Raman Tweezers Microspectroscopy of Functionalized 4.2 nm Diameter CdSe Nanocrystals in Water Reveals Changed Ligand Vibrational Modes by a Metal Cation

Randa Mrad, Sergei G. Kruglik, Nassim Ben Brahim, Rafik Ben Chaâbane, and Michel Negrerie

Laboratoire des Interfaces et Matériaux Avancés, Faculté des Sciences de Monastir, University of Monastir, Bd. de l’Environnement, 5019 Monastir, Tunisia

Laboratoire Jean Perrin, Sorbonne Université, CNRS, 75005 Paris, France

Laboratoire d’Optique et Biosciences, INSERM U1182, CNRS UMR7645, Ecole Polytechnique, 91128 Palaiseau, France

ABSTRACT: We demonstrated the possibility of acquiring Raman spectra of colloidal quantum dots (QDs) at low concentration in water with a size as small as 2.5 nm in diameter using Raman tweezers microspectroscopy. We measured the spectra of CdSe QDs capped with thioglycerol and with l-cysteine. This technique was applied to probe the interaction between Co\(^{2+}\) and Cys—CdSe QDs whose fluorescence emission is quenched in the presence of this metal cation. The quenching mechanism was so far hypothetical. The Raman spectra of Cys—CdSe QDs recorded in the absence and in the presence of Co\(^{2+}\) demonstrated the binding of Co\(^{2+}\) cations to the carboxylate groups of the l-cysteine ligand grafted on the surface of the 4.2 nm CdSe QDs. The frequency of modes for the grafted ligand is changed with respect to the free ligand in solution. Considering the vibrational coupling between the excitonic state and the ligand, we inferred that the binding of a metal cation to the grafted ligand modifies this coupling, so that exciton relaxation through crystal defects is favored. This result rationalizes the fluorescence quenching observed during the metal cation—QD interaction.

INTRODUCTION

Nanoparticles synthesized from semiconductor materials (very often chalcogenides) offer size-dependent and tunable optical properties governed by the size quantization effect. These quantum dots (QDs) were widely studied for numerous applications, especially as probes for imaging and for the detection of diverse analytes in solution. The QD-based optical sensors in solution take advantage of the properties, which are modulated through molecular interactions. The development of these QD-based optical sensors in solution takes advantage of the grafting of organic ligands at the surface of the semiconductor nanoparticles which allowed to solubilize the QDs and made them susceptible to molecular and ionic interactions with analytes. Since the first reported study of CdS QD fluorescence quenching by metal ions, semiconductor QDs of various materials (CdS, CdSe, CdTe, ZnTe), whose surface was functionalized with different grafted organic ligands, were reported to detect metal cations in water. The binding of metallic cations induces the fluorescence quenching of QDs but very few studies were aimed to rationalize the quenching mechanism.

The QD emission quenching may involve several effects which favor nonradiative recombination induced by ions. The defects at the QD surface have an essential role and the surface trapping of excitons can act as an efficient relaxation pathway. Indeed, the photophysical properties highly depend on the synthesis of QDs and on the nature and density of capping ligands, conditions which modulate surface defects and surface state emission bands. Because the interaction of QDs with metal cations (which selectively modulate fluorescence) involves the ligands which bind cations, the exciton relaxation must be influenced by a QD—ligand coupling. A general fundamental mechanism to explain fluorescence quenching induced by metal cations binding to colloidal QDs is still lacking, although a mechanism was proposed in particular cases, such as energy transfer, but not possibly extended as a general basic process. Here, we considered the exciton to ligand vibrational coupling. Our motivation was to probe the organic ligand vibrational modes upon the QD—cations interaction in nonresonant conditions to obtain their spectrum free from QD contribution.

The QD photophysics is usually probed by fluorescence but in that case the grafted ligands are silent. Because nanoma-

Received: July 16, 2019
Revised: September 16, 2019
Published: September 18, 2019
molecules,31 no Raman data was reported for grafted ligands as surface-enhanced Raman spectroscopy probes for adsorbed trapped silver nanoparticles in the range 15−400 BR/LN) cooled to 50 nm. The light was recorded by a back-illuminated near-infrared metal cation. RTM is a powerful technique which was applied change of their emission spectra in the presence of a particular interaction in water,26,27 whereas the use of a capillary fiber did not result in satisfactory spectral resolution.28 The use of Raman excitation in the QD absorption band induces fluorescence which precludes recording the ligand spectrum, unless the ligand itself quenches the QD emission,29 making the study of interactions in solution difficult. We used Raman tweezers microspectroscopy (RTM)30 to measure the Raman spectrum of the ligand grafted on QDs in parallel with the change of their emission spectra in the presence of a particular metal cation. RTM is a powerful technique which was applied to obtain spectra of exosomes of 100 nm size.30 Whereas trapped silver nanoparticles in the range 15−50 nm was used as surface-enhanced Raman spectroscopy probes for adsorbed molecules,31 no Raman data was reported for grafted ligands on ultrasmall semiconductor QDs in water. We investigated several QDs: CdS and CdSe capped with thioglycerol (TG; Figure S1) of different diameters in order to compare with the uniquely reported spectrum of TG-capped QDs in the solid state,32 noting that the solid state does not allow to study interactions with analytes. We then probed CdSe capped with l-cysteine which confers to the QDs the ability to bind Co2+ with a concentration-dependent fluorescence quenching.14 The present Raman data allow us to evidence vibrational changes induced by Co2+ cations bound to the carboxylate groups of the l-cysteine ligand grafted on the QD surface.

■ EXPERIMENTAL METHODS

Raman Tweezers Microspectroscopy. All Raman spectra were recorded using a home-built setup fully described elsewhere.30 Briefly, the excitation at 780 nm (~100 mW at sample) was provided by a continuous-wave Ti:sapphire laser (Spectra-Physics model 3900S) pumped by an Ar-ion laser. Raman scattering was generated through a water immersion objective (Olympus MA10, NA = 1.1) directly plunged into the sample solution (~100 μL) containing the nanoparticles. The focal point acting as an optical trap was located inside the solution droplet deposited on the CaF2 slide. The scattered Raman signal was collected in a backscattering geometry, with a long-pass filter before a 50 cm focal length spectrograph (Acton SpectraPro 2500i) equipped with a 400 groove mm−1 grating optimized for 850 nm. The light was recorded by a back-illuminated near-infrared charge-coupled device detector (Princeton Instruments SPEC-10 400BR/LN) cooled to ~120 °C with liquid nitrogen. The effective volume of Raman signal collection was estimated as a cylinder with a diffraction limited diameter of ~0.9 μm and length of ~3.5 μm (Vr = 2 μm3 = 2 fL), while the effective core volume of the optical trap was estimated30 to be on the order of Vtrap = 0.1 μm3. We note that, because of the very small size, the studied QDs were not completely immobilized, so that the optical trap served as a “particle concentrator” substantially enhancing the average Raman signal from the dispersed QDs. The spectral resolution of the setup is ~5 cm−1 with a 50 μm spectrometer slit width. Frequency calibration was performed using the Raman lines of toluene with an absolute accuracy of ±2 cm−1. Spectra were acquired using WinSpec software. The Raman spectra of TG and l-cysteine in the absence of nanoparticles were recorded in a backscattering configuration by placing the sample solution in a 1 × 1 × 4 cm3 quartz cuvette, with illumination by an air-immersion objective (Olympus MA10, M = 10×, NA = 0.4).

Synthesis of Capped CdSe Nanoparticles. The synthesis was adapted from previous protocols.15,14 Aqueous solution of cadmium acetate dehydrate (1.54 g) was first mixed with l-Cys (1.53 g) as the stabilizer in 100 mL of deionized water in a beaker under stirring. The pH of the resulting solution was adjusted to 11.2 by using aliquots of 1 M NaOH. Separately, an aqueous solution of Na2SeO3 (3 × 10−1 M) was prepared by introducing SeO2 into a NaOH solution. The Na2SeO3 solution was injected into the mixture of Cd2+ and Cys under vigorous stirring. Quickly, NaBH4 solution (10−2 M) was added to this N2 saturated solution under stirring. The molar ratio of Cd2+/Cys/Se−2 was set to 1:2:0.5. The precursors are converted to CdSe nanoparticles by refluxing the reaction mixture at 100 °C for 3 h under atmospheric conditions. The l-Cys-capped CdSe QDs are formed at this stage, accompanied by a yellow color. To isolate the synthesized CdSe nanoparticles, the final solution was concentrated down to 25 mL in an evaporator. The nanoparticles were then extracted by precipitation in isopropanol and the solution was stirred for 1 h. The precipitate was filtered and dried in a desiccator under vacuum. We calculated the molecular mass of individual QDs from their average diameter measured by high resolution transmission electron microscopy,15,14 assuming a spherical shape. From the volume of one crystal unit (hexagonal for CdS and cubic for CdSe), we estimated the number of atoms in one QD (e.g., 668 each of Cd and Se for a 4.2 nm size QD). QDs whose diameter is 4.2 nm possess a larger number of crystal units at the surface than within the core. Accordingly, we considered that one QD comprises ~100−150 capping ligands in average. From the number of atoms in one individual QD, its molecular mass is directly calculated together with the true molar concentration: MCGd−CdS = 2.84 × 106 g/mol and MCys−CdSe = 1.43 × 106 g/mol. The mass concentration of QDs in the colloidal suspension was 0.4 mg/mL for Raman measurements, yielding molar concentrations of 0.14 and 2.8 μM for TG−CdS and Cys−CdSe, respectively.

Spectrofluorometric Detection of Co2+ Ions with Cys−CdSe QDs. All reagents were purchased from Sigma-Aldrich Chemicals, except CoCl2·6H2O which was obtained from Riedel-de-Haën, and used without further purification. A solution of Cys−CdSe QDs was prepared from powder in deionized water at a concentration of 3 mg/100 mL, which corresponds to 0.2 μM based on its true molecular mass. The fluorescence spectra of this aqueous solution (2 mL) were recorded with a quartz cell (10 mm optical path) at room temperature with an excitation wavelength of 360 nm. The Co2+ ion solution was introduced as aliquots of increasing concentration 10 min before recording spectra. Steady-state fluorescence was measured with a Cary Eclipse spectrometer and absorption spectra were measured using a Shimadzu UV-1700 spectrophotometer.

■ RESULTS AND DISCUSSION

Detecting Vibrations of TG as the Ligand on Two Semiconductor Nanoparticles. We successfully recorded the Raman spectra of ligands grafted to very small colloidal
QDs at a low concentration in water thanks to the effect of optical trapping. We first recorded (Figure 1) the Raman spectrum of TG grafted to CdS and CdSe, which have diameters of 12.3 and 2.5 nm, respectively. We cannot measure the Raman spectrum of QDs in water without optical tweezers (e.g., using a 10X long-focal length objective). In “classical” Raman, the constraint for a high concentration of QD colloidal suspension results in a largely dominating Rayleigh scattering which precludes reliable measurement of the informative Raman signal on top of water background. The “concentrator effect” of optical tweezers allowed to considerably decrease the stationary QD concentration so that the Rayleigh scattering becomes negligible, making Raman measurements possible. The number of QDs in the probed focal volume (2 fL) was \(N_{QD} = 1.7 \times 10^7\) for TG–CdS QDs (0.14 \(\mu\)M; size 12.3 nm) and \(N_{QD} = 3.4 \times 10^5\) for Cys–CdSe QDs (2.8 \(\mu\)M; size 4.2 nm). These values can be compared to \(N_p = 0.3\) for 100 nm diameter liposomes. The absolute number of molecules to be probed (QD ligand or lipids) should be somewhat similar, taking into account the relative intensity of informative Raman bands with respect to the background water band at 1640 \(\text{cm}^{-1}\). This result agrees with the observed trend that smaller particles are more difficult to detect by optical trapping than large ones at the same concentration.

Two remarkable observations arise from the comparison of spectra. First, the spectrum of TG has changed after grafting to both QDs, as compared to free TG in solution. Second, the spectra of grafted TG are very similar for both CdS and CdSe semiconductor nanoparticles. Remarkably, the entire Raman spectra of our TG–CdSe and TG–CdS colloidal nanoparticles at low concentration in water and measured with the aid of optical trapping are identical to that obtained for the solid state 1.7 nm size TG–ZnS QDs in powder. This fact demonstrates that RTM provides the possibility to study molecular interactions in a diluted solution containing very small nanoparticles. By comparison, the use of a capillary fiber containing the QD suspension produced a spectrum of TG grafted to CdTe with very broad and overlapped bands which resemble neither the solid state QDs, nor free TG spectra. The spectrum of free TG, reported together with TG–ZnS, is also identical to ours, so that similar spectral changes are observed after TG grafting, either to the CdS, CdSe, or ZnS semiconductor core. We concluded that the insertion of the sulfur atom of TG within the crystal lattice of these semiconductor materials induces the same vibrational constraints on the grafted ligands. The generalization of this result to all thiols must still be verified.

After grafting, changes in the TG vibrational spectrum are observed for the stretching \(\nu(C=\text{S})\) (735 and 685 \(\text{cm}^{-1}\)) and bending \(\delta(C=\text{S})\) (362 \(\text{cm}^{-1}\)) vibrations. These modes involve the S atom which is directly inserted within the CdS/CdSe lattice at the QD surface. The assignment of S-containing vibrations is facilitated by the direct comparison of TG versus glycerol Raman spectra (Figure S2 of Supporting Information). The band at 871 \(\text{cm}^{-1}\) in free TG spectrum disappeared because of the formation of the S–Cd bond during CdS/CdSe QDs synthesis. The stretching \(\nu(C=\text{S})\) appears as two bands for free TG (685 and 735 \(\text{cm}^{-1}\)) probably due to the presence of conformers, the intensity of one of them being decreased for TG grafted as a result of constrained motion of the bound ligand. The intensity of the \(\delta(C=\text{S})\) deformation mode (362 \(\text{cm}^{-1}\)) decreased for bound TG.

Importantly, changes of the TG vibrational modes are observed even for bonds which do not directly interact with the QD surface. The \(\nu(C_{\alpha}=\text{C}_{\beta})\) at 786 \(\text{cm}^{-1}\) of free TG disappeared, with the appearance of modes at 827 \(\text{cm}^{-1}\), a downshift of other \(\nu(C=\text{C})\) modes, and an intensity decrease of the mode at 441 \(\text{cm}^{-1}\) [assigned to \(\delta(\text{COC})\)]. As a partial conclusion, several TG backbone vibrations (786, 1030–1100 \(\text{cm}^{-1}\)) are affected by the insertion of the S atom of TG within the CdS/CdSe lattice. Changes of ligand vibrational modes could also be observed by IR spectroscopy for the same samples in powder and for other kinds of QDs and ligands as well.

**Effect of Co\(^{2+}\) Cations Bound to l-Cys–CdSe Nanoparticles.** We focused on the spectral manifestations due to the interaction of a functionalized colloidal QD with the Co\(^{2+}\) cation and have chosen l-cysteine as the ligand, which is supposed to bind metallic cations to its carboxylate group,\(^{14,39,40}\) grafted to CdSe nanoparticles. Note that CdSe QDs devoid of L-cysteine are not soluble and cannot bind Co\(^{2+}\) in solution. First, the Raman spectrum of free l-cysteine in water (Figure 2a) is identical to that previously reported at the same pH and concentration.\(^{13,34}\) The capped CdSe nanoparticles have a 4.2 nm diameter and are fully soluble in water.\(^{14}\) Similar to the previous case of TG–CdS/CdSe QDs, the vibrational spectrum of l-cysteine changes after grafting to the semiconductor core (Figure 2b). The S-containing stretching \(\nu(C_{\text{SS}}=\text{C})\) (684 \(\text{cm}^{-1}\)) is downshifted, whereas the bending modes \(\delta(C=\text{SH})\) (935 and 994 \(\text{cm}^{-1}\)) disappeared due to the insertion of sulfur into the CdSe lattice. The backbone stretching \(\nu(C=\text{C})\) modes (620 and 874 \(\text{cm}^{-1}\)), \(\nu(\text{CCN})\) (909 \(\text{cm}^{-1}\)) and CH\(_2\) deformation (1021 \(\text{cm}^{-1}\)) are affected, as well as the carboxylate deformation (817 \(\text{cm}^{-1}\), which shifted to

![Figure 1. Raman spectrum of TG (a) (20% in water) and optical tweezers Raman spectra of TG grafted to CdS (b) and CdSe (c) solubilized nanoparticles in water. Raman excitation was set at 780 nm. Water contribution is subtracted.](image-url)
804 cm$^{-1}$ and its asymmetric $\nu_{as}(\text{COO}^-)$ stretching at 1592 cm$^{-1}$.

Now, in the presence of 10 $\mu$M Co$^{2+}$ in the solution, the overall profile of the Raman spectrum of grafted L-cysteine has again changed substantially (Figure 2c). Two spectral ranges are especially affected. First, the asymmetric stretching mode $\nu_{as}(\text{COO}^-)$ in the high-frequency range is upshifted by more than 20 cm$^{-1}$ (now centered at 1615 cm$^{-1}$) and the COO$^-$ deformation (516 cm$^{-1}$) of the carboxylate is enhanced, two effects that we attribute to the direct interaction of the Co$^{2+}$ cations with the Cys carboxylate group. Second, all the modes below 700 cm$^{-1}$ are strongly enhanced. Remarkably, the backbone deformation (CCN) mode (364–486 cm$^{-1}$) is enhanced, as well as the $\nu(\text{C–C})$ mode (600 cm$^{-1}$). Other backbone vibrational modes are also affected.

**Involvement of the Ligand Vibrations in QD Fluorescence Quenching.** The binding of Co$^{2+}$ to carboxylate of L-cysteine grafted on CdSe QDs affects many of its vibrational modes. Simultaneously, as observed for other capped QD–cation systems, the binding of Co$^{2+}$ affects the photophysics of Cys–CdSe by quenching its fluorescence (Figure 3a). When the emission intensity decreased as a function of the Co$^{2+}$ concentration, the shape of the spectrum, which comprises three gaussian individual bands arising from different relaxation processes, changes progressively (Figure 3b). The evolution and comparison of the normalized spectra indicate that the second band, of a larger relative intensity, are the most affected by quenching (Figure 3b, inset). The first Gaussian band, located at higher energy, corresponds to the core emission, whereas the two other bands at larger wavelengths are due to emission involving the exciton relaxation through trap defects, either at the surface or within the CdSe core (Cd or Se vacancies in crystal units) with different pathways. The emission due to surface trap states is thus more quenched by the binding of Co$^{2+}$.

The role of surface states and grafted ligands in exciton relaxation is well established. Surface defects associated with trap states favor nonradiative recombination, thus quenching, and are modulated by the grafted ligands (nature, density, stoichiometry). For example, the exchange after synthesis of an amine ligand for a thiol one in CdSe-induced trap states (Se vacancies) whose emission was enhanced while the band-edge emission decreased. More precisely, calculations showed that hybridization between electronic states of the QD and the capping ligands (electronic density delocalized over both) strongly facilitate the excitonic relaxation, and the electron–phonon interactions may modify the efficiency of relaxation mechanisms. In resonance conditions with QD absorption, the enhancement of ligand vibrational modes has evidenced an energy transfer from the CdSe core to its thiophenol ligand in the solid state. The vibronic coupling between a CdTe QD and its grafted ligand mercaptosuccinic acid was also demonstrated, the damping of the QD phonons depending upon the nature of the ligand. These results agree with theoretical computations indicating that excitonic delocalization induces a greater coupling...
between the exciton and vibrational modes of the ligands on QDs, leading to the nonradiative dissipation of electronic energy.4,24 As a consequence of this coupling, the vibrational frequencies of the grafted ligand are changed38,53 with respect to the free ligand, exactly as we observe here for the grafted L-cysteine compared to L-cysteine in solution.

In this context, the binding of a metal cation modifies the vibrational influence of a ligand on exciton relaxation, in agreement with several recent studies revealing the QD–ligand vibrational coupling, especially in colloidal CdSe QDs.24,25 Our present Raman data demonstrate that, without the need to be excited in resonance conditions with the absorption bands of the CdSe QDs, Co2+ bound to the grafted ligand L-cysteine changes its vibrational modes. The transition from the electronic state to molecular vibrations was proposed to occur through the overlap of wavefunctions and results in energy transfer to vibrations of the ligands.19,56 The change of frequencies of the grafted ligand are changed38,55 with respect to the free ligand, exactly as we observe here for the grafted L-cysteine compared to L-cysteine in solution.

ACKNOWLEDGMENTS
R.M. acknowledges a travel research fellowship “Bourse d’Altérance” from the Tunisian Government.

REFERENCES

DOI: 10.1021/acs.jpcc.9b06756