DAPI, blue): a-c) Bone marrow derived macrophages co-incubated with different medium concentrations of $[\text{ZrO}]^2^-[(\text{CLP})_{0.995}(\text{DUT})_{0.005}]^{2^-}$ IOH-NPs for 1 h with (a) medium without IOH-NPs, (b) medium supplemented with 5 µg/mL of IOH-NPs, and (c) medium supplemented with 50 µg/mL of IOH-NPs.

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