Glycosylated copper sulfide nanocrystals for targeted photokilling of bacteria in the near-infrared II window

Hou, Shuai; Mahadevegowda, Surendra Hittanahalli; Mai, Van Cuong; Chan - Park, Mary Bee Eng; Duan, Hongwei

2019

Hou, S., Mahadevegowda, S. H., Mai, V. C., Chan-Park, M. B., & Duan, H. (2019). Glycosylated copper sulfide nanocrystals for targeted photokilling of bacteria in the near-infrared II window. *Advanced Therapeutics*, 2(8), 1900052-. doi:10.1002/adtp.201900052

<https://hdl.handle.net/10356/138815>

<https://doi.org/10.1002/adtp.201900052>

This is the peer reviewed version of the following article: Hou, S., Mahadevegowda, S. H., Mai, V. C., Chan-Park, M. B., & Duan, H. (2019). Glycosylated copper sulfide nanocrystals for targeted photokilling of bacteria in the near-infrared II window. *Advanced Therapeutics*, 2(8), 1900052-. doi:10.1002/adtp.201900052, which has been published in final form at :10.1002/adtp.201900052. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.

Downloaded on 26 Apr 2023 21:47:04 SGT

Glycosylated Copper Sulfide Nanocrystals for Targeted Photokilling of Bacteria in the Near-Infrared II Window

Shuai Hou, Surendra H. Mahadevegowda, Van Cuong Mai, Mary B. Chan-Park,* and Hongwei Duan*

Dr. S. Hou, Dr. S. H. Mahadevegowda, V. C. Mai, Prof. M. B. Chan-Park, Prof. H. Duan
School of Chemical and Biomedical Engineering, Nanyang Technological University, 62
Nanyang Drive, Singapore 637459, Singapore
E-mail: mbechan@ntu.edu.sg; hduan@ntu.edu.sg

V. C. Mai
Nanyang Environment and Water Research Institute (NEWRI), Nanyang Technological
University, 1 Cleantech Loop, Singapore 637141, Singapore

Keywords: glycoconjugates, lectins, copper sulfide nanocrystals, photothermal therapy, photodynamic therapy

Abstract

Photothermal and photodynamic therapies have been established as alternative approaches to combating bacterial infections; however, the heat and reactive oxygen species generated by the photoagents act on both normal and bacterial cells. A targeting strategy is thus required to minimize side effects and enhance the antibacterial efficiency. Glycoconjugates specifically interacting with bacterial lectins have emerged as a new class of materials for targeting bacteria. In this paper, galactosylated plasmonic copper sulfide nanocrystals (Cu_{2-x}S NCs) were used to target *Pseudomonas aeruginosa* via galactose-LecA interactions and kill the bacteria by simultaneous photothermal and photodynamic therapy. Galactosylated Cu_{2-x}S NCs were obtained by functionalizing the nanocrystals with tri-thiogalactoside glycoclusters. We first demonstrate the excellent specificity of galactosylated nanoparticles towards LecA with a LecA-deficient *P. aeruginosa* strain as the control. Afterward, a laser in the near-infrared II window was used to kill the bacteria, and the critical role of targeted binding in efficient killing of bacteria was highlighted. This approach can be readily generalized to the targeting of other pathogens which have highly specific carbohydrate-binding lectins.

1. Introduction

New approaches to combating bacterial infections are in urgent need with the rise of drug-resistant bacteria.^[1] Conventional antibiotics are becoming less efficient, and only two new classes of antibiotics have been marketed during the last 50 years.^[2] Phototherapeutic approaches, particularly photothermal therapy (PTT)^[3-6] and photodynamic therapy (PDT),^[7-10] have attracted ever-increasing interest as alternative strategies for infection control. PTT uses photothermal agents to convert light energy into heat, leading to cell membrane damages, protein denaturation, and heat stress.^[3] PDT involves the generation of reactive oxygen species (ROS) which can destroy a broad spectrum of molecular targets such as proteins, lipids, and nucleic acids.^[7] Combination of PTT and PDT was reported to significantly enhance the therapeutic performance, due to the simultaneous actions of heat and ROS.^[11,12] Heat and ROS attack bacteria at multiple sites and cause irreversible damages to the most elementary bacterial components.^[13,14] Additionally, phototherapy can reduce the damage to healthy tissues by focusing the light only on the infection area.

The current trend of phototherapy is moving towards the use of light source in the near infrared (NIR) II window (1000-1700 nm), which is favorable for deeper penetration and higher power density tolerance in normal tissues. Although new materials are continuously developed for either PTT or PDT in the NIR II window,^[15,16] materials with a single component for simultaneous PTT and PDT at a single wavelength are still rare. Copper sulfide nanocrystals (Cu_{2-x}S NCs) have emerged as the material of choice for combined PTT and PDT.^[11,17-21] The high concentration of copper vacancies in the Cu_{2-x}S NCs gives rise to a strong localized plasmonic resonance, resulting in strong light absorption in the NIR II spectral region.^[22] In addition to the high photothermal conversion efficiency,^[17-21] light-induced copper ion release from Cu_{2-x}S NCs can initiate generation of hydroxyl radicals for

efficient killing of cells.^[17] They have been successfully employed as photoagents for the treatment of cancer^[17] and bacterial infections.^[11]

One of the inherent problems with phototherapy is the nonspecific cell interactions, as the functioning of heat and ROS does not make a distinction between normal and bacterial cells. It is thus necessary to add a targeting element to the photoactive nanomaterials, which not only protects the normal tissues but also enriches the nanomaterials for more efficient killing of the bacteria.^[23] Current targeting strategies involve the use of antibodies,^[24] aptamers,^[25] or small molecules such as vancomycin.^[26] Synthetic carbohydrates may be an alternative targeting element.^[27-29] Numerous strains of bacteria express carbohydrate-binding proteins, termed lectins, on their surfaces to mediate critical physiological and pathological activities, *e.g.*, adhesion to host cells, biofilm formation, and infection.^[30-33] The high specificity of carbohydrate-lectin recognition presents the opportunity to target bacterial lectins with glycoconjugates. Despite the low binding affinities of carbohydrate-protein interactions, arranging carbohydrate moieties on various scaffolds can enhance the binding affinity via the multivalency effect.^[34,35] Multivalent glycoconjugates, such as glycoclusters, glycopeptides, and glyconanoparticles, have been employed as lectin ligands for inhibition of bacterial adhesion and biofilm formation,^[36-40] decontamination of bacteria,^[41] and blockage of host cell invasion by bacteria.^[42] However, successful demonstrations of targeted killing of bacteria with glycoconjugates are still limited.^[27-29]

Here, we use galactosylated Cu_{2-x}S NCs to target the galactose-specific lectin LecA on the surface of *P. aeruginosa* and eradicate the bacteria via simultaneous PTT and PDT in the NIR II window. *P. aeruginosa* is a Gram-negative opportunistic pathogen causing severe infections at various sites within the body, such as the respiratory tract, urinary tract, and the skin. Although LecA proteins mainly locate in the cytoplasm of *P. aeruginosa*, they are also present on the outer membrane.^[43] The outer membrane LecA proteins have been suggested to mediate *P. aeruginosa* anchoring on host cells, and galactoside glycoclusters have been used

as anti-adhesives to prevent *P. aeruginosa* infections.^[44] We synthesized tri-thiogalactoside glycocluster functionalized Cu_{2-x}S NCs and proved the specific binding of the nanoparticles on *P. aeruginosa* via the LecA proteins. Afterward, a 1064-nm laser was used to kill the bacteria in the presence of the galactosylated Cu_{2-x}S NCs, and the important role of targeted binding in the killing of bacteria was highlighted by using the LecA-deficient bacterial strain as the control. Our results indicate that glyconanoparticles, which integrate the recognition function of carbohydrates and the therapeutic function of nanoparticles, may serve as a powerful platform for the treatment of bacterial infections.

2. Results and Discussion

2.1. Synthesis and Characterizations of Galactosylated Cu_{2-x}S NCs

We designed the tri-thiogalactoside cluster Gal₃-COOH (**Figure 1**) to target *P. aeruginosa*. D-galactose and its derivatives have long been known to bind *P. aeruginosa*; their binding affinities, however, vary drastically depending on the specific structure.^[43] We chose thiogalactose because its derivatives were reported to show a high binding affinity,^[43] and the thiol group enables the thiol-ene click chemistry linking thiogalactose into triads, which further increases the binding affinity. A polyglycol chain with a carboxyl group was conjugated to the nitrogen center of the glycocluster, facilitating the subsequent surface-functionalization of the Cu_{2-x}S NCs. Oleylamine coated Cu_{2-x}S NCs were prepared based on the thermal decomposition of copper precursors.^[18] To render the Cu_{2-x}S NCs hydrophilic, PEGylation with HS-PEG-NH₂ was carried out to transfer the nanoparticles from toluene to water. Finally, the amino-group-terminated Cu_{2-x}S NCs were conjugated with Gal₃-COOH via NHS/EDC chemistry to obtain the Gal₃-Cu_{2-x}S NCs (**Figure 1**). Tri-thioglucoside cluster functionalized Cu_{2-x}S NCs, Glc₃-Cu_{2-x}S, were also prepared for comparison.

TEM images of Gal₃-Cu_{2-x}S NCs show nanocrystals with a plate shape and a characteristic size around 40 nm (**Figure 2a** and **Figure S1**, Supporting Information).

Electrophoresis was performed to examine the surface-modification of the nanoparticles with the PEGylated Cu_{2-x}S NCs as the control (Figure 2b). PEGylated Cu_{2-x}S NCs moved towards the cathode owing to the positive surface charge (zeta potential: +25 mV). After conjugation with $\text{Gal}_3\text{-COOH}$, the electrophoretic mobility of $\text{Gal}_3\text{-Cu}_{2-x}\text{S}$ NCs was significantly reduced, consistent with the reduced zeta potential (+10 mV). The successful anchoring of galactose on the nanoparticles was further proved by incubating the nanoparticles with LecA (galactose-specific lectin) and ConA (mannose- and glucose-specific lectin). The recognition of nanoparticles and lectins can induce the formation of nanoparticle aggregates that are too large to move through the gel. Electrophoresis results show that $\text{Gal}_3\text{-Cu}_{2-x}\text{S}$ NCs specifically respond to LecA while the electrophoretic mobility after mixing with ConA was not affected. The $\text{Glc}_3\text{-Cu}_{2-x}\text{S}$ NCs, which exhibited similar electrophoretic mobility to $\text{Gal}_3\text{-Cu}_{2-x}\text{S}$ NCs, selectively respond to ConA rather than LecA. These results show excellent specificity of the glycosylated nanoparticles towards lectins, providing a solid foundation for targeting the galactose-specific *P. aeruginosa* lectin LecA.

The $\text{Gal}_3\text{-Cu}_{2-x}\text{S}$ NC solution shows a broad NIR absorption peak (Figure 2c), making them especially suitable for photothermal conversion in the NIR-II spectral window. We evaluated the photothermal conversion efficiency of the $\text{Gal}_3\text{-Cu}_{2-x}\text{S}$ NCs at 1064 nm. Calculating from the standard laser heating and cooling curves for two concentrations of Cu_{2-x}S (Figure 2d), we determined the photothermal conversion efficiency to be 49% (see Supporting Information for details). The photothermal efficiency of copper sulfides is reported to vary from 20% to 60%, due to differences in Cu/S ratio, nanoparticle size, and morphology.^[17-21]

Cu_{2-x}S NCs have been reported to induce the generation of hydroxyl radicals, which can be exploited to kill cancer cells and bacteria.^[17] We used hydroxyphenyl fluorescein (HPF) as a fluorescent indicator of the generation of hydroxyl radicals (Figure 2e). Cu_{2-x}S NC solution

after laser irradiation displayed stronger fluorescence intensity than the control (pure HPF solution incubated at room temperature). As laser irradiation also heats the solution, we also investigated the effect of pure heating on the fluorescence intensity. The temperature chosen for heating in a water bath was the highest temperature achieved during laser irradiation (Figure 2d). The fluorescence intensity after water bath heating was lower than the laser illuminated samples (Figure 2e), indicating that the laser-induced generation of hydroxyl radicals is not only due to thermal effect. It has been reported that hydroxyl radicals are generated by copper ions released from the Cu_{2-x}S NCs.^[17] Inductively coupled plasma mass spectrometry (ICP-MS) measurements revealed that the concentration of released copper ions after laser irradiation was around two times higher than that after pure heating (Figure S2, Supporting Information).

2.2. Binding of Cu_{2-x}S NCs to Bacteria

Next, we tested the binding of $\text{Gal}_3\text{-Cu}_{2-x}\text{S}$ and $\text{Glc}_3\text{-Cu}_{2-x}\text{S}$ NCs to *P. aeruginosa*. Two strains of *P. aeruginosa*, wild-type PAO1 and LecA-deficient mutants PW5313, were chosen for comparison. Dark-field microscopy was used to visualize the binding between the nanoparticles and bacteria (**Figure 3a-e**) by taking advantage of the scattering of blue light by the Cu_{2-x}S NCs. The bacterial solutions were incubated with $80\ \mu\text{g mL}^{-1}$ $\text{Gal}_3\text{-Cu}_{2-x}\text{S}$ or $\text{Glc}_3\text{-Cu}_{2-x}\text{S}$ NCs for 1 h, followed by removal of free Cu_{2-x}S NCs. $\text{Gal}_3\text{-Cu}_{2-x}\text{S}$ treated PAO1 bacteria show blue halos surrounding the bacterial cells (Figure 3a), suggestive of the binding of around the bacteria. The binding of the NCs to PAO1 was obstructed when the bacteria were first incubated with 1 mM $\text{Gal}_3\text{-COOH}$ molecules for 1 h, which would block the binding sites (Figure 3b), clearly proving that the NCs bound to the bacteria via the Gal_3 cluster. Moreover, when the bacteria were first labeled by the $\text{Gal}_3\text{-Cu}_{2-x}\text{S}$ NCs and then incubated in 1 mM $\text{Gal}_3\text{-COOH}$ solution for 1 h, the bound NCs were found to be washed away after centrifugation (Figure 3c), indicating that the NCs bound only to the surface of the

bacteria and were not internalized. There was no apparent binding of Gal₃-Cu_{2-x}S toward the LecA-deficient PW5313 bacteria, evidenced by the white contours originating from the light scattered by the bacterial cells (Figure 3d). Similarly, the binding capability of the Cu_{2-x}S NCs was lost after replacing the galactose moieties with glucose (Figure 3e).

These results are consistent with the quantitative data obtained by directly measuring the amount of Cu_{2-x}S bound to bacteria (Figure 3f). After the unbound Cu_{2-x}S NCs were removed by centrifugal filtration, the absorbance values at 1100 nm of the bacterial solutions were measured with UV-vis-NIR spectroscopy. The absorbance was measured at 1100 nm because the Cu_{2-x}S NCs show strong light absorption and the bacteria exhibit weak light scattering at this wavelength. As shown in Figure 3f, the amount of bound Gal₃-Cu_{2-x}S on PAO1 increases with increased feed concentration of Gal₃-Cu_{2-x}S with a roughly linear relationship. At the feed concentration of 80 µg mL⁻¹, around 3.5 µg Gal₃-Cu_{2-x}S NCs were attached to PAO1 (10⁷ CFU), while only 0.4 µg NCs were found to bind to PW5313 bacteria (10⁷ CFU). The amount of Glc₃-Cu_{2-x}S NCs on PAO1 was negligible.

It has been reported that there are mainly three types of lectins on the surface of *P. aeruginosa*, galactose-specific LecA, fucose/mannose-specific LecB, and galactose-specific T4P.^[45-47] Our results indicate that the primary target of Gal₃-Cu_{2-x}S NCs is LecA rather than T4P since there was no apparent binding between Gal₃-Cu_{2-x}S and the LecA-deficient mutants. Gal₃-Cu_{2-x}S NCs do not favor T4P lectins, most probably due to the strong hydrophobicity of the T4P lectins.^[45] The glucose control, Glc₃-Cu_{2-x}S NCs, showed almost no binding toward PAO1 because there are no glucose-specific lectins on the outer membrane of *P. aeruginosa*. Notably, although the Glc₃-Cu_{2-x}S NCs possess a weak positive charge (zeta potential: +10 mV), there was no significant nonspecific adsorption of positively charged nanoparticles on the negatively charged bacteria surface.

2.3. Photokilling of Bacteria by Gal₃-Cu_{2-x}S NCs

We exploited the LecA-binding properties of Gal₃-Cu_{2-x}S NCs for photokilling of *P. aeruginosa*. Free Cu_{2-x}S NCs unbound to bacteria were removed from the bacteria solutions incubated with Gal₃-Cu_{2-x}S, and then the bacterial solutions were transferred to centrifuge tubes for laser illumination (1064 nm, 10 min, 1 W cm⁻²). The bacteria viability was determined by streaking the treated bacteria on LB plates and incubating at 37°C for 12 h for CFU counting. Overall, PAO1 showed a much lower survival rate than PW5313 bacteria in both dark and laser conditions (**Figure 4a,b**). At the highest Gal₃-Cu_{2-x}S concentration, the viability of PAO1 bacteria was 47% in the dark and 0.0862% with laser irradiation, while the viability of PW5313 bacteria was 90% in the dark and 62% with laser irradiation. For both PAO1 and PW5313 bacteria, laser irradiation enhances the antibacterial ability of the Gal₃-Cu_{2-x}S NCs. Remarkably, almost all the PAO1 bacteria were killed after treating with 80 µg mL⁻¹ Gal₃-Cu_{2-x}S and laser irradiation, whereas there were still 47% of PAO bacteria alive at the same experimental condition but without laser irradiation. The killing efficiency of the NCs also depends on the power density of the laser used. At a lower laser power density of 0.5 W cm⁻², more bacterial cells were left alive with the same concentrations of the NCs (**Figure S3**, Supporting Information).

Live/dead staining was used as an alternative method to determine the bacterial viability immediately after laser irradiation. These results (**Figure 4c-f**) agree well with CFU counting, showing a greater extent of killing with laser irradiation than dark conditions and more killing of PAO1 than PW5313. With 80 µg mL⁻¹ Gal₃-Cu_{2-x}S NCs, the bacteria were almost all stained red, suggesting near-complete killing of PAO1 bacteria.

In the meanwhile, the cytotoxicity of the Gal₃-Cu_{2-x}S NCs against human dermal fibroblasts (HDF) was tested (**Figure S4**, Supporting Information) to assess the biocompatibility of the NCs in treating *P. aeruginosa* infections such as those in wounds. No obvious cytotoxicity was observed after incubating the cells with 80 µg mL⁻¹ Gal₃-Cu_{2-x}S

NCs for 24 h, and more than 96% of the cells survived. Laser irradiation (1064 nm, 10 min, 1 W cm⁻²) was also performed after removing unbound NCs, and subsequent viability assay revealed that there were still 87% of the cells survived, possibly due to negligible binding and uptake of the Gal₃-Cu_{2-x}S NCs to the cells.

Both photothermal and photodynamic effects should have contributed to the enhanced killing efficiency of bacteria with laser irradiation. We first assessed the role of the photothermal effect. IR camera imaging of the temperature profiles of the bacterial solutions at the end of laser irradiation (**Figure 5a**) indicates increasing temperature with increasing Gal₃-Cu_{2-x}S feed concentration. The maximum temperature rise for PW5313 solutions was only 9°C, much lower than the 28°C temperature rise of PAO1 solutions. As we used a continuous wave laser with low power intensity (1 W cm⁻²), the solution temperature measured with the IR camera should be very close to the temperature on the surface of the Cu_{2-x}S NCs. The surface temperature, at a conservative estimate, is only 6.6×10⁻⁴ K higher than the ambient solution temperature (see Supporting Information for discussion). The observed solution heating was therefore a result of accumulative heating, and optical intensities around 10⁴–10⁵ W cm⁻² are required to obtain a significant temperature increase on the nanoparticle surface. The temperature pattern shown in Figure 5a agrees very well with that in Figure 4a,b: a higher temperature always corresponds to lower bacteria viability. This correlation, however, is not itself evidence of the killing effect of pure laser heating. The final temperature of the bacterial solution treated with 40 µg mL⁻¹ Gal₃-Cu_{2-x}S was only 40.7°C, which is within the *P. aeruginosa* survival temperature range between 4°C and 42°C. The viability was measured to be 98.7% and 63.4% after pure heating at 40.7°C and 52°C for 10 min, respectively, which is much higher than the viability of 10.3% and 0.0862% after Gal₃-Cu_{2-x}S and laser treatment.

The above discussion indicates that the heat generated by laser irradiation is not sufficient to account for the observed killing of the bacteria, and photodynamic effects of Cu_{2-x}S NCs must have played a critical role. To probe the photodynamic effect of laser irradiated Cu_{2-x}S NCs in bacteria, the fluorogenic dye dichlorofluorescein diacetate (DCFDA) was used as an indicator of ROS generation. DCFDA responds to most types of ROS, but it is most sensitive to hydroxyl radicals. The dye was first incubated with the bacteria for 30 min to be deacetylated by esterases, which renders it fluorescent after oxidation by ROS. Afterward, the bacteria were mixed with Gal₃-Cu_{2-x}S NCs for LecA binding. After removing the unbound Gal₃-Cu_{2-x}S NCs and free dyes in the solution, the samples were irradiated by laser and then fluorescence images were taken (Figure 5b-g). There is a clear trend of increasing fluorescent intensity with increasing Cu_{2-x}S concentration, suggestive of elevated ROS level in POA1 bacteria induced by the nanoparticles with laser irradiation. In contrast, the PW5313 bacteria, with much less Cu_{2-x}S available to produce the hydroxyl radicals, show no detectable fluorescence even with 80 µg mL⁻¹ Gal₃-Cu_{2-x}S NCs. DCFDA staining also reveals that the killing of bacteria by Gal₃-Cu_{2-x}S NCs in dark conditions, as shown in Figure 4c, was also due to the generation of ROS (Figure S5, Supporting Information). The fluorescence intensity, however, is much weaker than that with laser irradiation. This is consistent with the finding that laser irradiation can accelerate the release of copper ions from the Cu_{2-x}S NCs (Figure S2, Supporting Information).

Binding of Gal₃-Cu_{2-x}S NCs on bacteria is especially important in the context of hydroxyl radical mediated killing of bacteria, evidenced by the fact that 80 µg mL⁻¹ Gal₃-Cu_{2-x}S NCs can kill around 50% of PAO bacteria even without laser irradiation, while only around 10% of PW5315 bacteria and 3% of HDF cells were killed at the same concentration of NCs. It should be noted that the overall concentration of copper ions released from the Cu_{2-x}S NCs is in the sub-ppm range in our study (Figure S2, Supporting Information), which

is two orders of magnitude lower than the concentration previously found to effectively kill bacteria or induce cytotoxicity.^[48,49] Only when the NCs are attached on the bacterium surface can they directly release copper ions into the bacteria and reach a high local concentration. Moreover, the lifetime of hydroxyl radicals in water is extremely short, often in the microsecond range,^[50] which indicate that the copper ions have to be in the proximity of the outer membrane or inside the bacteria to generate effective hydroxyl radicals to kill the bacteria.

3. Conclusion

We have shown that galactosylated Cu_{2-x}S NCs specifically bind to *P. aeruginosa* and effectively kill the bacteria with laser irradiation in the NIR II window. The high photothermal conversion efficiency and the ability to generate hydroxyl radicals make the Cu_{2-x}S NCs especially advantageous in the treatment of bacterial infections at a laser power density within the permissible exposure limit for the skin (1 W cm⁻² for a 1064 nm laser).^[52] Using a LecA-deficient strain as the control, we proved that LecA is an effective and specific target for binding and killing of *P. aeruginosa* with galactose-conjugates. Many other bacterial species also show specific interactions with certain carbohydrates, such as *Streptococcus pneumonia* binding to lactose or *N*-acetyl-lactosamine, *Mycobacterium tuberculosis* binding to heparin sulfate, and different strains of *Escherichia coli* expressing receptors for various glycans.^[30] Our approach can be readily generalized to the targeting and killing of these pathogens by integrating the recognition function of carbohydrates and the therapeutic function of nanoparticles.

4. Experimental Section

Synthesis of Glycoclusters: All reactions were performed using oven-dried glassware apparatus. The tri-thiogalactoside and tri-thiogluconoside glycoclusters were prepared from corresponding monovalent sugar thiols via thiol-ene click strategy. Thiol-ene click reactions

were performed using UV light of wavelength 365 nm under argon atmosphere. The glycoclusters were further functionalized with an ethylene glycol linker, and eventually the terminal amine group was converted into carboxylic acid by amine initiated succinic anhydride ring opening reaction. Silica gel (35-70 mesh) purchased from Merck Pte. Ltd. was used to perform column chromatography. ^1H , ^{13}C , and DEPT-135 spectra were recorded using a Bruker AV 300 MHz NMR instrument. In ^1H NMR spectra, solvent residual peaks were set to 4.79 for D_2O , 7.26 for CDCl_3 , and in proton-decoupled ^{13}C NMR spectra, the middle peak for the solvent residual peak of CDCl_3 was set to 77.16. The detailed synthesis procedures are described in the Supporting Information.

Synthesis of Cu_{2-x}S NCs: The copper diethyldithiocarbamate ($\text{Cu}(\text{DEDTC})_2$) precursor was prepared by mixing sodium diethyldithiocarbamate (5 mL, 10 mmol) with CuCl_2 (10 mL, 10 mmol) under magnetic stirring for 1 h. The $\text{Cu}(\text{DEDTC})_2$ precursor was obtained by filtration and dried at room temperature under vacuum. For the preparation of Cu_{2-x}S NCs, 2 mL of a toluene solution of the precursor was injected into a mixture of 2 mL of oleylamine and 8 mL of toluene with magnetic stirring at 120°C . The precursor was injected drop by drop over 5 min, and the mixture was reacted for another 20 min. The nanoparticles were collected by centrifugation and washed twice with ethanol. Finally, the nanoparticles were redispersed in toluene.

Surface Modification of Cu_{2-x}S NCs: The toluene solution of Cu_{2-x}S NCs (0.5 mL, 2 mg mL^{-1}) was mixed with a toluene solution of HS-PEG- NH_2 (5000 Da, 0.5 mL, 2 mg mL^{-1}). After brief sonication and mixing for 30 min, 1 mL of water was added and mixed vigorously until the Cu_{2-x}S NCs transferred to the water phase. The toluene layer was carefully removed, and the precipitates were collected by centrifugation (5000 rpm, 10 min) followed by two rounds of washing with water. Finally, the Cu_{2-x}S NCs were redispersed in 1 mL of water. $\text{Gal}_3\text{-COOH}$ or $\text{Glc}_3\text{-COOH}$ pre-incubated with EDC and NHS for 1h was added into the

Cu_{2-x}S solution. After overnight mixing, the glycosylated nanoparticles were collected by centrifugation, washed twice with water and redispersed in water.

Gel Electrophoresis: Surface modification of Cu_{2-x}S NCs was evaluated by gel electrophoresis (Thermo Scientific EC 300XL) in 0.25% agarose gels using 1X 3-(N-morpholino)propanesulfonic acid (MOPS, pH 7.4) as the running buffer at 100 V for 10 min.

Measurement of Photothermal Conversion Efficiency: The photothermal conversion efficiency of $\text{Gal}_3\text{-Cu}_{2-x}\text{S}$ NCs was determined from the heating-cooling curve, which was obtained by laser heating the $\text{Gal}_3\text{-Cu}_{2-x}\text{S}$ solution to a stable temperature and then switching off the laser to cool the solution to room temperature. The solution (2 mL) was kept in a quartz cuvette. The laser power density was 1 W cm^{-2} .

Characterization of Hydroxyl Radical Generation of Cu_{2-x}S : The copper sulfide nanoparticle solution was first irradiated with a laser, after which HPF was added, and the mixture was incubated for 1 h. The fluorescence spectra of the solutions were measured on a Horiba FluoroMax-3 fluorometer.

Bacteria Culture: Two strains of *P. aeruginosa*, wild-type PAO1 and LecA-deficient PW5313, were cultured in LB media. A single colony of the bacteria was picked and inoculated into 1 mL of LB media for overnight incubation at 37°C . Then 10 μL of the bacterial solution was diluted in 1 mL of LB media, and the bacteria were allowed to grow until the OD reached 1.

Quantification of Cu_{2-x}S Bound to Bacteria: 500 μL of the bacterial solutions in PBS (pH 7.4, with 1 mM CaCl_2) was incubated with Cu_{2-x}S at 37°C for 1h. Note that Ca^{2+} is required for galactose-LecA binding.^[51] Then the mixture was centrifuged three times with a centrifuge tube filter (pore size = 0.45 μm). Absorption values at 1100 nm were measured to determine the amount of Cu_{2-x}S bound to bacteria.

Dark-field Imaging of Bacteria: The bacteria solution treated with Cu_{2-x}S nanocrystals with different surface modification were centrifuged in a filter centrifuge tube (pore size = 0.45 μm), and then 2 μL of the solution was dropped on a glass slide and cover by a coverslip. The images were obtained on an inverted dark-field microscope (Olympus IX71) with a 100 $\times$ oil immersion objective.

Photokilling of P. aeruginosa: 100 μL samples of the bacterial solutions (10^7 CFU mL^{-1}) were treated with different concentrations of Gal₃-Cu_{2-x}S NCs at 37°C for 1h. After washing with the centrifuge tube filters, the samples (100 μL) were transferred to centrifuge tubes and illuminated by a NIR laser (1064 nm, 1 W cm^{-2}) for 10 min. A forward-looking infrared (FLIR) camera (A645sc, FLIR) was used to capture the temperature profiles of the solutions at the end of laser illumination. Then the samples were streaked to LB plates and incubated at 37°C for 16 h for CFU counting.

Live/Dead Staining: After treating the bacteria with 80 $\mu\text{g mL}^{-1}$ Gal₃-Cu_{2-x}S NCs under either dark or laser irradiation conditions, the samples were stained with the LIVE/DEAD BacLight bacterial viability kit for 15 min at room temperature. Fluorescence images were captured on an inverted fluorescence microscope (Olympus IX71) with a 20 $\times$ objective.

Monitoring ROS Generation in Bacteria: DCFDA was incubated with *P. aeruginosa* for 30 min. Then the Gal₃-Cu_{2-x}S solution was added for nanoparticle binding. After washing three times with PBS, the mixture was irradiated by laser for 10 min. Finally, the bacteria were observed at 100 $\times$ magnification with a fluorescence microscope.

Cytotoxicity Assay: HDF cells were harvested from the flask by trypsinization treatment, and the cell concentration was adjusted to 2×10^4 cells mL^{-1} . 200 μL of the cell dispersion was pipetted into a 96-well collagen-treated tissue culture plate to grow for 24 h (37°C, 5% CO₂). Calculated amounts of Gal₃-Cu_{2-x}S stock solution (1 mg mL^{-1}) prepared in DMEM was pipetted into the 96-well plate with seeded cells, which were further incubated for 24 h. Then

the culture media containing the NCs were removed, and the cells were rinsed twice with PBS. Half of the wells were irradiated by laser (1064 nm, 1 W cm⁻²) for 10 min to test the cytotoxicity in the presence of light. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (200 µL, 5mg mL⁻¹) was pipetted into each well, and the plate was incubated in the incubator for an additional 4 h. Then MTT was discarded, and dimethyl sulfoxide (DMSO) was added into each well, and the plate was kept in the shaker (at 150 rpm) until the intracellular formazan crystals were completely solubilized. The absorbance of each well at 570 nm was recorded using a microplate reader (BIO-RAD, Benchmark Plus). The percentage of the cell viability was calculated by the formula:

$$\text{Cell viability} = (\text{Average absorbance of treated cells})/(\text{Average absorbance of controls}) \times 100\%$$

Statistical Analysis: All data are presented as the mean $\pm$ standard deviation (SD). All tests were performed three times (n = 3) unless otherwise stated.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgments

S.H. and S.H.M. contributed equally to this work. This work was funded and supported by Ministry of Education-Singapore (MOE2013-T3-1-002 and RG49/16), and a Singapore MOH Industry Alignment Fund (NMRC/MOHIAFCAT2/003/2014). The *P. aeruginosa* strain PW5313 was kindly provided by Prof. Liang Yang.

Conflict of Interest

The authors declare no conflict of interest.

Received: ((will be filled in by the editorial staff))

Revised: ((will be filled in by the editorial staff))

Published online: ((will be filled in by the editorial staff))

References

- [1] P. W. Taylor, P. D. Stapleton, J. P. Luzio, *Drug Discovery Today* **2002**, 7, 1086.
- [2] A. R. Coates, G. Halls, Y. Hu, *Br. J. Pharmacol.* **2011**, 163, 184.
- [3] P. C. Ray, S. A. Khan, A. K. Singh, D. Senapati, Z. Fan, *Chem. Soc. Rev.* **2012**, 41, 3193.

- [4] M. C. Wu, A. R. Deokar, J. H. Liao, P. Y. Shih, Y. C. Ling, *ACS Nano* **2013**, 7, 1281.
- [5] B. Hu, L. P. Zhang, X. W. Chen, J. H. Wang, *Nanoscale* **2013**, 5, 246.
- [6] S. H. Kim, E. B. Kang, C. J. Jeong, S. M. Sharker, I. In, S. Y. Park, *ACS Appl. Mater. Interfaces* **2015**, 7, 15600.
- [7] N. N. M. Adnan, Y. Y. Cheng, N. M. N. Ong, T. T. Kamaruddin, E. Rozlan, T. W. Schmidt, H. T. T. Duong, C. Boyer, *Polym. Chem.* **2016**, 7, 2888.
- [8] M. Gao, Q. Hu, G. Feng, N. Tomczak, R. Liu, B. Xing, B. Z. Tang, B. Liu, *Adv. Healthcare Mater.* **2015**, 4, 659.
- [9] X. Yu, S. Wang, X. Zhang, A. Qi, X. Qiao, Z. Liu, M. Wu, L. Li, Z. L. Wang, *Nano Energy* **2018**, 46, 29.
- [10] B. Z. Ristic, M. M. Milenkovic, I. R. Dakic, B. M. Todorovic-Markovic, M. S. Milosavljevic, M. D. Budimir, V. G. Paunovic, M. D. Dramicanin, Z. M. Markovic, V. S. Trajkovic, *Biomaterials* **2014**, 35, 4428.
- [11] X. Dai, Y. Zhao, Y. Yu, X. Chen, X. Wei, X. Zhang, C. Li, *ACS Appl. Mater. Interfaces* **2017**, 9, 30470.
- [12] L. Li, Y. Liu, P. Hao, Z. Wang, L. Fu, Z. Ma, J. Zhou, *Biomaterials* **2015**, 41, 132.
- [13] M. Wainwright, T. Maisch, S. Nonell, K. Plaetzer, A. Almeida, G. P. Tegos, M. R. Hamblin, *Lancet Infect. Dis.* **2017**, 17, e49.
- [14] T. Maisch, *Photochem. Photobiol. Sci.* **2015**, 14, 1518.
- [15] J. Zhou, Y. Jiang, S. Hou, P. K. Upputuri, D. Wu, J. Li, P. Wang, X. Zhen, M. Pramanik, K. Pu, H. Duan, *ACS Nano* **2018**, 12, 2643.
- [16] Q. Chen, J. Chen, Z. Yang, L. Zhang, Z. Dong, Z. Liu, *Nano Res.* **2018**, 11, 5657.
- [17] S. Wang, A. Riedinger, H. Li, C. Fu, H. Liu, L. Li, T. Liu, L. Tan, M. J. Barthel, G. Pugliese, F. De Donato, M. S. D'Abbusco, X. Meng, L. Manna, H. Meng, T. Pellegrino, *ACS Nano* **2015**, 9, 1788.

- [18] Q. Tian, F. Jiang, R. Zou, Q. Liu, Z. Chen, M. Zhu, S. Yang, J. L. Wang, J. H. Wang, J. Hu, *ACS Nano* **2011**, 5, 9761.
- [19] Z. Liu, X. Liu, Y. Du, J. Ren, X. Qu, *ACS Nano* **2015**, 9, 10335.
- [20] L. Li, L. H. Rashidi, M. Yao, L. Ma, L. Chen, J. Zhang, Y. Zhang, W. Chen, *Photodiagn. Photodyn. Ther.* **2017**, 19, 5.
- [21] Q. Tian, J. Hu, Y. Zhu, R. Zou, Z. Chen, S. Yang, R. Li, Q. Su, Y. Han, X. Liu, *J. Am. Chem. Soc.* **2013**, 135, 8571.
- [22] Y. Liu, M. Liu, M. T. Swihart, *J. Phys. Chem. C* **2017**, 121, 13435.
- [23] J. Chen, S. M. Andler, J. M. Goddard, S. R. Nugen, V. M. Rotello, *Chem. Soc. Rev.* **2017**, 46, 1272.
- [24] R. S. Norman, J. W. Stone, A. Gole, C. J. Murphy, T. L. Sabo-Attwood, *Nano Lett.* **2008**, 8, 302.
- [25] Y. S. Kim, J. Chung, M. Y. Song, J. Jurng, B. C. Kim, *Biosens. Bioelectron.* **2014**, 54, 195.
- [26] A. J. Kell, G. Stewart, S. Ryan, R. Peytavi, M. Boissinot, A. Huletsky, M. G. Bergeron, B. Simard, *ACS Nano* **2008**, 2, 1777.
- [27] Z. Qi, P. Bharate, C. H. Lai, B. Ziem, C. Böttcher, A. Schulz, F. Beckert, B. Hatting, R. Mülhaupt, P. H. Seeberger, R. Haag, *Nano Lett.* **2015**, 15, 6051.
- [28] S. H. Mahadevegowda, S. Hou, J. Ma, D. Keogh, J. Zhang, A. Mallick, X. W. Liu, H. Duan, M. B. Chan-Park, *Biomater. Sci.* **2018**, 6, 1339.
- [29] A. M. Eissa, A. Abdulkarim, G. J. Sharples, N. R. Cameron, *Biomacromolecules* **2016**, 17, 2672.
- [30] A. Varki, R. D. Cummings, J. D. Esko, H. H. Freeze, P. Stanley, C. R. Bertozzi, G. W. Hart, M. E. Etzler, *Essentials of Glycobiology*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA **2008**.
- [31] N. Sharon, *FEBS Lett.* **1987**, 217, 145.

- [32] C. Chemani, A. Imberty, S. de Bentzmann, M. Pierre, M. Wimmerová, B. P. Guery, K. Faure, *Infect. Immun.* **2009**, *77*, 2065.
- [33] S. P. Diggle, R. E. Stacey, C. Dodd, M. Cámara, P. Williams, K. Winzer, *Environ. Microbiol.* **2006**, *8*, 1095.
- [34] C. Müller, G. Despras, T. K. Lindhorst, *Chem. Soc. Rev.* **2016**, *45*, 3275.
- [35] J. J. Lundquist, E. J. Toone, *Chem. Rev.* **2002**, *102*, 555.
- [36] R. U. Kadam, M. Bergmann, M. Hurley, D. Garg, M. Cacciarini, M. A. Swiderska, C. Nativi, M. Sattler, A. R. Smyth, P. Williams, M. Cámara, A. Stocker, T. Darbre, J.-L. Reymond, *Angew. Chem., Int. Ed.* **2011**, *50*, 10631.
- [37] C. Ligeour, O. Vidal, L. Dupin, F. Casoni, E. Gillon, A. Meyer, S. Vidal, G. Vergoten, J.-M. Lacroix, E. Souteyrand, A. Imberty, J.-J. Vasseur, Y. Chevolot, F. Morvan, *Org. Biomol. Chem.* **2015**, *13*, 8433.
- [38] M. Bergmann, G. Michaud, R. Visini, X. Jin, E. Gillon, A. Stocker, A. Imberty, T. Darbre, J. L. Reymond, *Org. Biomol. Chem.* **2016**, *14*, 138.
- [39] A. Barras, F. A. Martin, O. Bande, J. S. Baumann, J. M. Ghigo, R. Boukherroub, C. Beloin, A. Siriwardena, S. Szunerits, *Nanoscale* **2013**, *5*, 2307.
- [40] M. Khanal, F. Larssonneur, V. Raks, A. Barras, J. S. Baumann, F. A. Martin, R. Boukherroub, J. M. Ghigo, C. O. Mellet, V. Zaitsev, J. M. Garcia Fernandez, C. Beloin, A. Siriwardena, S. Szunerits, *Nanoscale* **2015**, *7*, 2325.
- [41] K. El-Boubbou, C. Gruden, X. Huang, *J. Am. Chem. Soc.* **2007**, *129*, 13392.
- [42] A. Novoa, T. Eierhoff, J. Topin, A. Varrot, S. Barluenga, A. Imberty, W. Römer, N. Winssinger, *Angew. Chem., Int. Ed.* **2014**, *53*, 8885.
- [43] N. Garber, U. Guempel, A. Belz, N. Gilboa-Garber, R. J. Doyle, *Biochim. Biophys. Acta* **1992**, *1116*, 331.
- [44] F. Zutton, C. Ligeour, O. Vidal, M. Wälte, F. Morvan, S. Vidal, Y. Chevolot, M. Phaner-Goutorbe, H. Schillers, *Nanoscale* **2018**, *10*, 12771.

- [45] N. C. Worstell, A. Singla, P. Saenkham, T. Galbadage, P. Sule, D. Lee, A. Mohr, J. S. I. Kwon, J. D. Cirillo, H. J. Wu, *Sci. Rep.* **2018**, 8, 8419.
- [46] J. C. Comolli, L. L. Waite, K. E. Mostov, J. N. Engel, *Infect. Immun.* **1999**, 67, 3207.
- [47] L. Saiman, A. J. Prince, *J. Clin. Invest.* **1993**, 92, 1875.
- [48] C. Gunawan, W. Y. Teoh, C. P. Marquis, R. Amal, *ACS Nano* **2011**, 5, 7214.
- [49] M. C. Cortizo, M. F. L. de Mele, *Biol. Trace Elem. Res.* **2004**, 102, 129.
- [50] P. Attri, Y. H. Kim, D. H. Park, J. H. Park, Y. J. Hong, H. S. Uhm, K. N. Kim, A. Fridman, E. H. Choi, *Sci. Rep.* **2015**, 5, 9332.
- [51] G. Cioci, E. P. Mitchell, C. Gautier, M. Wimmerova, D. Sudakevitz, S. Pérez, N. Gilboa-Garber, A. Imberty, *FEBS Lett.* **2003**, 555, 297.
- [52] ANSI, *American National Standard for Safe Use of Lasers*, Laser Institute of America, Orlando, FL, USA **2007**.

Figure 1. Schematic illustration of the surface modification process for the Cu_{2-x}S NCs and the molecular structure of the tri-thiogalactoside cluster $\text{Gal}_3\text{-COOH}$. The tri-thiogluco-side cluster $\text{Glc}_3\text{-COOH}$ has the same structure except that the galactose moieties are replaced by glucose.

Figure 2. Characterizations of the Gal₃-Cu_{2-x}S NCs. a) TEM image. b) Gel mobility of the PEGylated Cu_{2-x}S NCs (control), Gal₃-Cu_{2-x}S NCs (no treatment, incubated with 50 nM LecA for 30 min, and incubated with 50 nM ConA for 30 min), and Glc₃-Cu_{2-x}S NCs (no treatment, incubated with 50 nM LecA for 30 min, and incubated with 50 nM ConA for 30 min). c) Absorption spectrum of 20 µg mL⁻¹ Gal₃-Cu_{2-x}S NCs. d) Temperature evolution during laser irradiation (1064 nm, 1 W cm⁻²) for the first 20 min and subsequent cooling. Two concentrations of Gal₃-Cu_{2-x}S NCs (40 µg mL⁻¹ and 20 µg mL⁻¹) were tested with pure water as the control. e) Fluorescence intensity of the hydroxyl radical indicator HPF treated with different concentrations of Gal₃-Cu_{2-x}S at three conditions: laser irradiation (1 W cm⁻²), heating in a water bath, and incubation at room temperature. The control is pure HPF solution (10 µM) incubated at room temperature for 1 h. Data are presented as the means ± SD.

Figure 3. Binding test of the $\text{Gal}_3\text{-Cu}_{2-x}\text{S}$ and $\text{Glc}_3\text{-Cu}_{2-x}\text{S}$ NCs with two *P. aeruginosa* strains, wild-type PAO1 and LecA-deficient strain PW5313. a-e) Dark-field images of PAO1 treated with $\text{Gal}_3\text{-Cu}_{2-x}\text{S}$ (a), PAO1 treated first by 1 mM $\text{Gal}_3\text{-COOH}$ and then by $\text{Gal}_3\text{-Cu}_{2-x}\text{S}$ (b), PAO1 treated first by $\text{Gal}_3\text{-Cu}_{2-x}\text{S}$ and then by 1 mM $\text{Gal}_3\text{-COOH}$ (c), PW5313 treated with $\text{Gal}_3\text{-Cu}_{2-x}\text{S}$ (d), and PAO1 treated with $\text{Glc}_3\text{-Cu}_{2-x}\text{S}$ (e). The nanoparticle concentration for the imaging experiments was $80 \mu\text{g mL}^{-1}$. f) The amount of bound Cu_{2-x}S on bacteria with different feed concentrations of the nanoparticles. Data are presented as the means $\pm$ SD.

Figure 4. Killing of *P. aeruginosa* (PAO1 and PW5313). a,b) The bacterial viability of PAO1 (a) and PW5313 (b) treated with increasing concentrations of Gal₃-Cu_{2-x}S in the dark or with laser irradiation (1064 nm, 1 W cm⁻², 10 min). Data are presented as the means ± SD, n = 5. c-f) Fluorescence images of PAO1 and PW5313 treated with 80 μg mL⁻¹ Gal₃-Cu_{2-x}S, laser irradiated for 10 min, and then stained with propidium iodide (PI) and SYTO 9.

Figure 5. Photothermal and photodynamic effects. a) Temperature profiles of the bacterial solution after laser irradiation (1064 nm, 1 W cm⁻²) for 10 min. b-g) Fluorescence images of bacteria incubated with 10 μM DCFDA for 30 min, treated with increasing concentrations of $\text{Gal}_3\text{-Cu}_{2-x}\text{S}$, and then laser irradiated for 10 min.

Galatosylated copper sulfide nanocrystals target the LecA proteins of *P. aeruginosa* and kill the bacteria by simultaneous photothermal and photodynamic therapies. The combined targeting property of carbohydrates and the therapeutic function of the nanocrystals provide a promising platform for targeted killing of pathogens.

Keyword: Glycoconjugates

Shuai Hou, Surendra H. Mahadevegowda, Van Cuong Mai, Mary B. Chan-Park,* Hongwei Duan*

Glycosylated Copper Sulfide Nanocrystals for Targeted Photokilling of Bacteria in the Near-Infrared II Window

ToC figure

Supporting Information

Glycosylated Copper Sulfide Nanocrystals for Targeted Photokilling of Bacteria in the Near-Infrared II Window

Shuai Hou, Surendra H. Mahadevegowda, Van Cuong Mai, Mary B. Chan-Park,* and Hongwei Duan*

1. Evaluation of the photothermal conversion efficiency

Conservation of energy in the laser heating system can be described as

$$\sum_i m_i c_{p,i} \frac{dT}{dt} = Q_{\text{NC}} + Q_{\text{sys}} - Q_{\text{diss}} \quad (1)$$

where m and c_p are the mass and specific heat capacity of the system components, including the solution (2.00 g) and the quartz cuvette (5.66 g). T is the solution temperature, Q_{NC} is the energy input from Cu_{2-x}S NCs, Q_{sys} is the energy input from the cuvette containing a solvent in the absence of Cu_{2-x}S , and Q_{diss} is heat dissipation from the system to the surroundings.

The dissipated heat, Q_{diss} , is proportional to the temperature difference between the solution/cuvette and the surrounding environment (T_{surr}):

$$Q_{\text{diss}} = hS(T - T_{\text{surr}}) \quad (2)$$

where h is the heat transfer coefficient, and S is the exposed surface area of the cuvette.

When the temperature of Cu_{2-x}S NC solution reaches an equilibrium ($T_{\text{max,NC}}$), the energy input is equal to the energy dissipated:

$$Q_{\text{NP}} + Q_{\text{sys}} = Q_{\text{diss}} = hS(T_{\text{max,NC}} - T_{\text{surr}}) \quad (3)$$

Similarly, when pure water is heated at the same condition to equilibrium, we have

$$Q_{\text{sys}} = Q_{\text{diss}} = hS(T_{\text{max,H}_2\text{O}} - T_{\text{surr}}) \quad (4)$$

Combining Equations (3) and (4) would give

$$Q_{\text{NC}} = hS(T_{\text{max}} - T_{\text{max,H}_2\text{O}}) \quad (5)$$

Meanwhile, the heat adsorbed by the Cu_{2-x}S NCs, Q_{NC} , can also be expressed as

$$Q_{\text{NC}} = I(1 - 10^{-A})\eta \quad (6)$$

Comparing Equations (5) and (6), the photothermal efficiency η can be calculated by

$$\eta = \frac{hS(T_{\text{max,NC}} - T_{\text{max,H}_2\text{O}})}{I(1 - 10^{-A})} \quad (7)$$

where I is incident laser power in W, A is the absorbance of Cu_{2-x}S at 1064 nm.

The only unknown parameter in Equation (7) is hS , which can be obtained by considering the cooling process of the NC solution. When the light is turned off, the heat input terms become zero, and Equation (1) becomes

$$\sum_i m_i c_{p,i} \frac{dT}{dt} = -Q_{\text{diss}} = -hS(T - T_{\text{surr}}) \quad (8)$$

Rearranging Equation (8) would give

$$t = -\frac{\sum_i m_i c_{p,i}}{hS} \ln \frac{T - T_{\text{surr}}}{T_{\text{max,NC}} - T_{\text{surr}}} \quad (9)$$

Equation (9) is in the form of $t = -\tau_s \ln \theta$. By linear regression of the t vs. θ curve, hS can be determined.

2. A comparison between surface temperature of the Cu_{2-x}S nanocrystals and ambient solution temperature during laser irradiation

The localized increase in temperature around a Cu_{2-x}S nanocrystal depends on its absorption cross section, laser intensity, size of the nanoparticle, and thermal conductivities of both the nanoparticle and surrounding medium (*ACS Nano* **2010**, 4, 709-716).

The temperature increase on the surface of an individual nanoparticle in aqueous solution is:

$$\Delta T = \frac{\sigma_{\text{abs}} I}{4\pi R_{\text{eq}} \beta \kappa_{\text{water}}} \quad (10)$$

where σ_{abs} is the absorption cross section (m^2), I is the intensity of the incident light (W m^{-2}), R_{eq} is the equivalent radius of the particle, β is the thermal capacitance coefficient dependent on nanoparticle aspect ratio (AR) ($\beta = 1 + 0.96587 (\ln^2(AR))$) and κ_{water} is the thermal conductivity of water ($\kappa_{\text{water}} = 0.6 \text{ W m}^{-1} \text{ K}^{-1}$). For simplicity, the Cu_{2-x}S NC is taken as a 40 nm sphere, hence $\beta = 1$ and $R_{\text{eq}} = 20$ nm. The power intensity used in our experiments is $I = 1 \text{ W cm}^{-2}$, and an estimation of the absorption cross section for the Cu_{2-x}S nanocrystal is $\sigma_{\text{abs}} \approx 1 \times 10^{-14} \text{ m}^2$. Based on these parameters, we calculate theoretical temperature increases of $\Delta T = 6.6 \times 10^{-4} \text{ K}$ at the nanoparticle surface. These small increases in temperature on the surface of the nanoparticles are primarily a result of the low CW optical intensities used in these experiments (1 W cm^{-2}). To obtain a significant temperature increase on the nanoparticle surface, optical intensities would be required to be $\sim 10^4 - 10^5 \text{ W cm}^{-2}$, requiring pulsed laser sources. The observed solution heating must therefore be a result of accumulative heating, in agreement with similar analyses by Govorov et al. (*Nano Lett.* **2009**, 9, 1139-1146).

Figure S1. Size distribution of Cu_{2-x}S NCs determined from 67 particles in TEM images.

Figure S2. Concentration of copper ions released from Cu_{2-x}S NCs after different treatments (water bath heating and laser irradiation). Data are presented as the means $\pm$ SD.

Figure S3. The bacterial viability of PAO1 treated with increasing concentrations of Gal₃-Cu_{2-x}S and laser irradiation with different power densities. Data are presented as the means $\pm$ SD.

Figure S4. Viability of HDF cells after incubation with different concentrations of Gal₃-Cu_{2-x}S NCs for 24 h. Laser irradiation was performed after washing the incubated cells. Data are presented as the means $\pm$ SD.

Figure S5. Fluorescence images of PW5315 (a) and PAO1 (b) stained with 10 μ M DCFDA and treated with 80 μ g mL⁻¹ Gal₃-Cu_{2-x}S NCs without laser irradiation.

Scheme S1. Synthesis of tri-thiogalactoside cluster amine **3 from tri-O-allyl derivative¹ **1**:**

A solution of **1** (3.10 g, 9.07 mmol), galactoside thiol² **2a** (20.0 g, 54.89 mmol), 2,2-dimethoxy-2-phenylacetophenone (1.17 g, 4.54 mmol) in anhydrous MeOH (60 mL) was bubbled with argon for 40 mins. Then the reaction mixture was stirred and exposed to UV light of wavelength 365 nm for 8 h (¹H NMR for crude reaction mixture shows complete conversion of **1**). Then volatiles of reaction mixture were evaporated by rotary evaporator and the resultant mixture was dried for 2 h under vacuum. The obtained crude reaction mass was dissolved in anhydrous CH_2Cl_2 (100 mL) and TFA (24 mL) was added dropwise at 0 °C, and then reaction mixture was warmed to room temperature. After stirring the reaction mixture for 6 h, the volatiles were evaporated by vacuum and crude was purified over silica gel column with eluent 50% EtOAc in hexane (v/v) to 50% MeOH in EtOAc (v/v) to afford Boc deprotected TFA salt (12.49 g, 8.62 mmol, overall yield = 95% from compound **1**). The obtained TFA salt (4.8 g, 3.31 mmol) was dissolved in EtOAc (350 mL), washed with 5% aqueous NaHCO_3 (3x100 mL), washed once with brine solution (130 mL), organic layer was separated, dried with anhydrous Na_2SO_4 and volatiles were evaporated under reduced pressure to afford tri-thiogalactoside cluster amine **3** (3.97 g, 2.98 mmol, yield 90%).

¹H NMR (300 MHz, CDCl_3): δ 5.26 (d, J = 3.1 Hz, 3H), 5.04 (t, J = 9.9 Hz, 3H), 4.90 (dd, J = 10.0, 3.3 Hz, 3H), 4.37 (d, J = 9.9 Hz, 3H), 3.90-4.01 (m, 6H), 3.82 (t, J = 6.5 Hz, 3H), 3.34 (t, J = 5.8 Hz, 6H), 3.16 (s, 6H), 2.76 (s, 2H), 2.52-2.69 (m, 6H), 1.99 (s, 9H), 1.90 (s, 9H), 1.87 (s, 9H), 1.81 (s, 9H), 1.66-1.76 (m, 6H).

¹³C NMR (75 MHz, CDCl_3): δ 170.01, 169.92, 169.70, 169.22, 83.95, 74.03, 72.28, 71.55, 69.29, 67.05, 66.99, 61.18, 55.90, 29.61, 26.91, 20.54, 20.39, 20.39, 20.30.

HRMS (ESI): m/z calcd for $\text{C}_{55}\text{H}_{84}\text{NO}_{30}\text{S}_3$ $[\text{M}+\text{H}]^+$ 1334.4240, found 1334.4186.

Scheme S2. Synthesis of tri-thiogalactoside cluster 5 from amine 3: To a stirred solution of acid **4** (1.039 g, 2.697 mmol) in anhydrous CH_2Cl_2 (20 mL), was added EDC.HCl (1.13 g, 5.931 mmol) and HOBT (838 mg, 6.20 mmol) at 0°C and reaction mixture was stirred for 20 mins. Then a solution of amine **3** (1.80 g in 20 mL of CH_2Cl_2 , 1.348 mmol) was added to reaction mixture and allowed to stir at room temperature. After 3 days, the reaction mixture was diluted with CH_2Cl_2 (250 mL), washed with saturated NaHCO_3 solution (40 mL), and further washed twice with water (2x 150 mL) and brine solution (120 mL). The obtained crude was purified over SiO_2 gel column with eluent 50% EtOAc in hexane (v/v) to 100% EtOAc to afford coupled product **5** (1.60 g, 0.943 mmol, yield = 70%).

^1H NMR (300 MHz, CDCl_3): δ 7.73 (d, $J = 7.4$ Hz, 2H), 7.58 (d, $J = 7.3$ Hz, 2H), 7.25–7.39 (m, 5H), 6.75 (s, 1H), 5.38 (d, $J = 2.5$ Hz, 3H), 5.17 (t, $J = 9.9$ Hz, 3H), 5.02 (dd, $J = 10.0$, 3.1 Hz, 3H), 4.41 (dd, $J = 21.4$, 8.2 Hz, 6H), 4.01–4.21 (m, 6H), 3.87–3.91 (m, 6H), 3.66 (s, 6H), 3.36–3.53 (m, 13H), 2.60–2.77 (m, 6H), 2.11 (s, 9H), 2.02 (s, 9H), 1.99 (s, 9H), 1.94 (s, 9H), 1.72–1.89 (m, 6H).

^{13}C NMR (75 MHz, CDCl_3): δ 170.22, 170.11, 169.92, 169.44, 156.44, 143.86, 141.16, 127.60, 126.96, 124.97, 119.90, 84.23, 74.16, 71.70, 70.80, 70.59, 70.00, 69.41, 68.99, 67.17, 66.42, 61.29, 59.42, 47.12, 40.73, 29.82, 27.13, 20.70, 20.55, 20.55, 20.48.

HRMS (ESI): m/z calcd for $\text{C}_{76}\text{H}_{105}\text{N}_2\text{O}_{35}\text{S}_3$ $[\text{M}+\text{H}]^+$ 1701.5660, found 1701.5740.

Scheme S3. Synthesis of (Gal)₃-COOH from compound 5: To a stirred solution of galactoside cluster **5** (2.25 g, 1.32 mmol) in anhydrous CH₂Cl₂ (40 mL), was added piperidine (2.2 mL) at RT and reaction mixture was stirred for 18 h. Then volatiles were evaporated under reduced pressure, toluene was added to the crude and then volatiles were co-evaporated under reduced pressure, dried under vacuum and the obtained crude amine was purified over SiO₂ column with eluent 0.1% Et₃N in 1:1 Hexane in EtOAc (v/v) to 0.1% Et₃N in 20% MeOH in EtOAc (v/v) and the obtained amine was used for the next step. The obtained amine (1.50 g, 1.01 mmol) was dissolved in anhydrous dioxane (20 mL) and a solution of succinic anhydride (330 mg, 3.30 mmol in 20 mL) was added dropwise and heated at 80 °C for 6 h. Then reaction mixture was brought to RT and volatiles were evaporated under reduced pressure. The obtained crude was purified over SiO₂ column with eluent 100% EtOAc to 20% MeOH in EtOAc (v/v) to afford corresponding carboxylic acid (1.10 g, 0.696 mmol, confirmed by ¹H NMR spectra, overall yield = 68%). The obtained carboxylic acid (1.0 g, 0.633 mmol) was dissolved in anhydrous MeOH (20 mL), to this stirred solution was added methanolic solution of 1M NaOMe (8 mL) dropwise at RT. After stirring for 3 days, acidified to pH ≈ 4 with Dowex® 50WX8 hydrogen form, filtered to remove the resin, volatiles were evaporated under reduced pressure and dried under vacuum to afford **Gal₃-COOH** (558 mg, 0.519 mmol, yield = 82%).

¹H NMR (300 MHz, D₂O): δ 7.25-8.02 (m, -CO-NH-), 4.44 (d, *J* = 9.5 Hz, 3H), 4.06 (br s, 3H), 3.96 (d, *J* = 3.0 Hz, 3H), 3.48-3.80 (m, 42H), 3.36-3.41 (m, 2H), 3.14 (m, 2H), 2.52-2.87 (m, 10H), 1.91 (br t, *J* = 6.3 Hz, 6H).

¹³C NMR (75 MHz, D₂O) δ 177.08, 174.85, 171.90, 86.01, 78.86, 73.94, 70.41, 69.99, 69.71, 69.65, 69.33, 68.90, 68.81, 68.74, 68.39, 61.00, 60.02, 38.91, 30.28, 29.23, 28.87, 26.80.

HRMS (ESI): *m/z* calcd for C₄₁H₇₅N₂O₂₄S₃ [M+H]⁺ 1075.3872, found 1075.3871.

Scheme S4. Synthesis of tri-thioglucoside cluster **6** from compound **1**:

A solution of **1** (1.11 g, 3.25 mmol), glucoside thiol² **2b** (7.10 g, 19.50 mmol), 2,2-dimethoxy-2-phenylacetophenone (417 mg, 1.625 mmol) in anhydrous MeOH (25 mL) was bubbled with argon for 40 mins. Then reaction mixture was stirred and exposed to UV light of wavelength 365 nm for 6 h (¹H NMR for crude reaction mixture shows complete conversion of **1**). The volatiles of reaction mixture were evaporated by rotary evaporator and resultant mixture was dried for 2 h under vacuum. The obtained crude was dissolved in anhydrous CH_2Cl_2 (60 mL) and TFA (10 mL) was added dropwise at 0 °C, and then reaction mixture was warmed to room temperature. After stirring the reaction mixture for 6 h, the volatiles were evaporated by vacuum and the crude was purified over silica gel column with eluent 50% EtOAc in hexane (v/v) to 50% MeOH in EtOAc (v/v) to afford Boc deprotected TFA salt (4.37 g, 3.02 mmol, overall yield = 93% from compound **1**). The obtained TFA salt (2.08 g, 1.436 mmol) was dissolved in EtOAc (250 mL), washed with 5% aqueous NaHCO_3 (3x70 mL), washed once with brine solution (100 mL), organic layer was separated, dried with anhydrous Na_2SO_4 and volatiles were evaporated under reduced pressure to afford tri-thioglucoside cluster amine **6** (1.82 g, 1.364 mmol, yield 95%).

¹H NMR (300 MHz, CDCl_3): δ 5.12 (d, J = 9.3 Hz, 3H), 4.90-5.02 (m, 6H), 4.43 (d, J = 10.0 Hz, 6H), 4.16 (dd, J = 12.4, 4.8 Hz, 3H), 4.04 (dd, J = 12.3, 1.9 Hz, 3H), 3.64 (ddd, J = 17.5, 9.9, 7.6 Hz, 3H), 3.40 (t, J = 5.6 Hz, 3H), 3.22 (s, 6H), 2.54-2.74 (m, 6H), 2.26 (br s, 2H, NH_2), 1.99 (s, 9H), 1.97 (s, 9H), 1.94 (s, 9H), 1.92 (s, 9H), 1.70-1.85 (m, 6H).

¹³C NMR (75 MHz, CDCl_3) δ 170.49, 170.06, 169.34, 169.25, 83.64, 75.72, 73.77, 72.61, 69.76, 69.49, 68.23, 62.06, 56.03, 29.78, 26.94, 20.68, 20.65, 20.53, 20.51.

HRMS (ESI): m/z calcd for $\text{C}_{55}\text{H}_{84}\text{NO}_{30}\text{S}_3$ [$\text{M}+\text{H}$]⁺ 1334.4240, found 1334.4197.

Scheme S5. Synthesis of tri-thioglucoside cluster **7** from compound **6**:

To a stirred solution of acid **4** (1.15 g, 2.983 mmol) in anhydrous CH₂Cl₂ (15 mL), was added EDC.HCl (1.263 g, 6.588 mmol) and HOBT (931 mg, 6.891 mmol) at 0 °C and reaction mixture was stirred for 20 mins. Then a solution of amine **6** (2.0 g in 20 mL CH₂Cl₂, 1.498 mmol) was added to reaction mixture and stirred at room temperature. After 3 days, the reaction mixture was diluted with CH₂Cl₂ (250 mL), washed with saturated NaHCO₃ solution (40 mL), and further washed twice with water (2x 160 mL) and brine solution (130 mL). The obtained crude was purified over SiO₂ gel column with eluent 50% EtOAc in hexane (v/v) to 20% MeOH in EtOAc to afford coupled product **7** (2.20 g, 1.292 mmol, yield = 86%).

¹H NMR (300 MHz, CDCl₃): δ 7.73 (d, *J* = 7.4 Hz, 2H), 7.57 (d, *J* = 7.3 Hz, 2H), 7.36 (t, *J* = 7.3 Hz, 2H), 7.27 (t, *J* = 7.3 Hz, 2H), 6.73 (br s, 1H), 5.17 (d, *J* = 9.3 Hz, 3H), 4.94-5.06 (m, 6H), 4.45 (d, *J* = 10.0 Hz, 3H), 4.37 (d, *J* = 6.8 Hz, 3H), 4.19 (dd, *J* = 12.3, 4.7 Hz, 3H), 4.07 (dd, *J* = 12.3, 2.1 Hz, 3H), 3.93 (d, *J* = 5.2 Hz, 1H), 3.86 (s, 2H), 3.64 (br t, *J* = 11.3 Hz, 12H), 3.52 (br s, 3H), 3.45 (t, *J* = 5.7 Hz, 6H), 3.32-3.37 (m, 2H), 2.58-2.75 (m, 6H), 2.02 (s, 9H), 2.00 (s, 9H), 1.97 (s, 9H), 1.95 (s, 9H), 1.74-1.87 (m, 6H).

¹³C NMR (75 MHz, CDCl₃): δ 170.58, 170.15, 169.43, 169.35, 156.54, 143.99, 141.30, 127.72, 127.07, 125.09, 120.01, 83.77, 75.78, 73.83, 70.97, 70.73, 70.16, 69.89, 69.53, 69.12, 68.30, 66.60, 62.12, 59.54, 47.26, 40.86, 29.92, 27.03, 20.74, 20.73, 20.61, 20.59.

HRMS (ESI): *m/z* calcd for C₇₆H₁₀₅N₂O₃₅S₃ [M+H]⁺ 1701.5660, found 1701.5750.

Scheme S6. Synthesis of (Glc)₃-COOH from compound 7: To a stirred solution of glucoside cluster **7** (2.10 g, 1.23 mmol) in anhydrous CH₂Cl₂ (25 mL), was added piperidine (2.0 mL) at RT and reaction mixture was stirred for 18 h. Then volatiles were evaporated under reduced pressure, toluene was added to the crude and then volatiles were co-evaporated under reduced pressure, dried under vacuum and the obtained crude amine was purified over SiO₂ column with eluent 0.1% Et₃N in 1:1 Hexane in EtOAc (v/v) to 0.1% Et₃N in 20% MeOH in EtOAc (v/v) and the obtained amine was used for the next step. The obtained amine (800 mg, 0.54 mmol) was dissolved in anhydrous dioxane (20 mL) and a solution of succinic anhydride (189 mg, 1.89 mmol in 20 mL) was added dropwise and heated at 80 °C for 9 h. Then reaction mixture was brought to RT and volatiles were evaporated under reduced pressure. The obtained crude was purified over SiO₂ column with eluent 100% EtOAc to 20% MeOH in EtOAc (v/v) to afford corresponding carboxylic acid (560 mg, 0.354 mmol). The obtained carboxylic acid (525 mg, 0.332 mmol) was dissolved in anhydrous MeOH (14 mL), to this stirred solution was added methanolic solution of 1M NaOMe (8 mL) dropwise at RT. After stirring the reaction mixture for 3 days, acidified to pH $\approx$ 4 with Dowex® 50WX8 hydrogen form, filtered to remove the resin, volatiles were evaporated under reduced pressure and dried under vacuum to afford **Glc₃-COOH** (300 mg, 0.279 mmol, yield = 84%).

¹H NMR (300 MHz, D₂O): δ 7.34-7.79 (m, -CO-NH-, 2H), 4.49 (d, J = 9.8 Hz, 3H), 4.00 (s, 3H), 3.15-3.88 (m, manyH), 2.53-2.89 (m, 10H), 1.87-1.93 (m, 6H).

¹³C NMR (75 MHz, D₂O) δ 176.92, 174.10, 171.79, 85.44, 79.84, 77.21, 73.07, 72.29, 70.40, 69.93, 69.75, 69.62, 69.49, 69.31, 68.83, 68.37, 62.46, 60.89, 60.00, 38.97, 29.26, 29.20, 28.72, 26.71.

HRMS (ESI): m/z calcd for C₄₁H₇₅N₂O₂₄S₃ [M+H]⁺ 1075.3872, found 1075.3926.

References:

1. Compound **1** was synthesized by following the literature procedure: M. Segura, F. Sansone, A. Casnati and R. Ungaro, *Synthesis*, 2001, **14**, 2105–2112.
2. The 2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl thiol (**2a**) and 2,3,4,6-tetra-O-acetyl- β -D-glucopyranosyl thiol (**2b**) were synthesized using these literature procedures: (a) Q. V. Vo, C. Trenerry, S. Rochfort and A. B. Hughes, *Tetrahedron*, 2013, **69**, 8731-8737. (b) N. Floyd, B. Vijayakrishnan, J. R. Koeppe and B. G. Davis, *Angew. Chem. Int. Ed.* 2009, **48**, 7798 –7802.

¹H NMR spectrum of compound **3** (CDCl₃, 300 MHz).

¹³C NMR spectrum of compound **3** (CDCl₃, 75 MHz).

DEPT-135 spectrum of compound **3** (CDCl_3 , 75 MHz).

^1H NMR spectrum of compound **5** (CDCl_3 , 300 MHz).

¹³C NMR spectrum of compound **5** (CDCl₃, 75 MHz).

DEPT-135 spectrum of compound **5** (CDCl₃, 75 MHz).

¹H NMR spectrum of compound **(Gal)₃-COOH** (D₂O, 300 MHz).

¹³C NMR spectrum of compound **(Gal)₃-COOH** (D₂O, 75 MHz).

DEPT135 spectrum of compound **(Gal)₃-COOH** (D_2O , 75 MHz).

^1H NMR spectrum of compound **6** ($CDCl_3$, 300 MHz).

¹³C NMR spectrum of compound **6** (CDCl₃, 75 MHz).

DEPT-135 spectrum of compound **6** (CDCl₃, 75 MHz).

¹H NMR spectrum of compound **7** (CDCl₃, 300 MHz)

¹³C NMR spectrum of compound **7** (CDCl₃, 75 MHz).

DEPT-135 spectrum of compound **7** (CDCl₃, 75 MHz).

¹H NMR spectrum of compound **Glc3-COOH** (D₂O, 300 MHz).

¹³C NMR spectrum of compound **Glc₃-COOH** (D₂O, 75 MHz).

DEPT-135 NMR spectrum of compound **(Glc)₃-COOH** (D₂O, 75 MHz).