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Determination of the Fluorescence Quantum Yield of Quantum Dots: Suitable Procedures and Achievable Uncertainties

Markus Grabolle,[†] Monika Spieles,[†] Vladimir Lesnyak,[‡] Nikolai Gaponik,[‡] Alexander Eychmüller,*[,‡] and Ute Resch-Genger*[,†]

BAM Federal Institute for Materials Research and Testing, Richard-Willstaetter-Strasse 11, 12489 Berlin, Germany, and Physical Chemistry, TU Dresden, Bergstrasse 66b, 01062 Dresden, Germany

Despite the increasing use of semiconductor nanocrystals (quantum dots, QDs) with unique size-controlled optical and chemical properties in (bio)analytical detection, biosensing and fluorescence imaging and the obvious relevance of reliable values of fluorescence quantum yields for these applications, evaluated procedures for the determination of the fluorescence quantum yields (Φ_f) of these materials are still missing. This limits the value of literature data of QDs in comparison to common organic dyes and hampers the comparability of the performance of QDs from different sources or manufacturers. This encouraged us to investigate achievable uncertainties for the determination of Φ_f values of these chromophores and to illustrate common pitfalls exemplarily for differently sized water-soluble CdTe QDs. Special attention is dedicated to the colloidal nature and complicated surface chemistry of QDs thereby deriving procedures to minimize uncertainties related to these features.

Suitable labels and target-specific probes are at the core of fluorescence signaling, imaging, and sensing.[1,2] The spectroscopic properties of these fluorophores have a considerable influence on the detection limit and the dynamic range of a fluorescence method, on the reliability of the readout for a particular target or event, and on the suitability for multiplexing strategies, that is, parallel detection of different targets.[2] Of special relevance are the wavelength-dependent molar (decadic) absorption coefficient ε and the photoluminescence quantum yield Φ_f (termed here fluorescence quantum yield) that represents the number of emitted photons $N_{em}(\lambda_{ex})$ per number of absorbed photons $N_{abs}(\lambda_{ex})$, see eq 1, and characterizes a radiative transition in combination with the luminescence lifetime, the luminescence spectrum, the emission anisotropy, and the photostability.[2]

$$\Phi_f = \frac{N_{em}(\lambda_{ex})}{N_{abs}(\lambda_{ex})} \quad (1)$$

The product of ε at the excitation wavelength λ_{ex} or excitation wavelength interval (filter-based instruments) and Φ_f, termed brightness, controls the spectral sensitivity from the label side. The chromophore photostability determines the excitation intensity to be used and the number of possible measurement cycles. The overall importance of these quantities for the choice of optimum fluorescence tools is the ultimate driving force for the supply of data on ε, Φ_f, chromophore brightness, and occasionally also photostability by dye manufacturers.[3,4] The reliability of such values, however, is often limited as there exist no standardized protocols at present for the measurement of these key features, the methods used for the determination of these quantities are typically not detailed and in many cases only data in organic solvents are given. Accordingly, reliable procedures for the determination of the microenvironment-dependent key features ε, Φ_f, and photostability are of considerable relevance for the evaluation and comparability of the broad variety of chromophores ranging from molecular systems, nanometer- to micrometer-sized particles with size-independent optical features, to nanocrystal chromophores with size-dependent optical and physicochemical properties.[2] This situation is especially critical in the case of Φ_f values for the ever increasing variety of fluorescent labels. Compared, for example, to the relatively elementary determination of the ε value, the measurement of Φ_f is by far more challenging even for the simplest case, transparent, dilute dye solutions, and relative optical methods, because of the mandatory performance of both reliable absorption and emission measurements, the use of a quantum yield standard, and the correction of the measured emission spectra for instrument-specific contributions.[1,5] Despite of the obvious need for evaluated technical notes for the determination of this quantity, currently there exist only very few

* To whom correspondence should be addressed. E-mail: ute.resch@bam.de (U.R.-G.), alexander.eychmueller@chemie.tu-dresden.de (A.E.).
† BAM Federal Institute for Materials Research and Testing.
‡ TU Dresden.

(1) Lakowicz, J. R. *Principles of fluorescence spectroscopy*, 3rd ed.; Springer Science+Business Media, LLC: New York, 2006.
(2) Resch-Genger, U.; Grabolle, M.; Cavaliere-Jaricot, S.; Nitschke, R.; Nann, T. *Nat. Methods* **2008**, *5*, 763–775.
(3) Panchuk-Voloshina, N.; Haugland, R. P.; Bishop-Stewart, J.; Bhalgat, M. K.; Millard, P. J.; Mao, F.; Leung, W.-Y.; Haugland, R. P. *J. Histochem. Cytochem.* **1999**, *47*, 1179–1188.
(4) Berlier, J. E.; Rothe, A.; Buller, G.; Bradford, J.; Gray, D. R.; Filanoski, B. J.; Telford, W. G.; Yue, S.; Liu, J.; Cheung, C.-Y.; Chang, W.; Hirsch, J. D.; Beechem, J. M.; Haugland, R. P.; Haugland, R. P. *J. Histochem. Cytochem.* **2003**, *51*, 1699–1712.
(5) Rurack, K.; Resch-Genger, U. Determination of the Photoluminescence Quantum Yield of Dilute Dye Solutions (IUPAC Technical Note) submitted to the Fluorescence Task Force Group, IUPAC.

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overall accepted recommendations for the determination of Φ_f values for transparent, dilute solutions of small organic dyes with relative optical methods,[5−16] as well as few application notes from instrument manufactures,[17] yet no approved guidelines.

Within the last years, with quantum dots (QDs) made from II/VI and III/V semiconductors, a new class of fluorescent labels has finally come of age[18−20] that is increasingly employed, for example, for fluorescence assays, fluorescence imaging,[21−25] single molecule applications,[20] and as biosensors.[21] The spectroscopic and physicochemical properties of these chromophores, that are typically sophisticated core−shell (e.g., CdSe core with a ZnS shell) or core-only (e.g., CdTe) structures functionalized with a broad variety of different coatings, are governed by the constituent material, particle size, and size distribution (dispersity), and surface chemistry, specifically, the number of non-saturated dangling bonds favoring non-radiative deactivation.[26,27] Surface chemistry includes here inorganic passivation layers or shells of semiconductor material of larger band gap or, less common, also silica and organic capping ligands bound to surface atoms that additionally control QD solubilization. Accordingly, the application-relevant features of such QD labels depend to a considerable degree on particle synthesis and surface modification, as well as on the QD environment.[26] Because of the importance of the photoluminescence quantum yield for all types of fluorescence applications of QD fluorophores, many publications provide data on this quantity. Reported fluorescence quantum yields of properly surface-passivated QDs, that have been with a single exception[28] determined by relative optical methods,[14,16] are 0.65−0.85 for CdSe,[29,30] 0.1−0.4 for InP,[31−33] and 0.30−0.75 for CdTe and CdHgTe[34−36] as well as 0.26−0.70 and 0.1−0.8 for the NIR emitters PbS[37,38] and PbSe, respectively.[39,40] The differences between these values are determined by QD preparation and the quality of surface passivation as well as by the reliability of the method used for the determination of the Φ_f values. The latter is difficult to judge as in many cases this procedure is not described in detail.[41] In the few cases where a detailed procedure is provided typically valuable information is missing like, for example, the excitation wavelength, the absorbance at the excitation wavelength or the QD concentration, spectral correction of the measured emission spectra, and the fluorescence quantum yield of the quantum yield standard(s) used.[41]

This encouraged us to systematically investigate QD-related uncertainties for the determination of the fluorescence quantum yields of these fluorophores with a relative optical method thereby aiming at the provision of validated and simple protocols for the reliable determination of Φ_f values of such nanocrystalline labels. For this relative method, major instrument- and sample-related sources of uncertainty are illustrated and discussed and, for the first time, procedures to minimize such effects are presented. We chose here CdTe QD colloids of varying size stabilized with the frequently used monodentate ligand thioglycolic acid (TGA)[35] as CdTe is the most investigated QD emitting visible light that can be reliably prepared in high quality not only in organic solvents but also in water.[42,43]

INSTRUMENTATION AND MATERIALS

Steady State Absorption and Fluorescence. The absorption spectra were recorded on a Cary 5000 spectrometer. The accuracy

(6) Fery-Forgues, S.; Lavabre, D. *J. Chem. Educ.* **1999**, *76* (9), 1260–1264.
(7) Demas, J. N. *Opt. Radiat. Meas.* **1982**, *3*, 195–248.
(8) Velapoldi, R. A.; Tonnesen, H. H. *J. Fluoresc.* **2004**, *14*, 465–472.
(9) Velapoldi, R. A. Liquid standards in fluorescence spectrometry. In *Advances in Standards and Methodology in Spectrophotometry*; Burgess, C., Mielenz, K. D., Eds.; Elsevier: Amsterdam, 1987; pp 175−193.
(10) Eaton, D. F. *Pure Appl. Chem.* **1988**, *60*, 1107–14.
(11) Velapoldi, R. A.; Epstein, M. S. *ACS Symp. Ser.* **1989**, *383*, 98–126.
(12) Parker, C. A.; Rees, W. T. *Analyst* **1960**, *85*, 587–600.
(13) Testa, A. C. *Fluorescence News* **1969**, *4*, 1–3.
(14) Demas, J. N.; Crosby, G. A. *J. Phys. Chem.* **1971**, *75*, 991–1024.
(15) Melhuish, W. H. *J. Res. Natl. Bur. Stand. A* **1972**, *76*, 547–60.
(16) Rurack, K. Fluorescence quantum yields: methods of determination and standards. In *Standardization and Quality Assurance in Fluorescence Measurements I: Techniques*; Resch-Genger, U., Ed.; Springer-Verlag: Berlin Heidelberg, 2008; Vol. 5, pp 101−145.
(17) (a) http://www.iss.com/resources/tech3/ (accessed Sept 29, 2008). (b) http://www.jobinyvon.com/Fluorescence/Applications/Quantum-Yield (accessed Sept 29, 2008).
(18) Alivisatos, A. P. *Science* **1996**, *271*, 933–937.
(19) Weller, H. *Curr. Opin. Colloid Interface Sci.* **1998**, *3*, 194–199.
(20) Jovin, T. M. *Nat. Biotechnol.* **2003**, *21*, 32–33.
(21) Medintz, I. L.; Uyeda, H. T.; Goldman, E. R.; Mattoussi, H. *Nat. Mater.* **2005**, *4*, 435–446.
(22) Xing, Y.; Chaudry, Q.; Shen, C.; Kong, K. Y.; Zhau, H. E.; Chung, L. W.; Petros, J. A.; O'Regan, R. M.; Yezhelyev, M. V.; Simons, J. W.; Wang, M. D.; Nie, S. *Nat. Protoc.* **2007**, *2*, 1152–1165.
(23) Parak, W. J.; Pellegrino, T.; Plank, C. *Nanotechnology* **2005**, *16*, R9-R25.
(24) Nabiev, I.; Mitchell, S.; Davies, A.; Williams, Y.; Kelleher, D.; Moore, R.; Gun'ko, Y. K.; Byrne, S.; Rakovich, Y. P.; Donegan, J. F.; Sukhanova, A.; Conroy, J.; Cottell, D.; Gaponik, N.; Rogach, A.; Volkov, Y. *Nano Lett.* **2007**, *7*, 3452–3461.
(25) Michalet, X.; Pinaud, F. F.; Bentolila, L. A.; Tsay, J. M.; Doose, S.; Li, J. J.; Sundaresan, G.; Wu, A. M.; Gambhir, S. S.; Weiss, S. *Science* **2005**, *307*, 538–544.
(26) Dabbousi, B. O.; Rodriguez-Viejo, J.; Mikulec, F. V.; Heine, J. R.; Mattoussi, H.; Ober, R.; Jensen, K. F.; Bawendi, M. G. *J. Phys. Chem. B* **1997**, *101*, 9463–9475.
(27) Xie, R.; Kolb, U.; Li, J.; Basche, T.; Mews, A. *J. Am. Chem. Soc.* **2005**, *127*, 7480–7488.
(28) Steckel, J. S.; Coe-Sullivan, S.; Bulovic, V.; Bawendi, M. G. *Adv. Mater.* **2003**, *15*, 1862–1866.
(29) Wang, X.; Qu, L.; Zhang, J.; Peng, X.; Xiao, M. *Nano Lett.* **2003**, *3*, 1103–1106.
(30) Talapin, D. V.; Mekis, I.; Goetzinger, S.; Kornowski, A.; Benson, O.; Weller, H. *J. Phys. Chem. B* **2004**, *108*, 18826–18831.
(31) Xu, S.; Kumar, S.; Nann, T. *J. Am. Chem. Soc.* **2006**, *128*, 1054–1055.
(32) Talapin, D. V.; Rogach, A. L.; Mekis, I.; Haubold, S.; Kornowski, A.; Haase, M.; Weller, H. *Colloids Surf., A* **2002**, *202*, 145–154.
(33) Micic, O. I.; Smith, B. B.; Nozik, A. J. *J. Phys. Chem. B* **2000**, *104*, 12149–12156.
(34) Rogach, A. L.; Eychmüller, A.; Hickey, S. G.; Kershaw, S. V. *Small* **2007**, *3*, 536–557.
(35) Shavel, A.; Gaponik, N.; Eychmüller, A. *J. Phys. Chem. B* **2006**, *110*, 19280–19284.
(36) Zhang, H.; Zhou, Z.; Yang, B.; Gao, M. *J. Phys. Chem. B* **2003**, *107*, 8–13.
(37) Hinds, S.; Myrskog, S.; Levina, L.; Koleilat, G.; Yang, J.; Kelley, S. O.; Sargent, E. H. *J. Am. Chem. Soc.* **2007**, *129*, 7218–7219.
(38) Fernee, M. J.; Thomsen, E.; Jensen, P.; Rubinsztein-Dunlop, H. *Nanotechnology* **2006**, *17*, 956–962.
(39) Du, H.; Chen, C.; Krishnan, R.; Krauss, T. D.; Harbold, J. M.; Wise, F. W.; Thomas, M. G.; Silcox, J. *Nano Lett.* **2002**, *2*, 1321–1324.
(40) Lifshitz, E.; Brumer, M.; Kigel, A.; Sashchiuk, A.; Bashouti, M.; Sirota, M.; Galun, E.; Burshtein, Z.; Le Quang, A. Q.; Ledoux-Rak, I.; Zyss, J. *J. Phys. Chem. B* **2006**, *110*, 25356–25365.
(41) Resch-Genger, U.; Hoffmann, K.; Pfeifer, D. Simple Calibration and Validation Standards for Fluorometry. In *Ann. Rev. Fluorescence*; Geddes, C. D., Ed.; Springer Science Businesss Media, Inc.: New York, in press.
(42) Gaponik, N.; Talapin, D. V.; Rogach, A. L.; Hoppe, K.; Shevchenko, E. V.; Kornowski, A.; Eychmüller, A.; Weller, H. *J. Phys. Chem. B* **2002**, *106*, 7177–7185.
(43) Rogach, A. L.; Franzl, T.; Klar, T. A.; Feldmann, J.; Gaponik, N.; Lesnyak, V.; Shavel, A.; Eychmüller, A.; Rakovich, Y. P.; Donegan, J. F. *J. Phys. Chem. C* **2007**, *111*, 14628–14637.

of the intensity and the wavelength scale was previously controlled using certified absorption standards from Hellma GmbH. The fluorescence spectra were measured with a Spectronics Instruments 8100 spectrofluorometer recently described.[44] The wavelength accuracy of the emission and the excitation monochromator of fluorometer 8100 was obtained with a cuvette-shaped, low pressure mercury/argon discharge lamp CAL-2000 (Ocean Optics Inc.; pen-type lamp inside a metal cuvette with a small reflector in the center, model HR4000CG-UV-NIR). The wavelength- and polarization-dependent spectral responsivity $s(\lambda_{em})$ of the detection system was determined with a calibrated quartz halogen lamp placed inside an integrating sphere (calibrated wavelength dependence of the *spectral radiance* ($L_\lambda(\lambda)$) and a calibrated non-fluorescent reflection or white standard (calibrated wavelength dependence of the *spectral radiance factor* $\beta(\lambda)$) from Gigahertz-Optik GmbH for all the measurement conditions (i.e., slit widths of the emission monochromator, polarizer settings, etc.) employed,[44−47] see also the Supporting Information. In addition to the characterization procedures used by us, the Supporting Information contains a description of other procedures suitable for the characterization of fluorescence instruments in non-expert laboratories. Excitation correction curves required for the consideration of the wavelength- and polarization-dependent (relative) spectral irradiance $E_\lambda(\lambda_{ex})$ reaching the sample were obtained with a calibrated Si photodiode that is mounted inside an integrating sphere (Gigahertz-Optik GmbH) placed at sample position.[47] This correction,[44−46] see also Supporting Information, provides the basis for the comparison of the excitation and absorption spectrum of the standard dyes that is mandatory to confirm the independence of the quantum yield from the excitation wavelength or for the determination of the fluorescence quantum yield using different excitation wavelengths for sample and standard. The photonic nature of the exciting light was considered upon division of the corrected excitation spectrum by the corresponding photon energies thereby referencing the excitation correction to the *spectral photon irradiance* ($E_{p,\lambda}$ equaling $E_\lambda \times \lambda/(hc_o)$).[44−47]

All the fluorescence measurements were carried out with Glan Thompson polarizers placed in the excitation and the emission channel set to 0° and 54.7°. The absorption and fluorescence measurements were typically performed with 10 mm-quartz cuvettes (Hellma GmbH) using air saturated solutions at $T = (25 \pm 1)$°C. The concentration dependence of the absorption spectra of the quantum yield standards fluorescein 27 and rhodamine 6G was measured in 50 mm-, 10 mm-, and 1 mm-quartz cuvettes (Hellma GmbH). We used either matched cuvettes or cuvettes, the optical path length of which had been previously controlled. For the determination of the concentration dependence of Φ_f, quartz microcuvettes (Hellma GmbH) were employed. Here, the optical path length was 10 mm in the direction of the excitation beam and 2 mm in the direction of fluorescence detection (perpendicular to the excitation) to minimize reabsorption of fluorescence light at high sample concentrations. The fluorescence quantum yield of each CdTe QD was measured at two excitation wavelengths using two quantum yield standards. For each QD-standard pair and excitation wavelength, the quantum yield was determined twice starting from the stock solutions of the QDs and the standard. For two exemplarily chosen QDs, we performed six measurements against each of the respective two quantum yield standards used to obtain the standard deviations of the Φ_f measurements.

Materials. All the solvents employed were of spectroscopic grade and purchased from Sigma-Aldrich. Prior to use, all the solvents were checked for fluorescent impurities. The quantum yield standards rhodamine 101 (batch no. 019502), rhodamine 6G (batch no. 119202), fluorescein 27 (batch no. 059216), and coumarin 153 (batch no. 029303) were obtained from Lambda Physik GmbH and were of the highest purity commercially available. For all dyes, only fresh solutions were used to avoid additional uncertainties, for example, because of acid−base equilibria.[48] Thioglycolic acid stabilized CdTe QDs of different sizes were synthesized in aqueous solution according to a previously described procedure.[35] All the samples of CdTe QDs were taken occasionally from different synthetic batches to provide arbitrarily chosen rather than selected samples. No special treatments allowing to increase fluorescence quantum yields postpreparatively (e.g., via photochemical etching[42,49]) were applied to these samples.

Safety Considerations. Proper safety procedures for the handling, storage, and disposal of CdTe QDs should be observed.

RESULTS AND DISCUSSION

Determination of Φ_f. The determination of the fluorescence quantum yield of a fluorophore using a relative optical method consists of the following steps: (i) measurement of the absorption and emission spectrum of the sample, (ii) choice of a suitable fluorescence quantum yield standard absorbing and emitting within a similar wavelength region as the sample the quantum yield of which should be reliably known for the measurement conditions to be used (e.g., solvent/matrix, excitation wavelength, temperature, chromophore concentration),[16,41,50] (iii) choice of measurement conditions (e.g., excitation wavelength λ_{ex} and absorbance at λ_{ex}, identical instrument settings for sample and standard) and measurement of the corresponding absorption and emission spectra of sample and standard and the emission spectra of the corresponding solvents, that must be subtracted from the emission spectra of sample and standard to remove possible background signals (scattering and fluorescence from the solvent; dark counts at the detector),[44] and (iv) data evaluation and calculation of the relative fluorescence quantum yield according to eq 2.[5] In eq 2, the subscripts "x" and "st" denote sample and standard. F is the spectrally integrated photon

(44) Resch-Genger, U.; Pfeifer, D.; Monte, C.; Pilz, W.; Hoffmann, A.; Spieles, M.; Rurack, K.; Hollandt, J.; Taubert, D.; Schönenberger, B.; Nording, P. *J. Fluoresc.* **2005**, *15*, 315–336.

(45) Resch-Genger, U.; DeRose, P. Characterization of photoluminescence measuring systems (IUPAC Technical Note 2008) submitted to the Fluorescence Task Force Group, IUPAC.

(46) Resch-Genger, U.; Pfeifer, D.; Hoffmann, K.; Flachenecker, G. ; Hoffmann, A.; Monte, C. Linking fluorometry to radiometry with physical and chemical transfer standards: instrument characterization and traceable fluorescence measurements. In *Standardization and Quality Assurance in Fluorescence Measurements I: Techniques*; Resch-Genger, U., Ed.; Springer-Verlag: Berlin Heidelberg, 2008; Vol. 5, pp 65−100.

(47) Hollandt, J.; Taubert, R. D.; Seidel, J.; Resch-Genger, U.; Gugg-Helminger, A.; Pfeifer, D.; Monte, C.; Pilz, W. *J. Fluoresc.* **2005**, *15*, 301–313.

(48) Seybold, P. G.; Gouterman, M.; Callis, J. *Photochem. Photobiol.* **1969**, *9*, 229–242.

(49) Bao, H.; Gong, Y.; Li, Z.; Gao, M. *Chem. Mater.* **2004**, *16*, 3853–3859.

(50) Resch-Genger, U.; Hoffmann, K.; Nietfeld, W.; Engel, A.; Neukammer, J.; Nitschke, R.; Ebert, B.; Macdonald, R. *J. Fluoresc.* **2005**, *15*, 337–362.

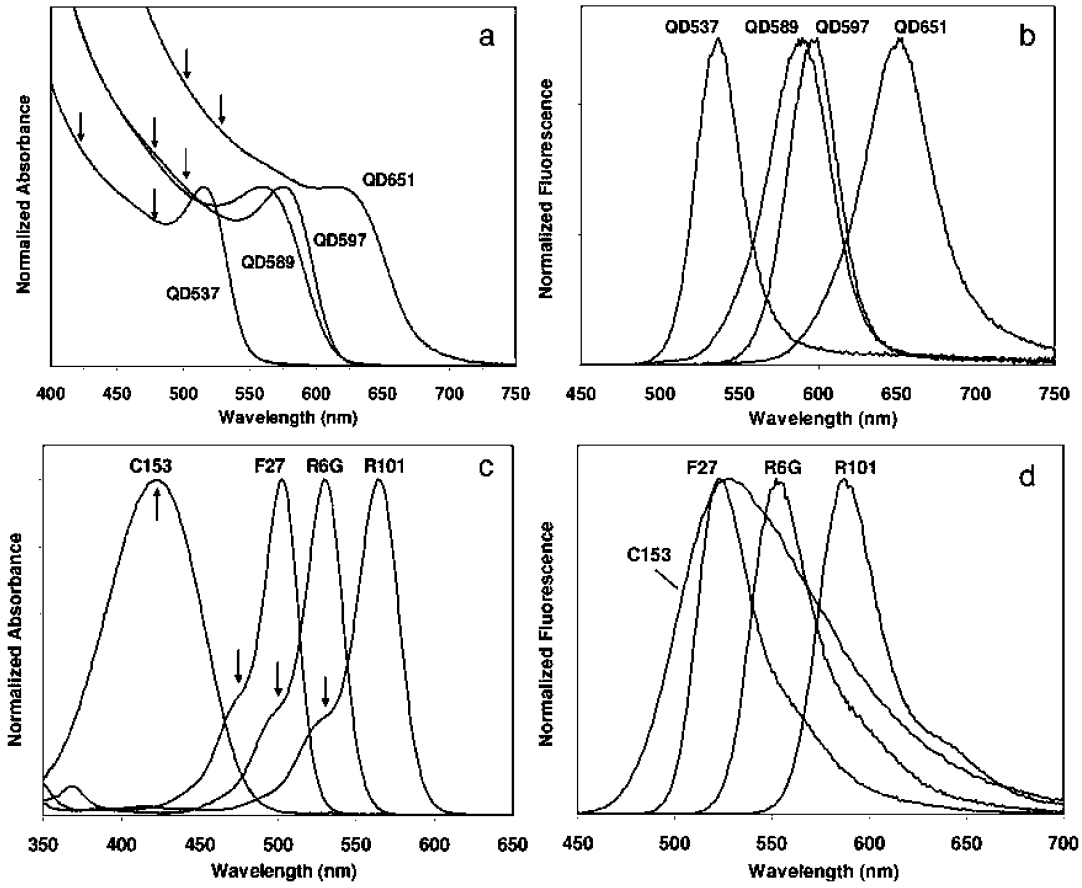

Figure 1. Absorption (a) and corrected emission (b) spectra of differently sized CdTe QDs in water and absorption (c) and emission (d) spectra of the fluorescence quantum yield standards, coumarin 153 (solvent ethanol), fluorescein 27 (solvent 0.1 M NaOH), rhodamine 6G (solvent ethanol), and rhodamine 101 (solvent ethanol). The chosen excitation wavelengths are indicated with arrows and are listed in Table 2.

flux $q_P(\lambda_{em})$ at the detector, i.e., the area under the emission spectrum $I_c(\lambda_{ex}, \lambda_{em})$ corrected for blank emission and the wavelength dependence of the instrument's spectral responsivity, see Supporting Information, that has to be multiplied with λ_{em} prior to integration (see eq 3) to account for the energy of the emitted photons.[12,44−46,51,52] Equation 2 contains also a refractive index correction term (n_i^2) that has to be applied if different solvents are used for sample and standard.[53] The absorption factor $f(\lambda_{ex})$, see eq 4, provides the fraction of the excitation light absorbed by the chromophore.[54] In eq 4, $A(\lambda_{ex})$ equals the absorbance, ε the molar decadic absorption coefficient, c the chromophore concentration, l the optical path length, and $T(\lambda_{ex})$ the transmittance. Thus, both instrument- and sample-related uncertainties can affect the reliability of the accordingly obtained fluorescence quantum yields.[16,41,44,46,50,55]

$$\Phi_{f,x} = \Phi_{f,st} \cdot \frac{F_x}{F_{st}} \cdot \frac{f_{st}(\lambda_{ex})}{f_x(\lambda_{ex})} \cdot \frac{n_x^2}{n_{st}^2} \quad (2)$$

$$F = \int_{\lambda_1}^{\lambda_2} q_P(\lambda_{ex}, \lambda_{em})\, d\lambda_{em} = (hc_0)^{-1} \int_{\lambda_1}^{\lambda_2} I_c(\lambda_{ex}, \lambda_{em}) \lambda_{em}\, d\lambda_{em} \quad (3)$$

$$f(\lambda_{ex}) = 1 - T(\lambda_{ex}) = 1 - 10^{-A(\lambda_{ex})} = 1 - 10^{-\varepsilon(\lambda_{ex}) cl} \quad (4)$$

Choice of Quantum Yield Standard and Excitation Wavelength. The absorption spectra and the corrected emission spectra of the CdTe QDs in water are shown in Figure 1, panels a and b, respectively. The absorption maxima of 516 nm (QD537), 560 nm (QD589), 576 nm (QD597), and 617 nm (QD651) correspond to particle diameters of 2.2, 2.9, 3.1, and 3.5 nm, respectively, as determined from recently published size-curves.[43] Figure 1 also contains the absorption and emission spectra of the organic dyes chosen as quantum yield standards (panels c and d). Criteria for the choice of these standards were comparable regions of absorption and emission, comparatively well-known fluorescence quantum yields and, at least in most cases, the possible excitation of the sample and the standard at an almost plateau-like region or at least at a wavelength where the slope in the absorption spectrum is considerably flat.[16,50] The quantum yield standards including the excitation wavelengths used, and the Φ_f values taken from the literature[56−64] are given in Table 1. Because of the considerable variation of some of these values and the need for the use of several quantum yield standards for this study to

(51) Ejder, E. *J. Opt. Soc. Am.* **1969**, *59*, 223–224.
(52) Chapman, J. H.; Kortum, G.; Lippert, E.; Melhuish, W. H.; Nebbia, G.; Parker, C. A.; Fresenius, Z. *Anal. Chem.* **1963**, *197*, 431–433.
(53) Mielenz, K. D. *Appl. Opt.* **1978**, *17*, 2875–2876.
(54) Braslavsky, S. E.; et al. *Pure Appl. Chem.* **2007**, *79*, 293–465; Glossary of Terms Used in Photochemistry, 3rd edition (IUPAC recommendations 2006).
(55) DeRose, P. C.; Early, E. A.; Kramer, G. W. *Rev. Sci. Instrum.* **2007**, *78*, 033107/1−033107/12.

Table 1. Evaluation of the Fluorescence Quantum Yield Standards Used for the Determination of the Φ_f Values of the CdTe QDs[a]

standard dye	solvent	Φ_f	reference dye	λ_{ex} (nm)	Φ_f (lit.)
rhodamine 101 (R101)	ethanol	n.d.			0.96[56] 0.95,[57] 0.97[58]
rhodamine 6G (R6G)	ethanol	0.95 (±0.01)	R101	490, 505, 530	0.95,[57] 0.94,[59] 0.88[60]
fluorescein 27 (F27)	0.1 M NaOH	0.87 (±0.015)	R6G	465, 480, 502	0.86,[58] 0.90,[58] 0.91,[62] 0.81[61]
coumarin 153 (C153)	ethanol	0.53 (±0.02)	F27	455, 465, 475	0.40,[57] 0.26,[64] 0.58[63]

[a] The fluorescence quantum yield of rhodamine 101 was taken from the literature ($\Phi_f = 0.96$) and used as reference. The Φ_f values of rhodamine 6G, fluorescein 27, and coumarin 153 were determined in air-saturated solution at $T = (25 \pm 1)$°C successively in the given order using the previously characterized dye in the column "reference dye" as standard employing the listed excitation wavelengths (column "λ_{ex}") thereby covering the excitation wavelength range to be used for the QDs. Within this range, the Φ_f values of all dyes were found to be independent of excitation wavelength. For ethanol and 0.1 M NaOH, we used refractive indices of 1.360 and 1.335 respectively. The given Φ_f values present excitation-wavelength averaged data (six measurements, three excitation wavelengths for each dye, two independent measurements per wavelength). The stated standard deviations were obtained from these six measurements. For comparison, selected Φ_f values from the literature (Φ_f (lit.)) are given. n.d.: not determined.

cover the absorption and emission range of the CdTe QDs the reliability of the Φ_f values of these dyes was controlled by the determination of this quantity for each dye in air-saturated solution at $T = (25 \pm 1)$°C at different excitation wavelengths (2 independent measurements per excitation wavelength) using the following pairs ("standard dye" vs "reference dye"; so-called chemical transfer standard dye approach[5]) and the accordingly obtained Φ_f values for each dye: rhodamine 6G versus rhodamine 101, fluorescein 27 versus rhodamine 6G, and coumarin 153 versus fluorescein 27, thereby employing the comparatively well characterized dye rhodamine 101 with its very consistent Φ_f values as ultimate reference.[56−58] The excitation wavelengths were chosen to cover the excitation wavelength regions subsequently used for the determination of the Φ_f values of the CdTe QDs thereby determining and accordingly including a potential wavelength dependence of the Φ_f values of the quantum yield standards. As for coumarin 153, no direct measurement of Φ_f at the excitation wavelength employed in the determination of the Φ_f of the QDs (422 nm) was possible with the reference dye fluorescein 27, see Figure 1c, the excitation wavelength independence of Φ_f was confirmed by a comparison of the absorption spectrum (the wavelength dependence of $f(\lambda_{ex})$) and the corrected excitation spectrum in photonic units (see, e.g., Supporting Information, Figure 1S). This was additionally controlled with measurements of the absolute fluorescence quantum yield of coumarin 153 at the chosen excitation wavelengths using a recently developed setup from Hamamatsu Inc. (absolute photoluminescence quantum yield measurement system C9920−02) providing Φ_f values of 0.53 independent of excitation wavelength. For all the quantum yield standards, Φ_f was found to be independent of excitation wavelength within the excitation wavelength regions used in this study taking into account typical uncertainties (relative standard deviations) of fluorescence quantum yield measurements from previous experiments (six independent measurements) with small organic dyes of ±5% (for $\Phi_f > 0.4$).[65] Table 1 summarizes the accordingly determined excitation wavelength-averaged Φ_f values including standard deviations. With values of ≤4%, the relative standard deviations of the Φ_f values are below the uncertainties stated above. To minimize standard-related uncertainties, only these averaged Φ_f values were used for the subsequent determination of the fluorescence quantum yields of the CdTe QDs. The suitability of the exploited chemical transfer standard dye approach[5] follows directly from the excellent agreement between the relative and absolute Φ_f values of Coumarin 153.

Instrument Properties to be Considered. Instrument properties that can affect the reliability and uncertainty of Φ_f values are the accuracy of the wavelength and absorbance scale of the absorption spectrometer, which can be both easily determined and controlled with the aid of commercial absorption standards,[66] and all the fluorometer quantities and parameters that can affect the spectral position, spectral shape, and intensity of measured fluorescence signals, see also Supporting Information.[44] This includes the wavelength accuracy of the instrument's excitation and emission channel,[67] the range of linearity of the instrument's detection system, and the wavelength- and polarization-dependent (relative) spectral responsivity of its emission channel ($s(\lambda_{em})$), termed also emission correction.[44−47,55] Uncertainties in the wavelength accuracy of spectrofluorometers that are typically in the order of 0.5 nm[45] can be neglected in most cases. To minimize uncertainties due to the different slit widths and shapes of the slit functions of the absorption and fluorescence spectrometer the excitation slits of the fluorescence instrument should be kept as narrow as possible. For samples and standards displaying an emission anisotropy r of about ≤ 0.05 polarizers are dispensable without strongly enhancing the measurement uncertainty.[44−46] However, for anisotropic emitters such as fluorophores in solid matrixes, fluorophores bound to macro-

(56) Arden, J.; Deltau, G.; Huth, V.; Kringel, U.; Peros, D.; Drexhage, K. H. *J. Lumin.* **1991**, *48−49*, 352–358.
(57) Drexhage, K. H. *J. Res. Natl. Bur. Stand., A* **1976**, *80A*, 421–428.
(58) Galanin, M. D.; Kut'enkov, A. A.; Smorchkov, V. N.; Timofeev, Y. P.; Chizhikov, Z. A. *Opt. Spektrosk.* **1982**, *53*, 683–690.
(59) Georges, J.; Arnaud, N.; Parise, L. *Appl. Spectrosc.* **1996**, *50*, 1505–1511.
(60) Rohwer, L. S.; Martin, J. E. *J. Lumin.* **2005**, *115*, 77–90.
(61) Magde, D.; Wong, R.; Seybold, P. G. *Photochem. Photobiol.* **2002**, *75*, 327–334.
(62) Chekalyuk, A.; Fadeev, V.; Georgiev, G.; Kalkanzhiev, T.; Nikolov, Z. *Spectrosc. Lett.* **1982**, *15*, 355–365.
(63) Kubin, R. F.; Fletcher, A. N. *Chem. Phys. Lett.* **1983**, *99*, 49–52.
(64) Jones, G., II; Jackson, W. R.; Halpern, A. M. *Chem. Phys. Lett.* **1980**, *72*, 391–395.
(65) Rurack, K.; Bricks, J. L.; Schulz, B.; Maus, M.; Reck, G.; Resch-Genger, U. *J. Phys. Chem. A* **2000**, *104*, 6171–6188.
(66) Travis, J. C.; Zwinkels, J. C.; Mercader, F.; Ruiz, A.; Early, E. A.; Smith, M. V.; Noeel, M.; Maley, M.; Kramer, G. W.; Eckerle, K. L.; Duewer, D. L. *Anal. Chem.* **2002**, *74*, 3408–3415.
(67) ASTM E 388-04 (2004, original version 1972); Spectral bandwidth and wavelength accuracy of fluorescence spectrometers. In Annual book of ASTM standards, vol 03.06.

Table 2. Spectroscopic Properties and Fluorescence Quantum Yields of CdTe QDs of Various Sizes Dissolved in Bidistilled (Milli-Q) Water: Wavelengths of the First Excitonic Absorption Maximum (λ_{abs}) and the Emission Maximum (λ_{em}), Excitation Wavelengths (λ_{ex}), and Quantum Yield Standards Used for the Determination of Φ_f and Obtained Φ_f Values[a]

quantum dot	Φ_f	standard deviation	λ_{abs} (nm)	λ_{em} (nm)	λ_{ex} (nm)	standard
QD537	0.267		516	537	422	coumarin 153
	0.303				475	fluorescein 27
QD589	0.348	0.020	560	589	476	fluorescein 27
	0.329	0.022			499	rhodamine 6G
QD597	0.360	0.026	576	597	479	fluorescein 27
	0.313	0.028			502	rhodamine 6G
QD651	0.695		617	651	505	rhodamine 6G
	0.726				527	rhodamine 101

[a] All the Φ_f measurements were performed in air-saturated solution at $T = (25 \pm 1)$°C at an absorbance of 0.04 at the excitation wavelength (matching absorbances of sample and standard). The Φ_f values of the quantum yield standards follow from Table 1. For bidistilled water, we used a refractive index of 1.333.

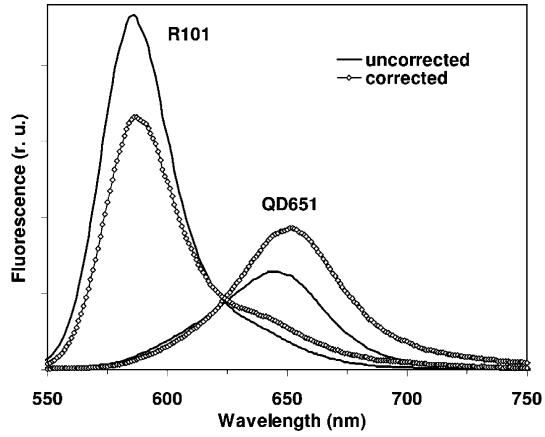

Figure 2. Comparison of an exemplarily chosen uncorrected and spectrally corrected emission spectrum of CdTe (QD651) and the corresponding quantum yield standard (rhodamine 101).

molecules, and rod-shaped QDs and other systems with a shape-introduced emission anisotropy[1,44,68,69] polarization effects can result in considerable uncertainties provided that no polarizers are used. To minimize such effects, generally, the use of polarizers in the excitation and emission channel is recommended. Here, magic angle conditions are to be favored as employed by us with the excitation polarizer set to 0° and the emission polarizer set to 54.7°.[70] Under these conditions, the detected emission intensities for samples in solution are independent of possible emission anisotropy.

The influence of $s(\lambda_{em})$ on emission spectra and fluorescence quantum yields is illustrated in Figure 2 comparing the uncorrected emission spectrum ($I_u(\lambda_{ex}, \lambda_{em})$) and corrected emission spectrum ($I_c(\lambda_{ex}, \lambda_{em}) = I_u(\lambda_{ex}, \lambda_{em})/s(\lambda_{em})$) of an exemplarily chosen QD (QD651) and the corresponding spectrum of the quantum yield standard (rhodamine 101).

Calculation of the fluorescence quantum yield from the uncorrected emission spectra of sample and standard shown in Figure 2 yields a Φ_f value of 0.40 compared to 0.73 as obtained from spectrally corrected spectra. This deviation of about 50% considerably exceeds the achievable uncertainties for Φ_f values summarized in Table 1. The size of the deviations between the integral fluorescence intensities derived from uncorrected and corrected emission spectra depends on the differences in the emission range of sample and standard.[50] Such deviations are more pronounced with a decreasing degree of spectral matching between standard and sample and typically increase at longer wavelengths as compared to the visible region around 500 to 600 nm since in the long wavelength region, the spectral responsivity of most detection systems displays a stronger wavelength dependence.[44] A straightforward and simple approach to the determination of $s(\lambda_{em})$ is the use of certified spectral fluorescence standards that can provide a comparability of (spectrally corrected) emission spectra better than 5%.[71−75]

Φ_f Values of CdTe QDs. The Φ_f values of the CdTe QDs in air-saturated distilled water are summarized in Table 2. These data have been determined at $T = (25 \pm 1)$°C using an absorbance of about 0.04 at the respective excitation wavelength for both the QD and the standard dye and employing two excitation wavelengths and two previously evaluated quantum yield standards, see Table 1. As follows from Table 2, with values in the order of 0.27 to 0.36 for the 3 CdTe QDs emitting below 600 nm and a fluorescence quantum yield of about 0.70 revealed by the biggest QDs, QD651, emitting above 600 nm, the fluorescence quantum yields are within the range of Φ_f values typically reported for

(68) Talapin, D. V.; Koeppe, R.; Goetzinger, S.; Kornowski, A.; Lupton, J. M.; Rogach, A. L.; Benson, O.; Feldmann, J.; Weller, H. *Nano Lett.* **2003**, *3*, 1677–1681.

(69) Koberling, F.; Kolb, U.; Philipp, G.; Potapova, I.; Basche, T.; Mews, A. *J. Phys. Chem. B* **2003**, *107*, 7463–7471.

(70) Mielenz, K. D.; Cehelnik, E. D.; McKenzie, R. L. *J. Chem. Phys.* **1976**, *64*, 370–374.

(71) Pfeifer, D.; Hoffmann, K.; Hoffmann, A.; Monte, C.; Resch-Genger, U. *J. Fluoresc.* **2006**, *16*, 581–587.

(72) Federal Institute for Materials Research and Testing (BAM) (2006) Certificates of analysis, Certified Reference Material BAM-F001, BAM-F002, BAM-F003, BAM-F004, and BAM-F001. Spectral fluorescence standard for the determination of the relative spectral responsivity of fluorescence instruments within its emission range. Certification of emission spectra in 1 nm-intervals. The corresponding product numbers from Sigma-Aldrich for the ready made standards are 97003-1KT-F for the Calibration Kit and 72594, 23923, 96158, 74245, and 94053 for BAM-F001, BAM-F002, BAM-F003, BAM-F004, and BAM-F005, respectively.

(73) Resch-Genger, U.; Pfeifer, D. Certification report, Calibration Kit Spectral Fluorescence Standards BAM-F001−BAM-F005, BAM (2006).

(74) DeRose, P. C.; Wang, L.; Gaigalas, A. K.; Kramer, G. W.; Resch-Genger, U.; Panne, U. Need for and metrological approaches towards standardization of fluorescence measurements from the view of National Metrology Institutes. In *Standardization and Quality Assurance in Fluorescence Measurements I: Techniques*; Resch-Genger, U., Ed.; Springer-Verlag: Berlin Heidelberg, 2008; Vol. 5, pp 33−64.

(75) Gardecki, J. A.; Maroncelli, M. *Appl. Spectrosc.* **1998**, *52*, 1179–1189.

these colloids.[43] The relative standard deviations of the Φ_f values of the QDs, derived from 6 measurements, that are between 6.5% (QD589) and 8% (QD597), slightly exceed the relative standard deviations obtained for the fluorescence quantum yield standards.[65] However, with the exception of QD651, all the QDs chosen for the characterization in this work reveal Φ_f values <0.4. The relative standard deviations found for QD589 and QD597 lay well within the typical uncertainties (relative standard deviations) of fluorescence quantum yield measurements derived from previous experiments for weaker emitting dyes (±10% found for $0.2 > \Phi_f > 0.02$).[65] This clearly demonstrates that with proper circumvention of the discussed sources of uncertainty of Φ_f measurements and consideration of the colloid nature of the QDs including QD-specific effects such as photobrightening, see next section, the same accuracy and reliability of Φ_f data can be achieved as for small molecular systems like organic dyes. The deviation between the Φ_f values of QD537 measured at 422 and 475 nm (relative deviation of 13%) that slightly exceeds the relative deviations of 6.5% and 8% determined for QD589 and QD597, respectively, can provide a hint for a small excitation wavelength dependence of Φ_f within the excitation wavelength interval chosen. Such a dependence of the fluorescence quantum yield may be ascribed to a possible contribution of non-emissive species in the colloidal solution (e.g., thiolate complexes of cadmium, small clusters of cadmium sulphide or cadmium telluride) to the absorption at lower wavelength. A clear assignment here, however, requires more systematic investigations that were beyond the scope of this study. For all the other CdTe QDs, the Φ_f values do not reveal an excitation wavelength dependence considering the measurement uncertainties provided.

QD-Specific Sources of Uncertainty. QD-specific properties that can possibly influence measured fluorescence quantum yields include photobrightening as well as an influence of QD concentration and excitation wavelength.

Photobrightening. Ensembles of QDs can reveal an increase in photoluminescence upon illumination termed photobrightening.[76,77] This process is reversible and the QDs return to their initial luminescence intensity after being kept in the dark.[2,78] After sufficient prolonged illumination finally photobleaching occurs, as is the case for organic dyes, indicated typically by a blue shift in absorption and emission. The mechanism of this phenomenon that is related to the QD quality (i.e., surface passivation) is not clear yet but seems to be most probably related to the light-induced saturation of defect states which are predominately located at the QD-surface.[2] Obviously, photobrightening that can depend on the wavelength of the exciting light and is typically most pronounced for UV excitation[79,80] can affect the measured fluorescence quantum yield. Thus, prior to Φ_f measurements with QDs it is always recommended to check on the occurrence of photobrightening, for example, by measuring a time trace of the fluorescence intensity at typically used measurement conditions (spectral irradiance reaching the sample, illumination time) for an exemplarily chosen sample that has been previously kept in the dark. Advantageously, in our case, CdTe QDs do not reveal photobrightening under the experimental conditions chosen as follows from the representative time trace shown in Figure 3.

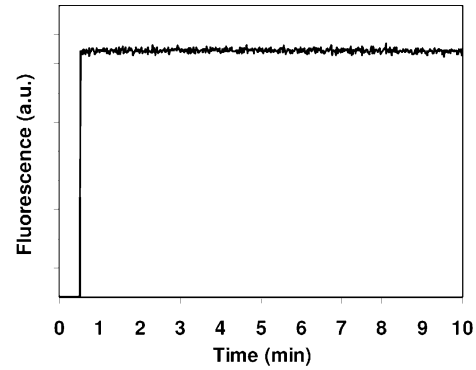

Figure 3. Time dependence of the fluorescence intensity under the illumination conditions used for the determination of Φ_f for an exemplarily chosen CdTe QD (QD589). Excitation was at 480 nm, emission at 589 nm.

Influence of QD Concentration. For QDs, environment effects on spectroscopic features are mainly governed by the accessibility of the core surface that depends on the ligands (and the strength of its bonding to QD surface atoms) and, for core−shell systems, also on the shell quality.[2,76,77] Especially the fluorescence quantum yield is strongly influenced by surface properties.[2,81] The bonding nature of organic ligands to the surface atoms of nanocrystals and the related ligand- and matrix-dependent adsorption−desorption equilibria have been only marginally investigated.[82,83] The latter processes can also result in concentration-dependent fluorescence quantum yields, especially for weakly bound ligands such as many monodentate compounds.[2,82,84,85] This can affect the reliability of the measured fluorescence quantum yields for such colloidal systems, especially since the absorbances, and thus the particle concentrations used for the determination of fluorescence quantum yields, can cover a comparatively broad region (e.g., absorbances from 0.05 up to at least 0.2). Moreover, the used absorbances are often even not provided. Similarly problematic in this respect can be the not well-defined surface chemistry of QDs that underwent ligand exchange, for example, for the transfer from a hydrophobic into a hydrophilic environment.

The influence of the QD concentration on the fluorescence quantum yield together with the corresponding absorption and emission spectra is shown in Figure 4 for two exemplarily chosen CdTe QDs of different size, here QD651 (top panel) and QD589 (bottom panel). For the Φ_f measurements, the absorbance of the standard was adjusted to match exactly the absorbance of

(76) Ziegler, J.; Merkulov, A.; Grabolle, M.; Resch-Genger, U.; Nann, T. *Langmuir* **2007**, *23*, 7751–7759.
(77) Grabolle, M.; Ziegler, J.; Merkulov, A.; Nann, T.; Resch-Genger, U. *Ann. N.Y. Acad. Sci.* **2008**, *1130*, 235–241.
(78) Asami, H.; Abe, Y.; Ohtsu, T.; Kamiya, I.; Hara, M. *J. Phys. Chem. B* **2003**, *107*, 12566–12568.
(79) Bentolila, L. A.; Weiss, S. *Cell Biochem. Biophys.* **2006**, *45*, 59–70.
(80) Stouwdam, J. W.; Shan, J.; Van Veggel, F. C. J. M.; Pattantyus-Abraham, A. G.; Young, J. F.; Raudsepp, M. *J. Phys. Chem. C* **2007**, *111*, 1086–1092.
(81) Yu, W. W.; Chang, E.; Drezek, R.; Colvin, V. L. *Biochem. Biophys. Res. Commun.* **2006**, *348*, 781–786.
(82) Ji, X.; Copenhaver, D.; Sichmeller, C.; Peng, X. *J. Am. Chem. Soc.* **2008**, *130*, 5726–5735.
(83) Munro, A. M.; Plante, I. J.-L.; Ng, M. S.; Ginger, D. S. *J. Phys. Chem. C* **2007**, *111*, 6220–6227.
(84) Medintz, I. L.; Berti, L.; Pons, T.; Grimes, A. F.; English, D. S.; Alessandrini, A.; Facci, P.; Mattoussi, H. *Nano Lett.* **2007**, *7*, 1741–1748.
(85) Kopping, J. T.; Patten, T. E. *J. Am. Chem. Soc.* **2008**, *130*, 5689–5698.

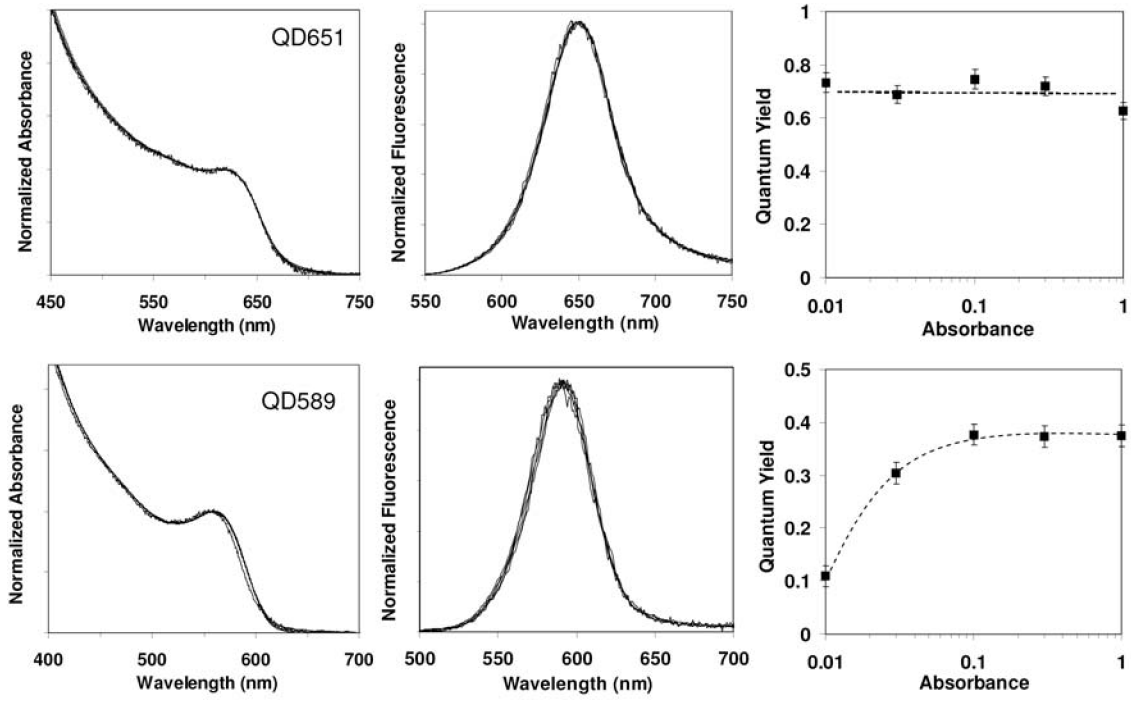

Figure 4. Influence of QD concentration on the normalized absorption (left) and normalized emission (middle) spectra and fluorescence quantum yields (right) of two CdTe QDs of different size: (a) QD651, excitation at 505 nm; (b) QD589, excitation at 478 nm. The absorbance refers to the absorbance at the respective excitation wavelength. For QD651 and QD589, the quantum yield standards rhodamine 6G and fluorescein 27 were used.

the QD at the excitation wavelength to account for the strong absorption of the exciting light beam at high sample concentrations. This is necessary because in conventional spectrometers the fluorescence read-out is focused mainly at the central part of the cuvette. Under these conditions, the use of eq 4 to account for differences in absorbance is only correct at a sufficiently low sample concentration (e.g., below an absorbance of 0.2, the uncertainty caused by non-matching absorbances of sample and standard is smaller than 1% for a 10 mm-cuvette). Reabsorption of fluorescence was minimized by the use of microcuvettes with an optical path length of 2 mm in the direction of the fluorescence detection. These concentration-dependent measurements rely on the assumption of concentration-independent Φ_f values of the chosen quantum yield standards, here rhodamine 6G (for QD651) and fluorescein 27 (for QD589). As organic dyes like fluoresceins or rhodamines are known to be prone to the concentration-dependent formation of non-fluorescent aggregates that could result in a diminution of the fluorescence quantum yield at high dye concentrations[2,86] the absorption spectra of both dyes were carefully examined for signs of dye aggregation such as spectral broadening or the occurrence of extra bands or shoulders. A comparison of the normalized absorption spectra of both standards obtained for absorbances of 0.025 (measured with 50 mm-cells) and 2.5 (measured with 1 mm-cells) at the main absorption band (Supporting Information, Figure 2S), yields matching spectra and reveals no hint for dye aggregation.

Intriguing is the fact that in the case of QD651 (Figure 4, top) the Φ_f values are more or less concentration-independent whereas the fluorescence quantum yield of QD589 (Figure 4, bottom) displays a considerable concentration dependence. This suggests an influence of the particle size on the concentration dependence of Φ_f. The fact that for the chosen ligand, TGA, Φ_f reveals only a pronounced concentration dependence for the smaller CdTe (QD589) with its higher surface-to-volume ratio points to ligand adsorption−desorption equilibria being responsible for this concentration dependence. Most probably, at lower QD concentration, the ligand TGA partially desorbs from the QD surface resulting in a reduction in fluorescence quantum yield.[87] This observation is confirmed by the inspection of two more batches of particles, namely, one CdTe-QD emitting at short wavelength (525 nm) and one emitting at 649 nm both capped with TGA (see Supporting Information, Figure 3S). Consistently with the results above, we found a strong diminution in the fluorescence quantum yield for the smaller particles. For very low QD concentrations (absorbances <0.002), eventually also the quantum yield of the long wavelength-emitting QD decreased. It is noted that dilutions to such low concentration levels are not common for Φ_f measurements but widely used for example in fluorescence microscopy, biolabeling, and imaging. The small initial increase in fluorescence seen in the first dilution steps (see also Supporting Information, Figure 3S) may be attributed to a partial agglomeration at high particle concentration which can result in a lower fluorescence because of self-quenching effects.

For a better understanding of these results it is mentioned that in addition to a relatively lower surface-to-volume ratio bigger CdTe QDs possess a comparatively thick sulfur-enriched surface

(86) Bindhu, C. V.; Harilal, S. S.; Nampoori, V. P. N.; Vallabhan, C. P. G. *Mod. Phys. Lett. B* **1999**, *13*, 563–576.

(87) Döllefeld, H.; Hoppe, K.; Kolny, J.; Schilling, K.; Weller, H.; Eychmüller, A. *Phys. Chem. Chem. Phys.* **2002**, *4*, 4747–4753.

shell which is formed as the result of a partial decomposition of TGA at the later stages of the synthesis as was confirmed by powder XRD,[42] synchrotron XPS,[88] and electrochemical methods.[89] This shell is built of material (CdS) with a larger bandgap than the core material, and it plays an additional role in the surface stabilization and in the improvement of the fluorescence properties.[42,43] Obviously, at moderate dilution levels (with absorbances in the range of or above 0.01) the shift of the equilibrium at the surface of the CdS shell and the partial ligand removal do not influence the inner interface between core and shell which results in the observed stability of the Φ_f of these larger core−shell QDs as opposed to smaller particles in which a CdS shell is not formed properly. At very low concentrations (e.g., below an absorbance of ca. 0.002) even in bigger QDs an efficient removal of surface ligands may cause a partial dissolution of the "naked" outer shell (e.g., because of the formation of soluble Cd-thiolate complexes[35]). This results in a propagation of surface defects through the shell to the core/shell interface and the subsequent reduction of Φ_f. We wish to stress here that a detailed investigation of this phenomenon should include not only dilution experiments of differently sized QDs but should extend to similar trials for QDs stabilized with other thiol-ligands (different binding energy to the surface), to effects of an addition of extra amounts of ligands to the diluted solutions, to the variation of the ionic strength and the pH of the aqueous solutions as well as to studies on other types of QDs and core shell nanostructures including those soluble in organic solvents. These experiments are currently being performed in our groups and will be the subject of a following publication.

Nevertheless, even from the results presented here one may conclude that such concentration effects, apparently depending on the ligand and the QD size can render the reliability of many Φ_f values of QDs reported in the literature questionable. Principally, such effects could be circumvented by very tightly bound bi- or preferably even multidentate ligands or cross-linked ligands[90] or by a passivating inorganic shell. However, in the case of CdTe, bidentate thiol ligands like 2,3-dimercaptopropanol are known to act as luminescence quenchers.[42] This observation of concentration-dependent Φ_f values also underlines the importance of a detailed description of the experimental procedure and parameters used for the determination of the fluorescence quantum yields of QDs. To recognize such a concentration dependence of Φ_f prior to the determination of the fluorescence quantum yield, dilution experiments are recommended to ensure that the absorption-weighted integral fluorescence remains constant.

Influence of Excitation Wavelength. For most organic dyes, Φ_f does not depend on the excitation wavelength as long as a single emissive species is present (i.e., a pure compound, no dimerization or aggregation) and the same lowest energy optical transition is excited.[1] However, for QDs with their size- and surface-dependent optical properties and their preparation-dependent particle size distribution such a dependence of Φ_f on the excitation wavelength is likely to be observed. Even a QD sample with a narrow size distribution still presents an ensemble of emitting nanoparticles with bigger particles absorbing and emitting differently from smaller particles with slightly varying fluorescence quantum yields.[42,91] Accordingly, for QDs, it needs to be controlled whether and to which extent the fluorescence quantum yield displays a dependence on the excitation wavelength. This is especially recommended for QDs revealing a rather broad particle size distribution as indicated by a comparable broad emission band and a relatively unstructured absorption spectrum if different excitation wavelengths are used within a series of experiments to be compared. In any case, if Φ_f values of QDs are reported also the chosen excitation wavelength should be given for comparability purposes.[41]

Whether and to which extent the Φ_f values of QDs depend on the excitation wavelength requires the knowledge of achievable uncertainties of Φ_f values for QDs as provided by us and can be controlled using the following approaches: (i) by measuring the relative fluorescence quantum yield of a QD at different excitation wavelengths as pursued by us here. Because of the typically narrow absorption bands of common quantum yield standards this approach requires the use of different standards with reliably known fluorescence quantum yields for the different excitation wavelengths. Method (ii) relies on the comparison of the integral fluorescence intensity of a QD obtained upon excitation at different wavelengths weighted with the (relative) spectral photon irradiance $E_{p,\lambda}(\lambda_{ex})$ reaching the sample (equaling the wavelength dependence of the excitation channel in photonic units) and the absorption factor $f(\lambda_{ex})$ at the corresponding excitation wavelengths.[44−46,51,52] In the case of an excitation wavelength-independent fluorescence quantum yield, the ratio of the (integral) fluorescence intensity F (see eq 3) and the product $E_{p,\lambda}(\lambda_{ex}) \times f(\lambda_{ex})$ measured at different excitation wavelengths should remain constant. Method (iii) presents the comparison of the absorption spectrum (here $1 - T(\lambda)$) and the corrected fluorescence excitation spectrum of dilute solutions (reference quantity spectral photon irradiance $E_{p,\lambda}(\lambda_{ex})$, see Section 2, Instrumentation, and Supporting Information),[44−47,51,52] ideally measured at different emission wavelengths.[44,46,48] Matching excitation and absorption spectra are to be expected only for a single emissive species with simple excited-state photochemistry. Deviations between the absorption spectrum and the corrected excitation spectrum are indicative of an excitation wavelength dependence of Φ_f.

For non-expert laboratories, only method (i) is recommended, thereby circumventing a tedious excitation correction. To reduce the uncertainty introduced by the mandatory use of different quantum yield standards the quantum yields of which reported in the literature can vary considerably, previous control measurements with the standards as performed by us are strongly recommended. As discussed in the section on the Φ_f values of CdTe QDs, for our CdTe QDs with their narrow size distribution, with this method, we observe only a hint for a small excitation wavelength dependence of Φ_f for QD537 within the excitation wavelength interval chosen.

(88) Borchert, H.; Talapin, D. V.; Gaponik, N.; McGinley, C.; Adam, S.; Lobo, A.; Möller, T.; Weller, H. *J. Phys. Chem. B* **2003**, *107*, 9662–9668.
(89) Poznyak, S. K.; Osipovich, N. P.; Shavel, A.; Talapin, D. V.; Gao, M.; Eychmüller, A.; Gaponik, N. *J. Phys. Chem. B* **2005**, *109*, 1094–1100.
(90) Susumu, K.; Uyeda, H. T.; Medintz, I. L.; Pons, T.; Delehanty, J. B.; Mattoussi, H. *J. Am. Chem. Soc.* **2007**, *129*, 13987–13996.
(91) Talapin, D. V.; Rogach, A. L.; Shevchenko, E. V.; Kornowski, A.; Haase, M.; Weller, H. *J. Am. Chem. Soc.* **2002**, *124*, 5782–5790.

CONCLUSIONS

With the simple procedure presented by us for the determination of the fluorescence quantum yields of nanocrystal labels, circumventing common technical and QD-related pitfalls discussed, Φ_f values of even moderately ($\Phi_f \sim 0.3$) emitting QDs can be determined with uncertainties of 7%. These uncertainties are comparable with the uncertainties achievable for small organic dyes previously reported by us and obtained in this study. As one of the major sources of uncertainty for the reliability of Φ_f values of QDs we identified ligand adsorption−desorption equilibria that are not only material-, ligand- and matrix-dependent, yet can seemingly also reveal a dependence on the QD size. To estimate the influence of such effects on resulting fluorescence quantum yields more systematic studies are needed with different QD materials and typically used ligands and solvents. This is similarly true for the systematic investigation of the excitation wavelength dependence of Φ_f values of QDs.

A first and straightforward step to improve the general reliability of reported Φ_f data for QDs is the supply of a detailed description of the relevant measurement parameters used including information on instrument characterization, choice of quantum yield standard (including employed Φ_f value), choice of excitation wavelength, and QD concentration or absorbance at the excitation wavelength. This should include also the use of at least some of the suggested tests on, for example, photobrightening and a possible excitation wavelength dependence and concentration dependence of Φ_f. Furthermore, although very difficult to achieve at present and not necessarily suited for all QD applications, a better defined surface chemistry should be generally favored like, for example, preferably no mixed ligands covering the QD surface, complete ligand exchange, and so forth. Here, whenever possible, the use of tightly bound multidentate ligands or cross-linked ligands, as well as the formation of inorganic passivating shells, could be helpful.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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