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Quantum dot-based assay for Cu^{2+} quantification in bacterial cell culture

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ABSTRACT

A simple and sensitive method for quantification of nanomolar copper with a detection limit of 1.2×10^{-10} M and a linear range from 10^{-9} to 10^{-8} M is reported. For the most useful analytical concentration of quantum dots, 1160 μg/ml, a $1/K_{sv}$ value of 11 μM Cu^{2+} was determined. The method is based on the interaction of Cu^{2+} with glutathione-capped CdTe quantum dots (CdTe–GSH QDs) synthesized by a simple and economic biomimetic method. Green CdTe–GSH QDs displayed the best performance in copper quantification when QDs of different sizes/colors were tested. Cu^{2+} quantification is highly selective given that no significant interference of QDs with 19 ions was observed. No significant effects on Cu^{2+} quantification were determined when different reaction matrices such as distilled water, tap water, and different bacterial growth media were tested. The method was used to determine copper uptake kinetics on *Escherichia coli* cultures. QD-based quantification of copper on bacterial supernatants was compared with atomic absorption spectroscopy as a means of confirming the accuracy of the reported method. The mechanism of Cu^{2+}-mediated QD fluorescence quenching was associated with nanoparticle decomposition.

Fluorescent semiconductor nanoparticles (NPs)[1] or quantum dots (QDs) are emerging as a powerful tool in nanotechnology, and the applications based on their properties are growing day by day [1].

During recent years, a variety of NPs have been used to develop analytical methods, some of them based on fluorescence quenching in the presence of different chemical species [2,3]. In particular, QDs are good candidates for analytical assays because they exhibit a Stern–Volmer quenching behavior [2–4]. This phenomenon has been related to a series of factors such as the chemical nature of the metal core and especially the capping agents surrounding the NP [2–5], both factors mostly determined by the synthetic method used. Most methods of QD synthesis involve high temperatures, anaerobic solutions, toxic reagents (beyond the metals and metalloids involved), large pH adjustments, and/or organic solvents—all conditions that complicate the procedure, affect NP toxicity, and increase production costs [6].

Considering these problems and the relevance of synthesis on QD properties, we recently developed a biomimetic synthetic method involving mild conditions resembling those found in biological systems [7,8]. This method uses the biological thiol glutathione (GSH) as capping and reducing agent to produce water-soluble QDs at low temperatures, aerobic conditions, and close to neutral pH. GSH as a capping agent is a well-documented molecule that enhances biocompatibility and solubility of different QDs such as CdTe, CdSe, and CdS [9–12]. The low production costs and unique properties of biomimetic QDs enhance their possible use for analytical assays in complex matrices such as culture media.

In this context, QD-based methods for quantification of different chemical species have been reported previously. Gattás-Asfura and Leblanc [13] reported the use of CdS QDs capped with different peptides for Cu^{2+} and Ag^{+} detection in aqueous solutions. They also tested the interference of different soluble cations on QD fluorescence, and no effects on fluorescence were observed. In addition, Zhang and coworkers [14] used cysteine-capped CdSe/CdS QDs for Cu^{2+} detection in vegetable samples; in this case, quenching was detected only with Cu^{2+} and no interferences were reported. Finally, Wang and coworkers [15] developed an arsenic quantification with GSH or mercaptoacetic acid (MA)-capped CdTe QDs, neither of which exhibited significant copper quenching. All of these

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[1] *Abbreviations used:* NP, nanoparticle; QD, quantum dot; GSH, glutathione; MA, mercaptoacetic acid; LB, Luria–Bertani; AAS, atomic absorption spectroscopy; RCF, relative centrifugal force; EDTA, ethylenediaminetetraacetic acid; MIC, minimal inhibitory concentration.

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antecedents indicate that QDs are a suitable, sensitive, and specific tool for detection of different metal ions. However, no QD-based detection assay has been developed for copper metal detection in biological culture media. A variety of metal ions are relevant to be studied and detected on cell growth cultures, especially when studying microorganisms that display an intricate relationship between metals and metabolism. One of these biologically relevant metals is copper, specifically the copper cation Cu^{2+}, which is essential for all living organisms and is required for redox reactions catalyzed by cellular enzymes, among other functions [16]. In addition, Cu^{2+} displays many industrial and technological applications because of its semiconductor properties [17,18].

To date, several analytical methods, such as colorimetry, atomic absorption spectrometry, and inductively coupled plasma atomic emission spectrometry, have been used for copper determination [19,20]. However, simple and inexpensive Cu^{2+} quantification methods in liquid media are required in studies of copper interaction with microorganisms, particularly in applications related to biomining and bioremediation and, most recently, with the capacity of some bacteria to biosynthesize copper NPs [18]. Such a method will contribute to the study of bacterial/Cu^{2+} interaction by allowing Cu^{2+} determination (i) when investigating whether metal resistance of bacterial strains is related to the ability to avoid copper uptake and decrease toxicity (a particularly relevant issue when examining new copper-resistant environmental isolates), (ii) when studying the ability of microorganisms to obtain Cu^{2+} from the environment for bioremediation strategies, (iii) when determining the effectiveness of bacterial bioleaching in the mining industry by determining Cu^{2+} solubilization from ores, and (iv) when investigating the production of copper sequestration by molecules produced by bacterial cells (phosphates, peptides, and polymers), among others.

In this context, determination of copper ion consumption by microorganisms in culture media using QDs will allow a fast and easy determination of metal absorption kinetics.

Based on this information and using biomimetically synthesized QDs, we have developed a rapid and inexpensive copper quantification assay using CdTe–GSH QDs. The method was validated in different reaction matrices and used to study bacterial copper consumption in both complex and well-defined growth media.

Materials and methods

Reagents

$MnCl_2$, $CaCl_2{\cdot}2H_2O$, KCl, NaCl, $MgCl_2{\cdot}6H_2O$, $ZnSO_4{\cdot}7H_2O$, CsCl, $HgCl_2$, $AgNO_3$, and $NiSO_4{\cdot}6H_2O$ were obtained from Merck.

$Co(NO_3)_2{\cdot}6H_2O$, $CdCl_2$, $NaAsO_2$, $Na_2AsO_4{\cdot}7H_2O$, $InCl_3$, $CuSO_4{\cdot}5H_2O$, Na_2SeO_3, K_2TeO_3, Li-acetate, TiO_2, GSH, and $NaBH_4$ were obtained from Sigma–Aldrich and used as received. Bacterial growth medium ingredients were purchased from Difco.

Cu^+ as copper(I) tris–thiourea sulfate complex was obtained by reducing 680 mM $CuSO_4{\cdot}5H_2O$ in the presence of thiourea (2.2 M) under aerobic atmosphere and heating at 55 °C for 10 min. The dissolved mixture was cooled, and a white precipitate indicative of Cu^+ formation was observed. The solution was filtered and recrystallized in 1.3 M thiourea solution.

Sea water samples were collected from Ventanas, Chile, and stored at room temperature. Tap water samples were taken directly from the Santiago water system and stored at room temperature.

QD synthesis

QD synthesis was carried out following a biomimetic protocol described previously by our group that produces highly fluorescent QDs that have already been characterized [7,8,10]. Two different sizes of QDs—with emission peaks at 510 (green) and 610 nm (red) when excited at 350 nm—were synthesized. The synthetic yield was determined by precipitating QDs with two volumes of isopropyl alcohol and drying and weighing the precipitate as described previously [7].

Quenching experiments

Green and red CdTe–GSH QDs (1160 μg/ml) were exposed to 0.1, 7.9, 15.7, and 157 nM Cu^{2+} solutions and fluorescence spectra after excitation at 350 nm was determined using a Synergy H1 M multiple-well plate reader (BioTek). Optimal concentration of QDs for Cu^{2+} quenching experiments was evaluated at 580, 1160, and 2380 μg/ml green CdTe–GSH QDs. To determine optimal copper incubation times, QD fluorescence decay was evaluated at different times after copper exposure using the conditions mentioned above.

Quenching effect of ions

Fluorescence of CdTe–GSH QDs (1160 μg/ml) was assayed in solutions amended with cadmium (Cd^{2+}), copper (Cu^+/Cu^{2+}), calcium (Ca^{2+}), sodium (Na^+), potassium (K^+), cobalt (Co^{2+}), zinc (Zn^{2+}), manganese (Mn^{2+}), magnesium (Mg^{2+}), indium (In^{3+}), arsenic (As^{3+} and As^{5+} oxyanions), selenium (Se^{4+} as the oxyanion selenite), tellurium (Te^{4+} as the oxyanion tellurite), lithium (Li^+), titanium (Ti^{4+}), cesium (Cs^+), mercury (Hg^{2+}), silver (Ag^+), and nickel (Ni^{2+}) at 1 μg/ml final concentrations. QD fluorescence in the presence of ions was determined as described above.

Effect of different aqueous culture media and water

Copper quenching on green CdTe–GSH QDs (1160 μg/ml) prepared in distilled water and Luria–Bertani (LB), R2A, and M9 media was determined. LB, R2A, and M9 media were prepared as described by Baev and coworkers [21], Massa and coworkers [22], and De Kievit and coworkers [23], respectively. LB and R2A are complex media using yeast extract, and M9 is a well-defined medium using glucose as carbon source. QDs were incubated with different Cu^{2+} concentrations ranging from 1 to 188 nM. Standard curves were constructed in distilled water and bacterial growth media by using 1, 1.97, 3.95, 7.9, and 15.7 nM Cu^{2+} and 19.7, 39.5, and 79 nM Cu^{2+}, respectively.

Cu^{2+} quantification assays

Cu^{2+} solutions (94 nM) were prepared in distilled, tap, and sea water. Copper content was determined with green CdTe–GSH QDs (1160 μg/ml) using conditions described above (see quenching experiments). The same experiment was performed in bacterial growth media (LB, R2A, and M9) but using 7.9- and 790-nM Cu^{2+} solutions. In all experiments, Cu^{2+} was also quantified by flame atomic absorption spectroscopy (AAS). All solutions for analysis by fluorescence quenching and by AAS were diluted before analysis to accommodate the linear range.

Copper and cadmium AAS detection

Copper and cadmium were detected employing an AA-6200 flame atomic absorption spectrometer (Shimadzu) at 324.7 and 228.8 nm, respectively. In cadmium AAS experiments, background correction was achieved using a deuterium lamp. Samples were diluted in distilled water for their determination.

Bacterial copper incorporation assay

Escherichia coli was grown in R2A medium at 37 °C with shaking until an OD_{600} of approximately 0.3 was reached. Then, 25 ml of bacterial cultures was amended with final concentrations of 0.5, 1, 3, and 6 mM Cu^{2+}. Aliquots were obtained after 20, 40, and 60 min of metal exposure. Obtained samples were centrifuged at 21380 RCF (relative centrifugal force) for 2 min in a Hettich centrifuge (model Mikro 200R). After that, copper was determined in supernatants by QD quenching assay as follows. Aliquots of supernatants were mixed with QDs at a 1160 μg/ml concentration in a final volume of 100 μl. After 10 min of incubation, the fluorescence was evaluated (excitation = 350 nm) using a microplate fluorescence reader. Fluorescence values were interpolated in the previously made calibration curve in R2A medium, and the concentration of Cu^{2+} was determined. Cu^{2+} content was normalized by protein concentration. For protein determinations, cell extracts were prepared by lysozyme treatment and proteins were determined by the Bradford method [24].

QD cadmium release

CdTe–GSH QDs were exposed for 15 min to 0, 157, and 790 nM Cu^{2+} in distilled water. To separate QDs from soluble cadmium, Cu^{2+}-treated QDs were centrifuged at 21380 RCF for 10 min and supernatants were collected for AAS cadmium determination.

Statistical analyses

All statistical analyses were done using GraphPad Prism 5.0 software. The Student's *t* test was used to establish statistical differences between two groups with a P value < 0.005.

Results and discussion

QD fluorescence quenching mediated by Cu^{2+}

With the aim to determine the effect of copper on the fluorescence of QDs produced by the biomimetic method, CdTe–GSH QDs were exposed to increasing concentrations of Cu^{2+} and the relative fluorescence was determined. Fluorescence decrease was observed as a copper concentration-dependent effect, reaching an almost total quenching at 157 nM Cu^{2+} (Fig. 1). Based on this result, we decided to evaluate QDs as a sensitive probe for Cu^{2+} detection.

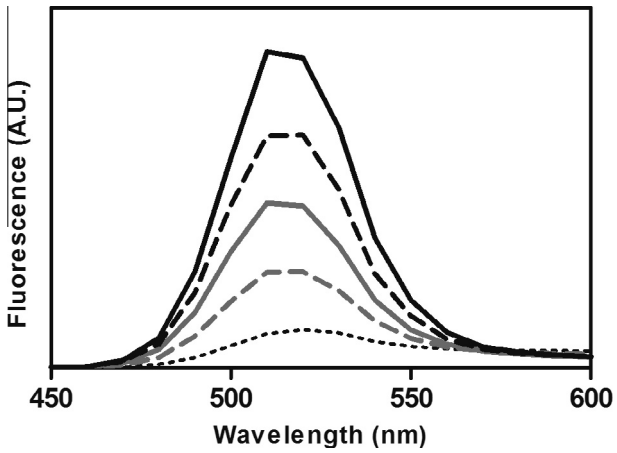

Fig.1. Copper-mediated fluorescence quenching of 1160 μg/ml CdTe–GSH QDs. Shown are fluorescence spectra of CdTe–GSH QDs in the presence of increasing concentrations of Cu^{2+} (from top to bottom): 0 (▬), 1 (▬ ▬), 7.9 (▬▬), 15.7 (▬ ▬), and 157 (· · ·) nM Cu^{2+}.

The effect of three QD concentrations (580, 1160, and 2380 μg/ml) in Cu^{2+} quantification was evaluated (Fig. 2A). These concentrations were chosen based on preliminary analysis of a wide range of QD concentrations from 72.5 to 2380 μg/ml (data not shown). Changes in fluorescence between 1 and 15.7 nM Cu^{2+} exhibited a linear Stern–Volmer behavior for all QD concentrations tested (Fig. 2B). This compares favorably with QD quenching methods for Cu^{2+} that others have reported [13–15].

Linear parameter R^2 values of standard curves for 580, 1160, and 2380 μg/ml QDs were 0.978, 0.987, and 0.985, respectively. The inverse of Stern–Volmer constant ($1/K_{SV}$) values were 4, 11, and 28 μM, respectively. $1/K_{SV}$ corresponds to the Cu^{2+} concentration when 50% of the fluorescence intensity is quenched, and it is used to discriminate between sensitivities in different experimental conditions. Chemically synthesized CdTe–GSH QDs were used for As^{3+} and Pb^{2+} detection by Wang and coworkers [15] and Mohamed Ali and coworkers [25], respectively, and they reported $1/K_{SV}$ values at the nanomolar (nM) scale, substantially better than our detection method, albeit involving lead instead of copper. Based on results obtained with standard curves indicating that 1160 μg/ml QDs present the best linearity (R^2 value) and good sensitivity ($1/K_{SV}$ constant), we decided to use this concentration to develop Cu^{2+} quantification assays. Although QDs at 580 μg/ml display a better sensitivity, the standard curve obtained with 1160 μg/ml presents a better linear correlation, which is a fundamental parameter for method accuracy. Although QDs at 580 μg/ml displayed a better analytical sensitivity (smaller $1/K_{SV}$), the 1160 μg/ml QD concentration was used for all quenching experiments because this concentration did not require QD precipitation and resuspension; instead, QD content was routinely followed with a fluorescence spectrometer (data not shown).

We also evaluated the effect of copper on fluorescence and pH of the QD solution over time. Results indicate that after 10 min of Cu^{2+} exposure, QD fluorescence stabilizes, particularly when copper concentrations are evaluated in the linear range (Fig. 3). In addition, no pH variations were observed, indicating that Cu^{2+} fluorescence quenching is not a consequence of pH (data not shown) as was described previously for other QDs [3].

Using these selected conditions, a Cu^{2+} detection limit (3σ) of 0.12 nM was determined. This value falls in the range reported for other Cu^{2+} quantification methods, validating the proposed assay [13–15]. The results obtained with our method were compared with those reported for other QD-based Cu^{2+} quantification assays. As described in Table 1, differences on capping agents and synthetic conditions affect metal sensitivity (detection limit) of QDs. In addition, Table 1 confirms that the proposed method involves the simplest synthetic conditions and displays excellent parameters as compared with other QD assays in the literature.

Ion interference on QD fluorescence

The effect on CdTe–GSH fluorescence of different ions regularly found in environmental and biological samples was evaluated to determine the selectivity of the proposed method.

QD fluorescence quenching was evaluated in the presence of 20 cations at 1 μg/ml concentrations. This concentration was chosen based on ion levels present as trace elements in most environmental and biological samples [26]. The results obtained indicate that the method is highly selective given that significant fluorescence quenching was observed only in the presence of Cu^{2+} (Fig. 4). Interestingly, other oxidants previously reported to decrease CdTe–GSH fluorescence, such as As^{3+}, did not show any statistically significant effect [6,7]. This can be the consequence of differences in synthetic conditions, which produce QDs with different properties (i.e.,

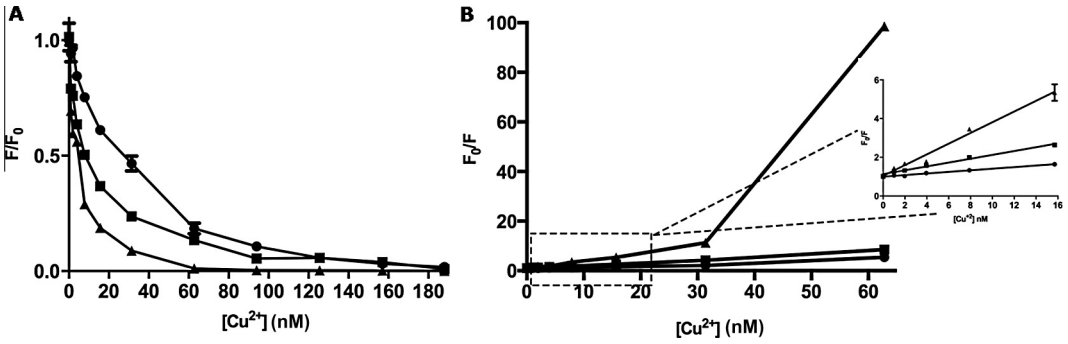

Fig.2. Effect of CdTe–GSH QD concentration on copper-mediated quenching curves. (A) Effect of QD concentrations—580 (▲), 1160 (■), and 2380 (●) μg/ml) on Cu^{2+}-mediated fluorescence decay. (B) Stern–Volmer plot of CdTe–GSH exposed to 1, 1.97, 3.95, 7.9, and 15.7 nM Cu^{2+}. R^2 and $1/K_{SV}$ values for 580, 1160, and 2380 μg/ml were 0.978, 0.987, and 0.985 and 4, 11, and 28 μM, respectively. Error bars here and elsewhere represent 1 standard deviation of three replicates.

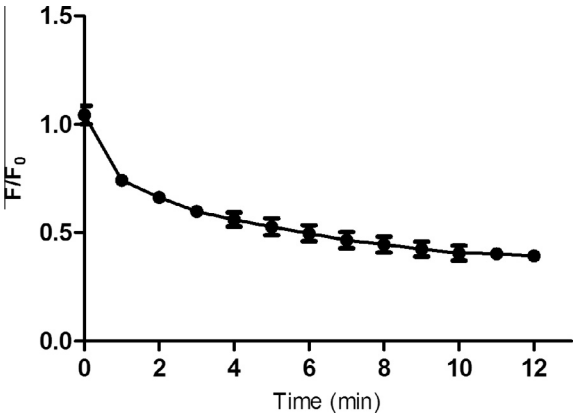

Fig.3. CdTe–GSH fluorescence measured at different Cu^{2+} exposure times.

metal/metalloid content, NP size, or NP structure) [6,7]. The fact that Ag^+ did not influence copper's fluorescence quenching with GSH–CdTe QDs can be contrasted with Gattás-Asfura and Leblanc's report of photoluminescent quenching of Cu^{2+} and Ag^+ with thioglycolic acid-coated CdS QDs [13]. This, therefore, highlight's a major difference between similar Cd/chalcogen QDs and the selectivity of the system we report here.

To evaluate the effect of another copper ion on QD fluorescence, quenching in the presence of Cu^+ was determined. Cu^+ ions in aqueous solution spontaneously react with oxygen being oxidized into Cu^{2+}, so we evaluated the copper(I) tris–thiourea sulfate complex. A small, statistically significant quenching effect was observed in the presence of Cu^+ (Fig. 4); however, even in solutions of the stabilizing complex we used, we saw indications of oxidation of Cu^+ to Cu^{2+} (based on solution color changes), and so the small Cu^+ quenching noted in Fig. 4 can reasonably be explained as quenching from Cu^{2+} created by atmospheric O_2 oxidation of Cu^+ to copper(II). This, therefore, indicates that the proposed method is selective for Cu^{2+} at the evaluated concentrations.

These results indicate that the proposed method is suitable for Cu^{2+} quantification in complex samples and could be used for Cu^{2+} detection in defined bacterial growth media.

Quantification of Cu^{2+} in water samples

To test the accuracy of the proposed method, 94-nM Cu^{2+} solutions were prepared in different water matrices and quantified using QDs and AAS. This concentration was chosen because it is a value close to those used in the standard curve developed in distilled water. Based on these results, a recovery percentage, which relates the solution concentration of metal added and then quantified by each method, was calculated (Table 2). Samples quantified in distilled water and tap water reported 102 and 105% recoveries, respectively, whereas sea water shows a 154% recovery (Table 2). These results are comparable to those determined by using AAS. In addition, recovery results are comparable to those reported with other copper quantification methods based on QDs [13–15]. Sea water results obtained by our method and AAS can be explained by the high copper content naturally present in oceans [27]. In addition, differences observed in sea water between our method and AAS could be consequence of precipitation of insoluble compounds with ions present in sea water or adsorption by ferrous sulfide, hydrated ferric oxide, hydrated manganese dioxide, apatite, clay, and/or organic matter [27,28].

Altogether, quantification results on different water matrices confirm the accuracy of the QD proposed method (excluding sea water) and also confirm that the method is not affected by the presence of ions on complex water solutions as tap water.

Quantification of Cu^{2+} in bacterial growth media

To assess the possibility of using this simple, fast, and inexpensive method for Cu^{2+} quantification in bacterial cell culture, Cu^{2+}

Table 1
Copper detection methods based on QDs.

QDs	Size (nm)	Capping agent	Synthetic conditions	Detection limit (M)	Reference
CdSe–CdS	>3	L-Cysteine	Inert atmosphere; anhydrous toluene; >90 °C	3×10^{-9}	[14]
CdSe	5.5	16-Mercaptohexadecanoic acid	Inert atmosphere; methanol/trimethyl ammonium hydroxide; >90 °C	5×10^{-9}	[30]
ZnS	8–10	L-Cysteine	Inert atmosphere; >90 °C	7.1×10^{-6}	[31]
CdTe/CdSe	2–10	Mercapto propionic acid	Inert atmosphere; >90 °C	2×10^{-8}	[32]
CdTe	3.4	None	Air atmosphere; >90 °C	1.5×10^{-7}	[33]
CdTe	4–8	Glutathione	Air atmosphere; aqueous solvent; 90 °C	1.2×10^{-10}	[7][a]

[a] Biomimetic synthetic method.

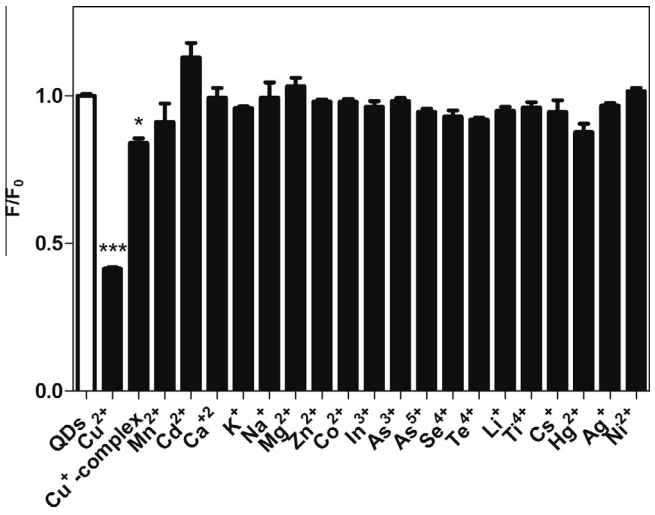

Fig.4. Effect of different ions on CdTe–GSH fluorescence. QDs were exposed separately to different ions (1 μg/ml), and fluorescence was measured as described in Materials and Methods. All determinations were done in triplicate. *Significant P value < 0.05; ***Significant P value < 0.001.

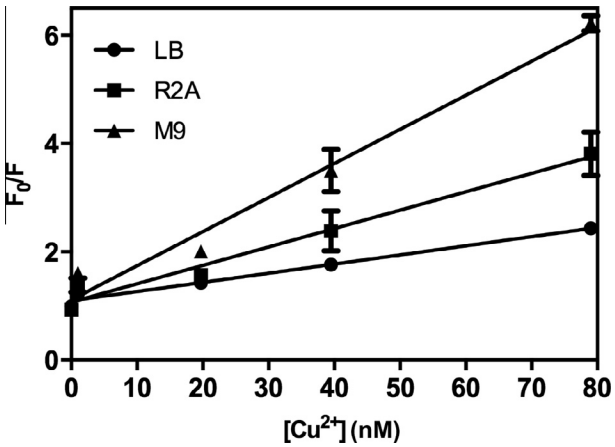

Fig.5. Stern–Volmer plots of CdTe–GSH exposed to different Cu^{2+} concentrations in LB, R2A, and M9 media.

Table 2
Copper determination in water samples.

Sample	Cu^{2+} (nM)[a]	Recovery (%)
Distilled water	96 ± 0.5	102
Sea water	144 ± 0.9	154
Tap water	99 ± 1.2	105
DW–AAS[b]	94.1 ± 0.02	100.1
SW–AAS[b]	459 ± 0.02	488
TW–AAS[b]	92.8 ± 0.001	98.7

Note: DW, distilled water; SW, sea water; TW, tap water.
[a] Mean value was calculated from three different determinations.
[b] AAS determination of Cu^{2+} in water samples.

Table 3
Copper determination in bacterial growth media.

Sample	Cu^{2+} added (nM)	Cu^{2+} determined (nM)[a]	Recovery (%)
LB medium	79	69 ± 0.3	87
	790	772 ± 0.3	98
R2A medium	79	77 ± 0.2	97
	790	786 ± 0.2	99
M9 medium	79	76 ± 0.5	96
	790	157 ± 0.5	19.9
AAS–LB[b]	79	89 ± 0.3	112
	790	835 ± 0.3	106
AAS–R2A[b]	79	80 ± 0.2	101
	790	789 ± 0.2	100
AAS–M9[b]	79	76 ± 0.2	96
	790	739 ± 0.3	94

Note: LB, Luria–Bertani.
[a] Mean value was calculated from three different determinations.
[b] AAS determination of Cu^{2+} in bacterial growth media.

detection was evaluated in three bacterial growth media: M9, R2A, and LB. Calibration curves were developed for each medium. LB, R2A, and M9 calibration curves presented R^2 values of 0.9824, 0.9551, and 0.9894, and $1/K_{SV}$ constants of 56.0, 27.5, and 15.6 nM, respectively. On the one hand, R^2 values indicate that copper quenching displays a linear behavior in all growth media, validating the use of the proposed method. On the other hand, differences in F_0/F and $1/K_{SV}$ constants indicate that specific calibration curves are required for Cu^{2+} determinations in each single culture medium and suggest that medium complexity is intrinsically related to the sensitivity of the method (Fig. 5).

The accuracy of the method on growth media was evaluated by quantifying Cu^{2+} solutions (79 and 790 nM) prepared in M9, LB, or R2A. Quantification results via fluorescence quenching were compared with those of AAS, indicating that the QD proposed method exhibits equivalent or slightly better accuracy for Cu^{2+} determination in LB and R2A media; however, copper recoveries in M9 medium for the higher addition level were unacceptable (Table 3). In the case of M9, a blue copper precipitate was observed in the presence of Cu^{2+} at the highest Cu^{2+} concentration examined. Therefore, Cu^{2+} is not totally available to quench QDs, decreasing recovery percentage at least at the higher addition level (Table 3). To determine whether differences observed in fluorescence quenching among the three growth media used in this work are a consequence of the presence of medium components that could act as chelating agents, the effect of incorporating ethylenediaminetetraacetic acid (EDTA) on the copper quantification assay was determined (see Fig. S1 in online supplementary material). No significant differences in copper QD quenching were observed in the presence or absence of a chelating agent when LB, R2A, or M9 medium was used (the only differences observed are those corresponding to the effect of EDTA on QDs). This result indicates that differences in quenching behavior are not related to the presence of chelating agents and are probably the consequence of other molecules in the media such as organic components and/or inorganic salts present on culture media.

These results indicate that LB and R2A media are good candidates for Cu^{2+} quantification in bacterial cultures, particularly at concentrations in the range between 1 and 79 nM.

E. coli Cu^{2+} uptake assay

E. coli Cu^{2+} incorporation curves were constructed for three metal concentrations (Fig. 6A). Curves obtained with both quantification methods were almost identical and describe the same metal uptake behavior, validating the use of the QD proposed method.

E. coli was treated with different Cu^{2+} concentrations and supernatants were examined at different times in order to evaluate bacterial copper affinity and incorporation kinetics (Fig. 6B). Although these analyses were routinely carried out at the three concentrations described and shown in Fig 6A, the highest Cu^{2+} concentrations plotted in Fig. 6B were also examined. Although 6 mM Cu^{2+} is above the minimal inhibitory concentration (MIC) for *E. coli,* the setup of that experiment involved using bacterial cultures at the exponential phase (OD_{600} ~ 0.3–0.4, as indicated in Materials

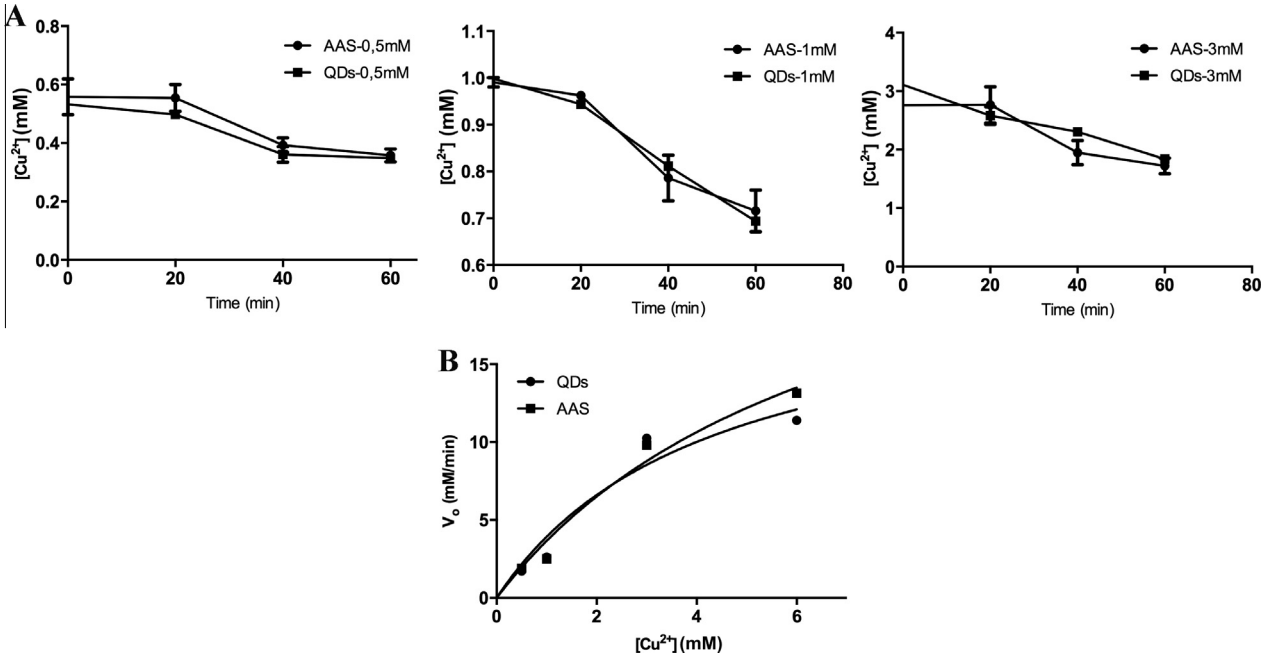

Fig.6. Bacterial copper consumption determined by the QD-based quantification method. (A) Cu^{2+} content in culture supernatants of *E. coli* grown in R2A medium and amended with different metal concentrations (0.5, 1, and 3 mM). Culture supernatants were obtained after 20, 40, and 60 min of Cu^{2+} exposure, and copper was quantified simultaneously using the QD-based method and AAS. (B) Michaelis–Menten plot for *E. coli* copper incorporation.

and Methods) and not the freshly inoculated media used for MIC determinations. This meant that a higher population of cells was present in the culture and the copper resistance was higher than that determined by the MIC. As a consequence, exposing bacterial cultures to 6 mM copper did not produce relevant cell death or lysis (as evidenced by OD_{600} measurements; not shown), allowing the successful determination of bacterial metal uptake. These results were also confirmed by AAS, albeit with a slightly larger deviation from QD results (Fig. 6B).

Using these data, we determined the *E. coli* Cu^{2+} incorporation rate (V_0) and the apparent affinity constant (K_M^*) as described previously by Schüler and Baeuerlein [29] to characterize iron transport systems in *Magnetospirillum gryphiswaldense*.

The K_M^* value obtained for *E. coli* cultures was 4.29 mM/mg protein, and the V_0 value increased at higher Cu^{2+} concentrations, reaching an apparent V_{max}^* value of 20.72 mM/min/mg protein (Table 4). Our results fit with a typical saturation behavior for substrate uptake, in this case from bacterial culture. Kinetic efficiency was determined from V_{max}^*/K_M^*, obtaining a value of 4.83 min^{-1}. All of these parameters can be used to compare different Cu^{2+} uptake phenotypes in bacterial cultures in terms of metal culture affinity and incorporation rate. To validate Cu^{2+} apparent kinetic parameters determined by the QD proposed method, AAS analyses were performed and similar values were determined (Table 4), confirming the accuracy of the QD proposed method in culture media.

Cu^{2+} quenching mechanism

One possible explanation for the observed copper quenching of QDs could be related to nanostructure destabilization and breakdown. If QDs are being decomposed by Cu^{2+} interaction with the NP, cadmium should be released into solution as a consequence of QD decomposition. Cadmium release experiments measured by AAS indicate that QDs liberate cadmium when exposed to Cu^{2+}. Green QDs (580 µg) release up to 20 µg/ml cadmium after 15 min of exposure to 157, 395, and 790 nM Cu^{2+}. These results suggest that fluorescence quenching observed in CdTe–GSH QDs in the presence of Cu^{2+} is a consequence of NP decomposition possibly mediated by metal/thiol interaction or maybe by cadmium chemical displacement by copper ions to form CuTe, as has been proposed for CdSe QDs [5].

Because green CdTe–GSH QDs display higher sensitivity for copper ions (0.4 and 0.58 F/F_0 values for green and red QDs, respectively), and because it was reported previously that green QDs display higher levels of GSH content as compared with red QDs [7], it is possible that fluorescence quenching observed in biomimetic CdTe–GSH QDs is related to metal/thiol interactions.

Table 4
Copper incorporation in *E. coli* cultures.

Method	K_M^* (mM)	V_{max}^* (mM/min)	V_{max}/K_M (1/min)
QDs	4.29	20.72	4.83
AAS	6.98	29.20	4.18

Conclusions

Currently, accurate copper detection methods involve expensive equipment or colorimetric methods and usually do not display the selectivity or detection limit required. A useful method with a detection limit of 1.2×10^{-10} M and a linear range between 10^{-9} and 10^{-8} M has been described. For the most useful analytical concentration of QDs, 1160 µg/ml, the $1/K_{sv}$ value is 11 µM Cu^{2+}. Quantification of trace copper concentration present in bacterial growth media was reported in this work. Green CdTe QDs capped with GSH present the better performance in copper quantification compared with red CdTe QDs. Copper QD fluorescence quenching may be associated with cadmium release from NPs in solution. Results here indicate that our method is highly selective given that no statistically significant interference was determined with the other 20 common ions present in environmental and biological samples, resulting in a fast and sensitive way to quantify the metal. Copper(I)'s slight interference may have been due to Cu(I) to Cu(II) oxidation. In this context, the biomimetic synthesis process is a simple and cost-effective approach to synthesize these probes with

unique properties. These successes encourage us to find other QD synthesis approaches that could render QDs capable of detecting other biological ions and open a new window on the development of QD-based probes for detection of trace elements in different matrices such as culture media for investigation purposes or different sources of water for health inspection.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ab.2014.01.001.

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