Highly Stable Bio-templated InP/ZnSe/ZnS Quantum Dots for Specific Monitoring of Bacterial Membrane Proteins

Hanie Yousefi1,2, Laxmi Kishore Sagar3, Armin Geraili1, Dingran Chang1, F. Pelayo García de Arquer3, Connor D. Flynn4, Seungjin Lee3, Edward H. Sargent3, Shana O. Kelley1,2,4,5,6,7

1Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON M5S 3M2, Canada, 2Department of Biomedical Engineering, Northwestern University, 60208 USA, 3The Edward S. Rogers Sr. Department of Electrical and Computer Engineering, University of Toronto, Toronto, ON M5S 3G4, Canada, 4Department of Chemistry, University of Toronto, Toronto, ON M5S 3M2, Canada, 5Department of Chemistry, Northwestern University, Evanston, IL, 60208 USA, 6Department of Biochemistry and Molecular Genetics, Northwestern University, Chicago, IL, 60611 USA 7 Chan Zuckerberg Biohub Chicago, Chicago, Illinois 60607, United States

Abstract:
Despite their unique optical and electrical characteristics, traditional semiconductor quantum dots (QDs) made of heavy metals or carbon are not compatible with many biomedical applications. Cytotoxicity and environmental concerns are key limiting factors that prevent their widescale transition from laboratory research to real-world medical applications. Recently, advanced InP/ZnSe/ZnS QDs have emerged as excellent alternatives to traditional QDs due to their lower toxicity and optical properties; however, they fall short of traditional QDs with respect to their versatility for bioconjugation (i.e., surface chemistry limitations causing unstability in aqueous environments). In this work, we construct a road map for generating, for the first time, highly efficient bio-templated InP/ZnSe/ZnS-aptamers (QDAPT) with long-term stability and high selectivity for applications in targeting bacterial membrane proteins. Our QDAPTs show fast binding reaction kinetics (less than 5 minutes), high brightness, and high shelf-life stability (3 months) after biotemplation in aqueous solvents. We also demonstrate the detection of bacterial membrane proteins on common surfaces using a hand-held imaging device, which attests to the great potential of this system for incorporation into future biomedical technologies.

Introduction:
The development of bio-functional, tunable, and non-toxic nanomaterials has the potential to revolutionize precision medicine (e.g., biomedical imaging, therapeutics, and drug delivery). While zero-dimensional semiconductor quantum dots (QDs) have been explored as biomedical materials for the last two decades due to their unique optical and electrical characteristics, their immediate and downstream safety and material viability have hindered their potential uses in real-world applications. Heavy metals (e.g., Cd, Pb) have been widely used in the synthesis of state-of-the-art QDs and have demonstrated suitability for many applications such as small molecule tracking1. In addition, these materials have been modified and bio-functionalized to be utilized for detecting viruses2 and bacteria3, in vitro cell imaging4,5, and in vivo biomarker analysis6-10. On the other hand, carbon-based QDs are the second largest group of established nanomaterials that have been proposed for various biomedical applications11,12. Although they possess superior photoluminescent properties, heavy-metal and carbon-based QDs disappoint when assessed for toxicity (e.g., cytotoxicity, environmental damage), cost, and
performance\textsuperscript{13, 14}. Because of the dangers of heavy metal exposure and photodegradation-induced cytotoxicity of carbon-based QDs, many concerns have been raised regarding their environmental, human health, and physical property considerations\textsuperscript{14}. InP-based QDs have emerged in recent years as non-toxic alternatives to traditional heavy metal QDs; however, they typically failed to exhibit the same extraordinary characteristics of traditional QDs such as optical properties and stability in aqueous solvents, leaving them undesired for practical applications\textsuperscript{15, 16}. Only recently have InP/ZnSe/ZnS QDs been optimized to exhibit optical properties, such as Photoluminescence Quantum Yield (PLQY) and External Quantum Efficiency (EQE), that near their traditional predecessors; these QDs were constructed by carefully controlling shell composition and thicknesses, ligand exchanges, and passivation strategies\textsuperscript{17-21}, allowing them to compete as promising alternatives and find their way into the fabrication world in electronics and displays\textsuperscript{22, 23}.

However, from a chemistry perspective, InP/ZnSe/ZnS QDs are extremely susceptible to environmental conditions such as surface ligands, humidity, and solvent composition, leading to a loss in their PLQY, solubility, and colloidal properties. Limited options for ligand chemistry and solvent choice lead to a lack of bioconjugation strategies for InP/ZnSe/ZnS QDs that typically require aqueous environments, placing a barrier to their potential uses in biomedical applications. Although several solutions have been proposed for InP QD bioconjugation (e.g., covering the surface with blocking agents like BSA), they limit the functionalization of InP QDs with molecular agents (e.g., DNA, antibodies)\textsuperscript{24}. As InP/ZnSe/ZnS QDs have not yet found their way into biomedical applications, cytotoxicity studies exploring their viability for \textit{in vivo} and \textit{in vitro} applications are limited. However, recent studies closely investigated the cytotoxicity of InP/ZnS or InP/ZnSe QDs and reported their safety in terms of biodistribution and intrinsic toxicity and reported their superior safety over the traditional QDs\textsuperscript{25-27}. Therefore, the utilization of these environmentally-benign, bright QDs in biomedical applications has taken one step closer to reality. Here, we construct a road map for the synthesis and bio-functionalization of InP/ZnSe/ZnS QDs with aptamers, taking advantage of the DNA secondary structure and steric hindrance (induced on large surface area of QDs) to produce colloidal stable bioconjugates that maintain their exceptional photoluminescent properties. We further demonstrate the stability of InP/ZnSe/ZnS/aptamer (QDAPT) constructs in aqueous solvents and ambient conditions for 3 months and also use the dots for detection of bacterial membrane proteins on common surfaces (aluminum and glass) used in healthcare settings such as hospitals. In addition, we show the highly efficient activity of the QDAPT constructs, such as extreme specificity and fast reaction times (less than 5 minutes), that confer InP/ZnSe/ZnS QDs the potential to be used as non-toxic, efficient, and reliable alternatives to traditional QDs. This breakthrough will open doors to many biomedical research avenues and facilitate the transition of these QDs to real-world applications.

Here, we developed water-soluble, environmentally-benign InP/ZnSe/ZnS QDs, carrying peptide-binding aptamer probes, with narrow spectra red emission. The synthesis mechanism for these QDs is shown in Scheme 1. First, indium acetate is mixed with oleic acid and octadecene (ODE) in tris(trimethylsilyl) phosphine (TMS-P) and tri-n-octylphosphine (TOP) at 250 °C for 1 hour to seed the InP core (Scheme 1.a, left). Next, the Zn shell is introduced to the dots with trioctylphosphine selenide (TOP-Se) and Zn at 300 °C for 1 hour to form the InP/ZnSe core/shell (Scheme 1.a, middle). Finally, the dots are transferred to tributylphosphine sulfide (TOP-S) and...
Zn where the second shell formation occurs. The final product – InP/ZnSe/ZnS (core/shell/shell) – is grown until they have reached the red spectra. The dots are then stabilized by oleate ligands and hexane (Scheme 1.a, right).

Many biomedical applications that could employ QDs – in our work, aptamer conjugation and bacterial detection – would require that the dots be highly stable and soluble in aqueous solutions as they must function on the cell membrane interface and undergo aqueous chemical reactions to prepare the probes (e.g., aptamer conjugation)\(^28\). Therefore, we performed optimization experiments to lay out pathways for transferring the dots to water. After investigating possible approaches, we settled on a two-step ligand and solvent exchange to achieve the highest quality, stabilized dots. First, 6-mercapto-1-hexanol (MCH) is used to replace the oleates, after which the dots are precipitated and transferred to ethanol (Scheme 1.b, left). Next, the MCH is replaced with aptamer probes. We hypothesized that by taking advantage of the steric hindrance caused by secondary structure of the aptamers as well as their high negative back-bone charge, the dots would exhibit high stability in aqueous buffers like Phosphate Buffered Saline (PBS) (Scheme 1.b, left). After optimizing the transfer process, we demonstrated detection and differentiation of MRSA on common surfaces such as aluminum and glass using a hand-held UV camera, enhanced with image processing software (Scheme 1.c)
Scheme 1. Schematic of the QDAPT system for bacteria detection. a) Schematic depicting the synthesis of InP/ZnSe/ZnS core/shell/shell quantum dots stabilized by oleate ligands. Red Emitting InP core dots were synthesized with addition of indium acetate in oleic acid and octadecene (ODE) in tris(trimethylsilyl) phosphine (TMS-P) and tri-n-octylphosphine (TOP) at 250 °C for 1 hour. The Zn shell was then introduced to the dots with trioctylphosphine selenide (TOP-Se) and Zn at 300 °C for 1 hour. After the formation of the ZnSe shell, dots were transferred to tributylphosphine sulfide (TOP-S) and Zn for the second shell formation. The dots were grown until they reached the red spectra. The dots were stabilized by oleate ligands. b) Ligand exchange, transfer to polar solvent, and aptamer conjugation. To transfer the dots generated in hexane to a polar solvent, we performed ligand exchange to 6-mercapto-1-hexanol (MCH) and transferred the dots to ethanol. We introduced phosphorothioated aptamer probes in 1% volumetric ratio (99% ethanol, 1% water) and vigorously mixed the solution to allow the aptamers to replace the MCH probes. Taking advantage of the steric hindrance of the aptamers on the surface of the QDs, we transferred the buffer from ethanol to PBS. c) Application of a handheld imaging device and software to monitor bacterial presence on common surfaces.
Results and Discussion:

InP/ZnSe/ZnS quantum dot synthesis, ligand exchanges, and solvent exchanges.
As described previously, we synthesized highly efficient, red-emitting InP/ZnSe/ZnS QDs by tuning their growth to the desired spectrum (red, 630 nm) with a synthesis time of 20 minutes. We measured solid-state optical absorption in order to monitor the excitation photoluminescence (PL) of the dots and capped the dots with oleate ligands dispersed in hexane (Figure 1.a). To further characterize the PL of the final oleate capped dots, we measured red emission in liquid state screening for red excitation at 400 nm emission. Figure 1.b shows the PL maximum value at 628 nm for the QDs/oleate couple. Using transmission electron microscopy (TEM), we looked closely at dot shape after each step of the fabrication process. TEM of QDs/oleates revealed that the InP/ZnSe/ZnS dots were irregular spheres with uniform size (~ 7nm), (Figure 1.c, top). We also measured dispersity and optical PL stability after each step (Figure 1.c bottom). Next, we transferred the dots to polar solvents and explored various ligand exchange options. We first aimed for DMF as the polar solvent and examined the possibility of using benzoic acid and zinc bromide as ligands (Supplementary table 1). Although we achieved successful exchange, we observed issues with dot instability after long-term storage and dot color retention. Next, we selected MCH as a ligand to transfer the dots from hexane to ethanol; using MCH, we successfully changed the ligand, precipitated the dots, and transferred them to ethanol. Figure 1.d demonstrates the post ligand-transfer TEM imaging and dispersity and PL stability (Figure 1.d, bottom). The final product, InP/ZnSe/ZnS QDs, is highly stable and bright in ethanol, with an excitation peak of 630 nm measured in liquid-state (Figure 1.e).
**Figure 1.** Synthesis and characterization of InP/ZnSe/ZnS core/shell/shell quantum dots. a) **Controlling the dots’ size and solid-state PL spectra.** Nanoparticle growth was monitored by measuring their absorption in the red spectra; the shell seeding was stopped by the introduction of oleates. b) **Photoluminescence spectra determination.** The emission spectra of the quantum dots were measured to determine the peak intensity of the dots. c) **TEM and theoretical structure of the QDs with their initial oleate ligand dissolved in non-polar solvent (Hexane).** d) **TEM images of the QDs after ligand and solvent exchange to polar solvent (ethanol).** e) absorption and photoluminescence of the QDs after final ligand exchange in MCH and ethanol.

**QDs-Aptamer conjugation.**

Although the dots were dissolved in ethanol, introduction of any amount of water caused the dots to precipitate and lose their color intensity (Figure 2.a.1). We tested different ratios of water to ethanol to confirm the instability of the dots in aqueous solutions (Supplementary Figure 1). Therefore, we decided to introduce a new ligand (aptamer) before the solvent exchange was complete. Following our previous protocols, we decided to conjugate the QDs with phosphorothioated aptamers(G*), the number of G* groups was previously optimized⁴,²⁹. These
phosphorothioated aptamers would replace the MCH and provide steric hindrance at the QD-liquid interface. Initially, we attempted a one-step approach for simultaneous ligand and solvent exchange (Figure 2a.2); as seen in the image, the addition of the aptamer helped to maintain the dot PL intensity but the colloidal behavior of the QDs changed (coagulated) upon immature introduction of the dots to water (Figure 2.a.2, right). We hypothesized the enhancement in dispersity of the dots in second approach (Figure 2.a.2) compared to first approach (Figure 2.a.1) could be due to the fast replacement reaction of the MCH with aptamers and the steric hindrance of the aptamers helping the QDs to disperse in an aqueous solution. Next, we sought to take advantage of the steric hindrance of the aptamers to help stabilize the dots by breaking the conjugation and buffer exchange process into two steps: 1. Ligand exchange (MCH with aptamer) in ethanol 2. Attempt solvent exchange, and transfer QDs-aptamers from ethanol to PBS (Figure 2.a.3). As seen in Figure 2.a.3, dots were successfully transferred to PBS without loss in PL intensity and dispersity.

Conjugate optimization.
The MCH capped InP/ZnSe/ZnS QDs are expected to have no significant surface charge compared to DNA-capped QDs30. We used these properties to confirm the DNA binding to the QDs by testing the products with agarose gel electrophoresis (0.6% agarose). After adding 100 mL TBE (45 mM Tris-borate, 1 mM EDTA) to 0.6 grams agarose in a flask and swirling it, we heated the solution in a microwave to combine the agarose/buffer mixture. After casting the gel in a gel tray, we added loading dye to the DNA sample. The free QDs– those capped with MCH and with no aptamer– were expected to move slowly in the matrix of the agarose gel (Figure 2.c, right), while the QDAPTs were expected to run on the gel toward the positively charged electrode (Figure 2.c, middle). We then sought to optimize the DNA:QDs ratio that allows all the DNA to be absorbed to the QD and avoid having free unbound DNA. We narrowed down the window for full consumption of DNA to 200-600 nM DNA (where 300nM of DNA suggests full consumption and the free DNA bond disappears on the gel row) (Figure 2.c, middle). Figure 2c, bottom, confirms the dots’ dispersity and high PL intensity after ligand exchange and buffer transfer. Next, we pursued to optimize the incubation time for the aptamer and QDs. We incubated the dots for 1-4 days and tested the conjugation efficiency (Supplementary figure 3). The experiment resulted in QDs-aptamer conjugates with the same quality, which suggested that the overnight incubation of the probes is sufficient to perform the ligand displacement with aptamer.

Post-conjugate Photoluminescence characterization.
We stored the QDAPTs which were dispersed in PBS at room temperature overnight and compared their PL intensity with free QDs (Figure 2.d) to confirm that the dots kept their PL after
conjugation. The emission peak for the dots stayed constant at 630 nm demonstrating the successful ligan exchange and stability of the PL intensity and sipersity of the QDS.

**Long-term stability of the QDAPT constructs.**
The conjugation of the dots with the aptamers resulted in highly stable final products that do not require special storage conditions (room temperature and ambient conditions do not cause the dots to precipitate), while the freshly generated dots required highly controlled environments to avoid disturbing their dispersity and solubility. To further characterize the stability of the QDAPT constructs, we stored the conjugates for three months and compared their physical and PL characteristics to a fresh batch of QDAPT conjugates; in doing this, we did not observe any differences in dispersity, color, or PL intensity. Figure 2.e shows the PL intensity comparison for fresh and three-months-old conjugates that exhibits no significant variation. This represents a significant milestone in the development of InP QDs, as it enables these QDAPT constructs to be used in real-world applications with long shelf-life storage requirements.

**QDAPT dispersity and size measurements.**
Using Dynamic Light Scattering (DLS), we confirmed that QDAPT constructs were not adsorbing to each other through surface charges; this was expected given the high negative charge of the aptamers and the steric hindrance between the probes (Figure 2.f). DLS measured the average QDAPT diameter to be approximately $8.577 \pm 1.392$ nm. We imaged the QDAPT constructs with TEM (Figure 2.f, top) and confirmed stability in QDAPT morphology. Using TEM, we observed
the majority of particles to be within the reported standard deviation of the DLS data; discrepancies may be due to reflection error caused by aptamers occupying the dots’ surface.

**Figure 2. Quantum dots transfer to aqueous solvent and ligand exchange for aptamer conjugation.** a) The sensitivity of the dots to abrupt solvent exchange leads to
susceptibility to precipitation of the colloids. a.1) The MCH-QDs are stable in ethanol, but the introduction of water causes precipitation of the dots. a.2) Simultaneous solvent and ligand exchange (aptamer capping and transfer to water) partially stabilize the dots but lead to solution coagulation, suggesting successful ligand exchange but dispersion instability. a.3) Steric hindrance can stabilize the dots in aqueous solvents. We introduced phosphorothioated aptamer probes in a 1% volumetric ratio (99% ethanol, 1% water) and vigorously mixed the solution to enable the aptamers to replace the MCH probes. Taking advantage of the steric hindrance of the aptamers on the surface of the QDs, we transferred the buffer from ethanol to PBS. b) Optimized step-by-step procedure to transfer the dots from initial oleate ligands and non-polar solvents to DNA ligands and aqueous solvents. 1: The dots are generated in hexane (non-polar) solvent with oleates as ligands. 2: Subsequently, the ligand was replaced with MCH and transferred to ethanol. 3: Keeping the dots in ethanol, we introduced 1% water containing phosphorothioated aptamer. 4: After the successful replacement of MCH with aptamer, we introduced the dots to PBS. (Supplementary table 1 includes other ligand exchange alternatives by which the dots did not remain stable) c) Agarose gel electrophoresis demonstrates full ligand exchange and DNA conjugation. We sought to eliminate free aptamers in the conjugate solution as they can interfere with the sensitivity of the detection. After exploring purification steps such as filtration and centrifugation, we aimed to control the aptamer ratio while performing the conjugation. We introduced the dots to various concentrations of the aptamer. Agarose gel demonstrates different ratios of DNA (100, 200, 300, 400, 500, 600 nM). [Supplementary information includes limitations for centrifugation and filter purification] d) Photoluminescence measurement of the dots pre- and post-DNA conjugation and buffer exchange. e) Stability of the conjugates after three months incubation under ambient conditions f) Dynamic Light Scattering (DLS) to estimate dot size after conjugation and TEM of the DNA-conjugated dots.

Targeting bacterial membrane proteins and fluorescence microscopy of labeled cells.
The chosen aptamer sequence was previously selected for Penicillin-Binding-Protein 2a Bacterial Protein (PBP2a)\(^{31}\). PBP2a exists on the outer membrane of MRSA, and by targeting it, we could differentiate between MRSA and methicillin susceptible staphylococcus aureus (MSSA) which has a similar exterior protein with a minor dissimilarity from MRSA. PBP2a has a lower affinity for binding β-lactams (penicillins, cephalosporins, and carbapenems)\(^{32}\). This allows for resistance to all β-lactam antibiotics and obviates their clinical use during MRSA infections. After culturing the bacteria and separating the cells from culture media, we set the cell concentrations at 10\(^5\) CFU/mL and used the source for the rest of the experiments. Supplementary figure 4 depicts the secondary formation of the MRSA aptamer and includes the sequence information for the PBP2a aptamer used for this work. We incubated the QDAPT constructs with the cells for 10 minutes and imaged the cells with fluorescence microscopy. Using brightfield settings, we first located the cells and then switched to the red filter to localize the QDAPT (Figure 3.a). We used E. coli as a control for assessing the specificity of the aptamer binding to the cells (Figure 3.a, left), and we successfully localized MRSA cells with PBP2a-QDAPT (Figure 3.a, right).

TEM images of the QDAPT probes attached to MRSA cells.
After the incubation of MRSA cells with QDAPT probes, we imaged the cells with TEM to locate the QDAPT on the cell surface. We successfully observed the probes on the bacterial outer membrane, where PBP2s peptides are expected to be located (Figure 3.b). The coupled cells in the image are MRSA cells that often clump in solution media.

Figure 3. Bacteria detection using the aptamer capped QDs. a) Microscope images of the dots attaching to the target cells (MRSA - right) and no non-specific binding towards non-target cells (E.coli – left) b) TEM images of the MRSA cells covered with QDs on their exterior membrane (PBP2a-Aptamer binding).

QDAPT-target binding kinetics.
Development of an on-surface detection approach requires a robust system that can provide results in a matter of minutes. In this work, the speed of aptamer binding to the bacteria surface dictates this timeline, which is affected by the aptamers’ individual binding kinetics, dissociation rate ($k_d$), number of aptamers on the surface of each QD, and the availability of the target protein on the surface of the bacteria. In order to take a closer look at the staining efficiency of the cells with QDs, we used flow cytometry ranking to study the kinetics of the reactions in terms of incubation time (QDAPT and MRSA) and concentration dependence (QDAPT concentration) which is depicted in Figure 4. To study the incubation time effects on the binding, we mixed the QDAPT and MRSA cells in solution and monitored the cells staining every minute (Figure 4.a). As seen in Figure 4.a&c, 94% of the saturation reaction happens in the first 6 minutes, with 37% of it occurring within the first minute. This observation demonstrates the potential for on-surface imaging of the cells with short incubation times after application of the QDAPT probes (i.e., 2-10 minutes).

We also studied the concentration dependence of the QDAPT probes on staining the cells in order to optimize the amount needed for maximum coverage of the bacteria cell surfaces (Figure 4.b). We used 100 pM-5 nM of the QDAPT constructs with $10^5$ CFU/mL of bacteria in solution, incubated for 10 minutes, and ranked in flow cytometry for their staining. As seen in Figure 4.b&d, a significant shift in staining efficiency (99% of saturation reaction for 2nM vs. 2% for 500 pM occurred when 2uL of the QDAPT construct was added with concentrations ~ 1nM, which implies a $10^6$:1 QDAPT: bacteria ratio.
Figure 4. Investigation of binding kinetics. a) Time dependence of the aptamer-cells binding event. b) Concentration dependence of the cell staining efficiency. 2uL of the QDAPT constructs (~1nM) for staining 10^5 CFU/mL bacterial cells. c) Rapid binding of bacteria cells with QDAPT. The time dependence experiments show 37% saturation in the first
minute and 94% saturation after 8 minutes. d) MRSA staining efficiency and QDAPT concentration optimization. The concentration dependence experiment results demonstrate that 99% of the saturation reaction occurs when 2nM of QDAPT is added, while only 2% of the reaction happened by adding 500 pM of the QDAPT.

Lastly, we sought to characterize the QDAPT-target binding efficiency towards non-target bacteria cells (gram-negative and gram-positive) to ensure the probes can specifically bind to their target—in this case, MRSA. We selected a list of eight bacteria, including MSSA, with similar exterior protein structures for this test (Figure 5). We cultured the cells, removed the cells from culture media, diluted them in a buffer to $10^5$ CFU/mL, and incubated them with 1nM of the QDAPT constructs. Using flow cytometry, we ranked the stained and unstained cells and their red fluorescence intensity. As demonstrated in Figure 5, there was no significant staining of the QDAPT constructs with non-target cells, including MSSA.

![Figure 5. Selectivity of the QDAPT staining bacterial cells.](image)

Selective binding of the QDAPT to target cells (MRSA) vs. three gram-negative and four gram-positive non-target bacteria (including MSSA with minor cell exterior differentiation from MRSA)

**On-surface bacteria detection using QDAPT.**

To demonstrate bacteria detection on surfaces, we chose two common surfaces used in hospital interiors: aluminum and glass. We defined a protocol for bacteria preparation and drop-casting the cells on surfaces, let them dry to mimic a natural bacteria state, and sprayed QDAPT constructs on the surfaces where bacteria were deposited.

Starting with glass surfaces shown in Figure 6.a, we drop-casted 1 µL of MRSA at $10^5$ CFU/mL (10³ CFU deposited) and *E.coli* (non-target) cells in distinguishable rows, and imaged them under a Chemidoc imaging system to observe their autofluorescence before any QDAPT application (Figure 6.a.1). We then removed the glass slides from Chemidoc and applied QDAPT constructs to the cells and re-imaged them (Figure 6.a.2). Comparing Figures 6.a 1 and 2 demonstrates the difference in fluorescence intensity of the cells with and without the QDAPTs. We then gently rinsed the glass surface by letting water flow on the surface for 10 seconds and re-imaged the slide (Figure 6.a.3) to examine the binding efficiency of the QDAPTs to target, as well as the non-target bacterial cells. The rows with MRSA bacteria remained stained while the rows with *E.coli* cells decreased in fluorescence intensity after the rinse. Figure 6.a.4 depicts the fluorescence intensity differences in MRSA vs. *E.coli* cells detected on glass surfaces.
We then repeated the same experimental process on aluminum surfaces (Figure 6.b). Surprisingly, the MRSA and E.coli cells did not exhibit measurable autofluorescence on aluminum surfaces after drop-casting the cells (Figure 6.b.1). The issue was resolved after applying QDAPT constructs to the cells (Figure 6.b.2), as the imaged surface clearly revealed the cell locations. We re-imaged the surface after a gentle rinse for 10 seconds (Figure 6.b.3), and analyzed the fluorescence intensity values, which showed a significant difference between the target (MRSA) and non-target (E.coli) cells (Figure 6.b.4).

Figure 6. Performance of QDAPT constructs for detecting bacteria on surfaces (glass and aluminum) using a Chemidoc imaging chamber a) Bacteria detection on glass. a.1) Bacteria cells (MRSA and E.coli in columns 1,3,5 and 2,4,6, respectively) deposited and dried on the glass surface exhibit autofluorescence. a.2) Addition of QDAPT to the cells (target (MRSA) and non-target (E.coli)) a.3) Rinsing the surface partially removes the QDAPTs from the non-target cells, leaving the specifically bound QDAPT on the surface a.4) Analysis of the image (section a.3) shows statistically significant differences in fluorescence of detected cells b) Bacteria detection
on aluminum. b.1) Bacteria (MRSA and *E.coli* in columns 1,3,5 and 2,4,6, respectively) deposited on the aluminum surface exhibited no autofluorescence (different from observations of bacteria on glass). b.2) QDAPT applied to the cells b.3) Gently rinsing the aluminum surface for 10 seconds removes non-specifically bound QDAPT from the surface, leaving QDAPT residues on the target cells (MRSA). b.4) Fluorescence analysis of the rinsed surface (from section b.3) demonstrated successful (statistically significant) detection of the bacteria on the surface.

**On-surface bacteria detection using hand-held imaging system.**

To demonstrate potential for real-world application of QDAPTs in the detection of bacteria on common surfaces, the accompanying imaging device should be portable, hand-held, user-friendly, and affordable. Therefore, we partnered with Optisolve to tailor a UV exposing imaging system for use in direct imaging of the QDAPT bacteria. **Supplementary Figure 6** shows the UV light hallow and the tablet camera of the Optisolve system. We prepared and drop-casted 1uL of bacteria cell solutions (target: MRSA and non-target: *E.coli*) in distinguishable rows on the glass surface and sprayed QDAPT on the cells, followed by imaging with the tablet camera (**Figure 7.a.1**). Using enhancement software that can be built into the tablet, we isolated the red channel and reduced the background intensity of the photo to process the image for enhanced sensitivity (**Figure 7.a.2**). **Figure 7.a.3** details the fluorescence intensity measurements of the target and non-target bacteria incubated with the QDAPTs which shows no significant differences between the two groups. After acquiring the initial images of the glass slides, we gently rinsed the surfaces for 10 seconds to remove excess QDAPT and repeated the imaging with the UV-camera. **Figure 7.b.1** shows the raw image taken from the surface and **Figure 7.b.2** shows the same image modified with the processing software by separating red channel and enhancing brightness. Fluorescence intensity measurement on the contaminated areas reveals significant differences between the two groups of target and non-target bacteria (**Figure 7.b.3**).
Figure 7. Performance of QDAPT probes for detecting bacteria on glass surfaces using Optisolve hand-held imaging system. a) Bacterial contaminated and QDAPT incubated surfaces. a.1) Raw image acquired from the Optisolve camera. a.2) Processed image acquired from the Optisolve camera. a.3) Pre-rinse fluorescence analysis of the target (MRSA) and non-target (E.coli) cells incubated with QDAPT. b) Post-rinse bacteria detection using QDAPT and Optisolve camera. b.1) Raw image acquired with Optisolve camera. b.2) Processed image acquired with Optisolve camera. b.3) Fluorescence analysis of the image acquired with the Optisolve camera shows successful detection of target bacteria (MRSA). c) The Optisolve setup including UV hollow source and the tablet (left), and the imaging setup for surface imaging (right).

Conclusions:
Quantum dots have been around for several decades with their main applications in electronics and main building blocks. The traditional heavy metal distinc optical and physical properties have made them interesting candidates for use in biomedical applications yet their intrinsic cytotoxicity have not allowed for implication in real-world applications. Safer alternative materials have been recently used to generate the QDs
with same properties and this work, we for the first time, showed a pathway to synthesis InP/ZnSe/ZnS QDs and transfer them to aqueous solution and demonstrated their successful use in detection of surface bacterial contamination. More investigation on surface chemistry modification for the new highly efficient QDs can open door to many biomedical applications without concern for cytotoxicity and environmental adverse effects.

Materials and Methods:

**Chemicals.** Indium (III) acetate (In(OAc)$_3$, 99.99%), oleic acid (tech. 90%), tris(trimethylsilyl)phosphine ((TMSi)$_3$P), 95%), selenium powder (Se, 99.999%), 6-mercapto-1-hexanol (MCH), hexane (reagent grade, >99%), isopropyl alcohol (IPA, anhydrous, 99.8%) were purchased from Sigma-Aldrich. Zinc stearate (ZnO, 12.5-14%) and 1-octadecene (ODE, tech. 90%) were purchased from Alfa Aesar. Tri-n-octylphosphine (TOP, 97%) and sulfur powder (S, 99%) were purchased from Strem Chemicals. Anhydrous ethanol, toluene and n-octane were purchased from Caledon. All the chemicals and solvents above were used without further purification unless stated otherwise. Caution: Tris(trimethylsilyl)phosphine is pyrophoric, needs to be stored under air and moisture free environment. All glassware was dried in an oven overnight at 120°C before use. All manipulations were performed using standard Schlenk or glovebox techniques under dry nitrogen.

**InP/ZnSe/ZnS core/shell/shell QD synthesis:** InP/ZnSe/ZnS QDs were synthesized using a modified approach$^{33}$. 

**6-mercapto-1-hexanol ligand exchange process:** The ligand exchange was performed using a modified approach$^{34, 35}$. 25 mg of purified InP/ZnSe/ZnS QDs dispersed in 3 mL hexane were taken in a test tube. MCH (1 mmol, 140 µL) was swiftly added and the resulting solution was vortexed vigorously for 15 minutes. InP/ZnSe/ZnS QDs were precipitated by adding a 1 mL of toluene and centrifuged at 6000 rpm for 3 min. The supernatant was discarded and the precipitate was dispersed in ethanol with sonication and filtered with a 0.22 µm PTFE syringe filter.

**Solid-state absorption measurements:** The optical absorption measurements were performed with a Perkin-Elmer Lambda 950 UV-Vis-NIR spectrophotometer. The solutions were placed in a quartz cuvette with a 1 mm path length. The absorption measurements were done by dispersing QDs in n-octane or hexane. QD films were deposited on glass substrates to measure the solid-state film absorption.

**Liquid absorption measurement:**
The optical absorption of QDs solution was measured by Molecular Devices SpectraMax M2 microplate reader and analyzed with SpectraMax software.

**TEM Measurements of InP/ZnSe/ZnS oleate and MCH capped:** All transmission electron microscopy (TEM) images were acquired on a Hitachi HF 3300 electron microscope operating at 300 keV. TEM samples were prepared by drop-casting a purified solution of CQDs from hexanes onto a 300-mesh copper grid with a carbon film (SPI supplies). ImageJ was employed to generate the size of the dots.

**Agarose gel electrophoresis:**
100 mL TBE (45 mM Tris-borate, 1 mM EDTA) was added to 0.6 gr agarose and transferred to a flask. After swirling the solution, it was heated up in a microwave to melt the agarose/buffer mixture. After casting the gel in a gel tray by cooling the prepared solution in the tray, we added loading dye to the DNA sample to see how far it went inside the gel.

**Bacteria Preparation:**
MRSA was cultured in TSB liquid broth with 4 µg/mL Oxacillin overnight for 16 hours, other bacteria were cultured in TSB liquid broth. A second “sub-culture” was performed for 4 hours, and cells were collected from the culture media by centrifugation at 14,000 g for 3 minutes. The supernatant was discarded, and the cells were re-dispersed in PBS. Cell concentration was adjusted to 10⁵ CFU/mL using UV/VIS spectrometry by measuring OD₆₀₀ and converting it to CFU/mL according to previously calculated ratios.

**Bacteria Imaging via Microscopy:**
Bacteria cells were cultured and transferred to PBS, and their concentration was set at 10⁵ CFU/mL. 100 µL of the cells were incubated with 2µL of the QDAPT (at 1nM) and left to react for 10 minutes. 15 µL of the solution was transferred to a glass slide and covered with a microscope cover slip. Using a fluorescent Axio Observer.Z1 microscope (Carl Zeiss, Germany) with a modified 625 nm filter the QDAPTS bound to the cells were imaged.

**TEM of QDs-Aptamer and QDs-MRSA:**
QD-aptamers were imaged using the Talos L120C Transmission Electron Microscope (TEM). Samples were diluted in ultrapure water and drop casted onto copper TEM grids. Further ultrapure water rinses were used to remove excess salt.
QD-MRSA samples were imaged using the Talos L120C TEM. Samples were diluted into ultrapure water and immediately drop casted onto copper TEM grids. Further ultrapure water rinses were used to remove excess salt.

**DLS Measurement:**
The size distribution of QD and QD-aptamers was estimated using the Zetasizer Nano ZS. Samples were diluted into ultrapure water using small transfer volumes to minimize solvent differences.

**Flowcytometry ranking experiments and data analysis:**
Three sets of experiments were designed to show the binding kinetics of the bacteria cells with QDAPT as well as their selectivity. In this study, flow cytometry was initially used to show the rapid binding of MRSA with QDAPT as well as the optimized concentration of the QDAPT in order to cover the cells. Finally, the flow cytometer (cytoFLEX S) was used to illustrate the selectivity of the developed probe for MRSA against various bacteria. For all sets of experiments, samples of bacteria and different concentrations of QDAPT were prepared as described in the previous sections. Subsequently, samples were mixed and transferred to flow cytometry tubes to be tested by the flow cytometer. Acquired data were plotted as histograms and analyzed using FlowJo software (Version 10.7.1). To analyze the saturation reaction progress, first, we calculated the median of each measurement. Median values were divided by the median of the saturation point to achieve the final saturation reaction percentage.

**Bacterial contaminated surface preparation:**
10⁵ CFU/mL of cells were prepared in PBS, and 4 µL of the solution was drop-casted to the surfaces and let to be dried for 15 minutes before further imaging or addition of QDAPT.

References:


