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## Summary

*Quantum dots* (QDs) are increasingly widespread in medicine and environmental research. Because of their specific optical characteristics, QDs can be detected by fluorescence analyses, even in complex media, such as environmental or tissue samples. Their unique properties make them useful for a variety of applications, such as for the use as fluorescent markers for cells, as contrast agents in deep tissue and tumour imaging, in biosensing or photodynamic therapy, and for targeted drug delivery. As a result, QDs could potentially be used as detectable and clearly identifiable “nanotracers” to mark or detect specific targets or to be able to draw general conclusions about the fate of engineered nanoparticles (ENPs) in environmentally relevant media, e.g. in wastewater. QDs mainly consist of metallic semiconductor compounds, such as cadmium selenide (CdSe), cadmium telluride (CdTe), lead sulphide (PbS) or indium phosphide (InP), which can have toxic effects on cells and organisms. Research is therefore being conducted on non-toxic carbon-based QDs, amongst others. QDs are already being used in products, such as TV screens and novel solar cell technologies. However, with an increasing number of applications and thus an increase in production volumes, potential exposure is also intensifying. Consequently, risks to humans and the environment are increasing as accidental release and resulting negative effects cannot be ruled out. To date, however, only limited data exist on possible environmental and health risks.

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# Applications of Fluorescent quantum dots for medical and environmental science applications

## Introduction

Quantum dots (QDs) are nanocrystals made of semiconductor materials and belong to engineered nanoparticles (ENPs). Because of their unique optical properties, QDs are increasingly used as high-contrast and photostable dyes in both medicine and environmental research. Amongst others, QDs are used as fluorescent marker substances in medical cell biology, for example, to make certain cell types visible in tissues.<sup>1</sup> They are also used in environmental samples to be able to draw general conclusions about the fate of ENPs in wastewater.<sup>2</sup> Since many first-generation QDs still contain heavy metals such as cadmium or lead, their use in the medical field is currently subject to strict laboratory conditions and for *ex situ* diagnostics only, and not for direct use on patients.<sup>3,4</sup> In environmental research, too, QDs are currently only used under laboratory conditions. In the future, heavy metal-free QD types could then be used as fluorescent tracers in field studies. Because of their mobility and interaction with complex media (e.g. soil, water, plants), it is possible to study the general environmental behaviour and fate of ENPs in the environment in more detail.<sup>5,6,7,8,9</sup> In medicine and for biological applications, QDs must be water-loving (hydrophilic), biocompatible, and functionalisable with biomolecules and/or drugs; however, the development of QDs without heavy metals and the development of efficient strategies for surface functionalisation (e.g. with specific antibodies for cancer diagnostics) remains a major challenge.

This dossier provides an overview of the specific properties, surface modifications, and the fields of application of QDs in medicine and environmental research.

## Properties and specifics of quantum dots

QDs are nanocrystals with a core-shell architecture. Their size varies depending on the material composition and manufacturing method (synthesis). They have a core diameter of 1 to 10 nanometres (nm). These ENPs usually consist of one or more layers of inorganic semiconductors such as indium phosphide (InP), gallium nitride (GaN), cadmium selenide (CdSe) or cadmium telluride (CdTe), to which organic ligands are bound. These ligands serve to increase the so-called colloidal stability and act as “anchor structures” for further surface functionalisation. Using suitable surface modification, the environmental behaviour of QDs can be “tailored” depending on the area of application.<sup>10</sup> Depending on the original material, particle size, particle size distribution (dispersity), and surface modification, QDs have specific characteristics that can be optically detected. Such detection is done through fluorescence, as QDs emit a specific wavelength in the visible light spectrum or the near-infrared range after excitation with light. The fluorescence of individual QDs is similar to the uniqueness of a “fingerprint”. Their characteristic narrow emission spectrum and their large “Stokes shift” (i.e. a separation of the absorption and emission spectrum), both of which are size-dependent and therefore tunable to each other over the reaction time of the particles during their synthesis, enable these unique optical properties. Using fluorescence microscopy, QDs can specifically be detected in complex media, such as environmental or tissue samples, because of their luminosity or unique fluorescence properties (the “fingerprint”).

Because QDs are also photochemically robust (photostable), they facilitate better localisation and tracking of complex biological processes than would be possible with conventional organic dyes. This makes QDs a good addition to currently used organic dyes.<sup>11</sup> The simple yet bright luminous properties are another advantage of QD technology, and clearly distinguish QDs from

organic dyes. Currently used organic dyes often exhibit a photo-induced chemical degradation, i.e. photobleaching (an undesirable effect for traceability), which occurs especially with prolonged exposure to high light intensities. As a result, the instability of organic dyes hinders time-lapse imaging and therefore also the tracking of complex biological processes. QDs, on the other hand, remain photostable and therefore detectable over a comparatively longer period of time. However, they, too, can only withstand a certain photon density before losing their optically active properties before disintegrating. Long and intensive exposure to light can therefore cause the QD's metallic particle core to disintegrate, releasing toxic metals (in ionic form).<sup>12</sup> To anticipate possible toxic effects to humans and the environment, research on non-toxic carbon-based QDs is already being conducted, amongst others.<sup>13</sup>

The optical properties of organic dyes can be fine-tuned. The majority of common fluorophores, such as rhodamines or cyanines, are resonance dyes that are well characterised and often used. However, as a result of their unfavourable "Stokes shift", their lifetime is often very short (a few minutes) because of the occurring bleaching effects. Consequently, they are not suitable for long-term studies under the fluorescence microscope. The "Stokes shift" of QDs, however, requires broad-band excitation (for comparison, see Figure 1).

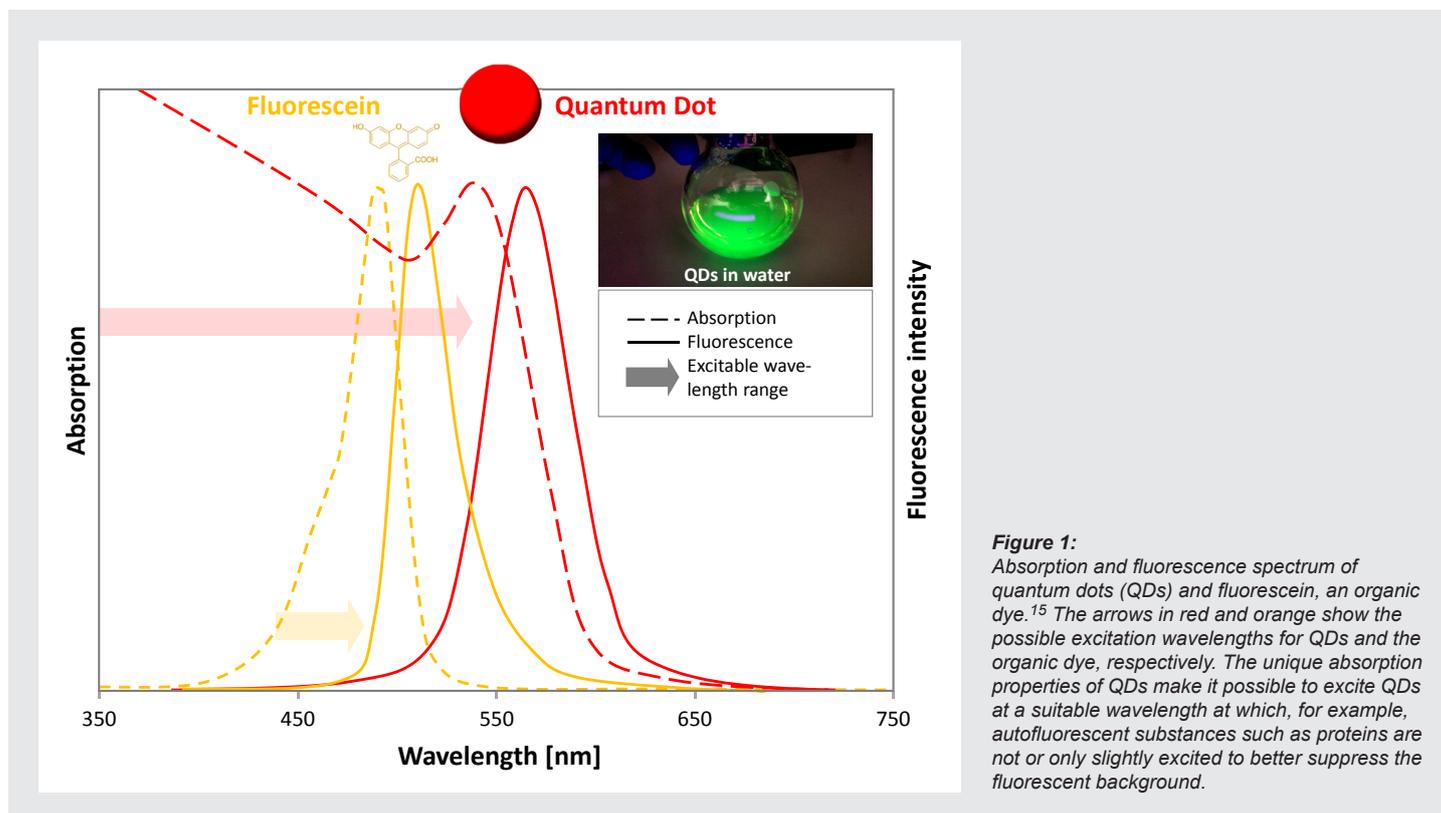
For QDs to interact with certain cellular target structures, biomarkers must be applied to the QD surface. For instance, it is possible to bind known antibodies or interacting peptides to the surface of a QD to detect specific cancer cells and make them visible under the microscope by fluorescence detection. Surface chemistry to bind such markers is an established procedure i.e. "bio-conjugation".<sup>14</sup> Nevertheless, such conjugation can also result in undesirable changes in the fluorescence properties. Functionalisation based on thiol or hydrogen sulphide compounds could improve QDs without changing the original structure of the marker molecules. QDs are therefore a promising addition to organic dyes where targeted marking of cells and their detection through imaging methods (cell targeting) is concerned. Specific cell targeting is also the prerequisite for targeted drug delivery to certain cell structures.<sup>14</sup>

## Medical and biological applications

Biocompatibility is essential for all biomedical applications of QDs (see Figure 2 on the next page), and coating materials (also called *capping layers* or *coating*) can modify the surface properties of QDs to give them dispersity, colloidal stability, photostability, and thus also biocompatibility.

Figuratively, QDs can be seen as a "molecular lantern", for example to make biochemical processes visible through fluorescence microscopy. This concept is an extremely interesting application of QDs in medicine and also in environmental analysis. In both contexts, however, the affinity of a QD to the target analyte is the fundamental prerequisite. Especially for QDs, very specific applications in diagnostic imaging are now available. Nevertheless, luminescence (*photoluminescence quantum yield*) is always a compromise between particle size, chemical composition, and the chosen dispersion media e.g. aqueous solutions, all of which affect the fluorescence properties.

As already mentioned, QDs can also be conjugated with specific peptides, antibodies, and other small molecules. These target a specific cell type, a specific cell structure or a specific tissue<sup>1</sup> and make observation with the help of imaging techniques, such as fluorescence microscopy, possible. Current and future biological and medical QD applications thus include the use of QDs as diagnostic and therapeutic tools, e.g. as fluorescent markers for cells, as contrast agents in deep tissue and tumour imaging, in biosensing or photodynamic therapy, and for targeted drug delivery.



**Figure 1:** Absorption and fluorescence spectrum of quantum dots (QDs) and fluorescein, an organic dye.<sup>15</sup> The arrows in red and orange show the possible excitation wavelengths for QDs and the organic dye, respectively. The unique absorption properties of QDs make it possible to excite QDs at a suitable wavelength at which, for example, autofluorescent substances such as proteins are not or only slightly excited to better suppress the fluorescent background.

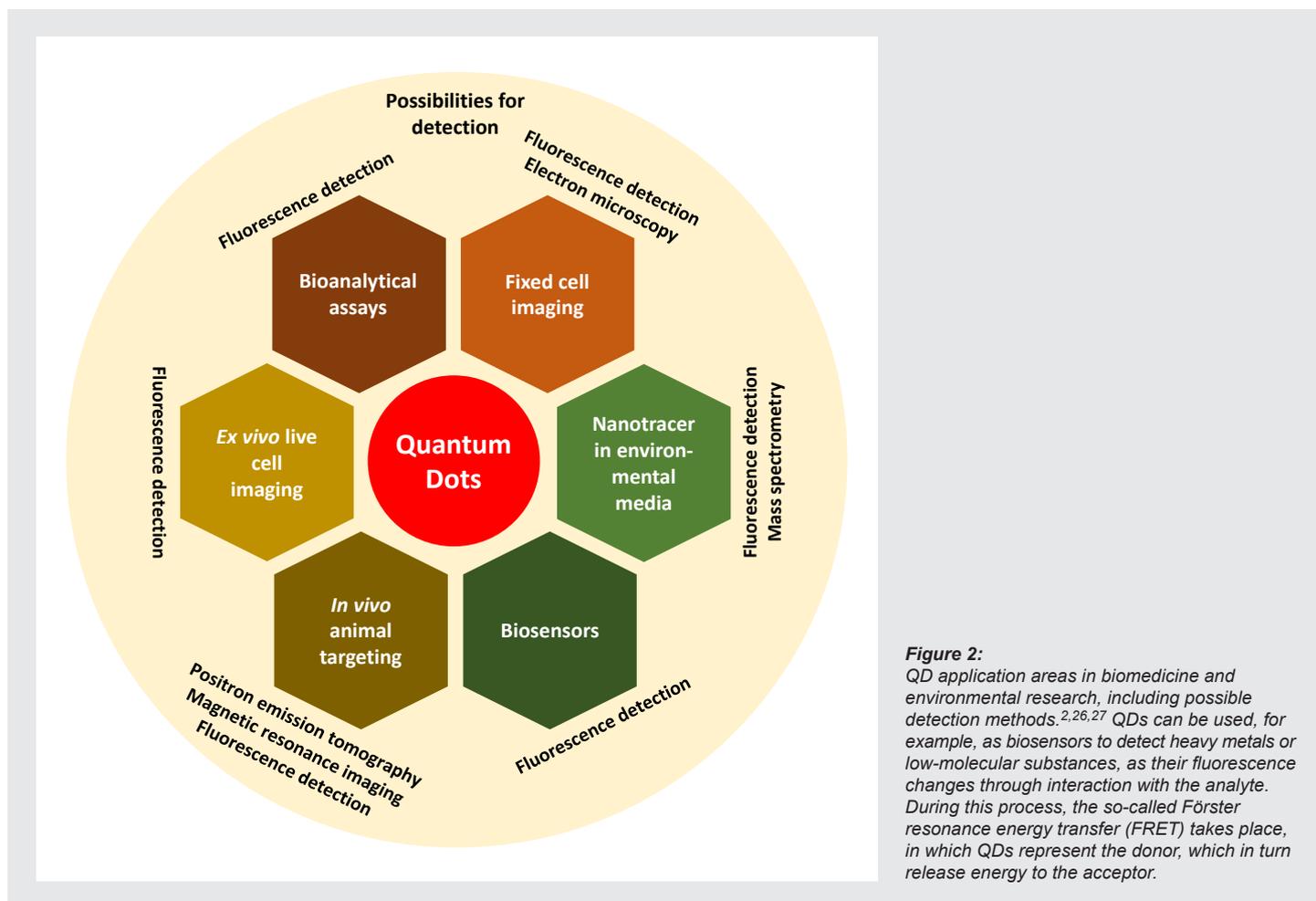
Diagnostics using imaging techniques that use fluorescence is an essential and widely used method in biomedicine. QDs can thus be used as fluorescent markers in bioimaging, as has been demonstrated in tests on mice.<sup>16</sup> Experiments in cell cultures have also shown that surface-modified QDs can be used for the specific detection and visualisation of tumour cells.<sup>17</sup> Successful application of QDs for tumour biomarker detection and tumour cell imaging has great potential for use in early cancer detection as well as tumour removal because of possible accurate visual tracking.<sup>1</sup> QDs can also be excellent markers for analysis in immunohistochemistry and immunocytochemistry when using epifluorescence microscopy or confocal microscopy, for example to support diagnostics in biopsy samples (*ex vivo*) in histology.<sup>18</sup> In diagnostics, QDs can be used either as carriers for the immobilisation of biological recognition elements or as markers for the generation, transmission, and amplification of signals; and even with small sample quantities, when compared with organic dyes, their unique optical properties offer a measurable signal.<sup>19,20</sup>

QDs also show great promise as therapeutic agents for cancer treatment, for instance in the

context of photodynamic therapy (PDT). Here, QDs act as figurative antennae to absorb light and transfer the energy via energy transfer to the closely linked photosensitiser to then initiate the production of *reactive oxygen species* (ROS), which in turn are to specifically damage cancer cells. Photothermal therapy (PTT) is a new technique in cancer treatment in which QDs, following laser irradiation, can efficiently convert light energy into heat to inhibit tumour growth.<sup>21</sup> Initial tests on mice show suitability for the specific removal of tumours.<sup>22</sup> Because of their unique properties, QDs can also play multiple roles in the development of drug delivery systems: they can serve as a means to monitor the administration of the drug or act as a carrier (so-called “nanocarrier”) that transports the drug to the target site to control the dosage of the drug in the target organ.<sup>23</sup> In the future, QDs may also play a role in so-called “point-of-care” diagnostics.<sup>25,24</sup> Meanwhile, the idea of personalised medicine has advanced from research stage into current therapy: with the help of adapted dosages, to the point of individually adapted combinations of substances, it is now possible to respond to a patient’s specific allergies, medical history, and their current clinical situation in the sense of “point-of-care”.

## Health effects

The unique properties of QDs make them useful for a variety of applications. Nevertheless, most QDs consist of compounds with heavy metals, such as CdSe, CdTe or PbS, where the effect of the particles after uptake into the organism can be toxic. For example, QDs can be taken up into the cytoplasm via endocytosis.<sup>1</sup> Their presence in the cell results in the production of reactive oxygen, which in turn can induce oxidative stress and thus be harmful.<sup>28</sup> Although this property is utilised in photodynamic therapy (PDT) to specifically damage cancer cells, the possibility that other cells in the body may also be damaged cannot be ignored. Similarly, QDs can penetrate cell membranes and thus have a possible growth-inhibiting effect on the cell or on the organism, as they influence the cell cycle.<sup>29</sup> Because of their small size, QDs could be used to cross biological barriers, such as the blood-brain barrier, and deliver drugs with precision to the central nervous system. Although this application has great potential, it is currently still the subject of intensive research because of great uncertainties concerning toxicity and long-term effects.



**Figure 2:** QD application areas in biomedicine and environmental research, including possible detection methods.<sup>2,26,27</sup> QDs can be used, for example, as biosensors to detect heavy metals or low-molecular substances, as their fluorescence changes through interaction with the analyte. During this process, the so-called Förster resonance energy transfer (FRET) takes place, in which QDs represent the donor, which in turn release energy to the acceptor.

Toxicity is therefore an essential factor that limits clinical application of QDs *in vivo* or in patients. Currently, intensive research is being conducted to develop heavy metal-free or pure carbon-based QDs. Silicon-based QDs, for example, are a promising alternative to heavy metal-based QDs, as they show no signs of toxicity in animal studies, even several months after treatment.<sup>14</sup> Even though silicon is biocompatible, it is not biodegradable. As a result, it tends to accumulate in organs and has potentially harmful effects that can also emerge at a much later stage. However, long-term studies in primates have so far shown no adverse effects, this indicates that such QDs are safe for humans and could therefore be approved for clinical use in the near future.<sup>14</sup> At present, intensive research is also being conducted on graphene quantum dots (GQDs), which have gained attention in biomedicine in terms of the “safe and sustainable by design” (SSbD) concept thanks to their higher biocompatibility and low cytotoxicity, when compared with other QDs.<sup>30</sup>

## Environmentally relevant applications

Because of their unique properties, QDs qualify as detectable and clearly identifiable so-called “nanotracers”, which are used to closely investigate the final fate of ENPs in the environment through fluorescence analyses.<sup>2,31</sup> The idea of such QD-based nanotracers is to be able to draw fundamental conclusions about the general environmental behaviour of ENPs (e.g. aggregation, sedimentation, particle–particle interactions) in the course of laboratory investigations, since fluorescent QDs, unlike many conventional ENPs such as silicon dioxide or titanium dioxide, can be clearly traced in complex environmental media. Using surface modification, the QDs can be encapsulated in silicon dioxide or titanium dioxide nanoparticles to better track conventional and “hard-to-detect” ENPs.<sup>32</sup>

QD-based nanotracers can be clearly distinguished from naturally occurring nanomaterials because of their spectroscopic “fingerprint”, and they can also be observed over a longer period because of their photostability. By tracking QDs using imaging techniques, it is possible to gain insights into how nanoparticles behave in environmental samples over long periods of time. Fluorescence spectroscopy can also be used to draw conclusions about interactions with natural, organic substances such as proteins, fulvic or humic acids, whereby such potential transformation processes, in turn, play a decisive role where mobility as well as toxicity of nanoparticles is concerned.<sup>5,33</sup>

## Environmental impact

Despite the anticipated increase in industrial production and associated increased release into the environment, there is still comparatively little information on possible release into the environment (e.g. through landfill of QD waste from the semiconductor industry). If QDs are released into the environment unintentionally, toxicity effects such as the release of toxic metal components of QDs can occur, which in turn can lead to damage of microorganisms.<sup>34</sup> In addition to the toxicity to individual cells or organisms, QDs also have the potential to bioaccumulate. Because of their chemical stability (persistence), they can thus also accumulate in higher organisms and reach higher levels of the food chain via trophic transfer. This poses not only a potential danger to the organisms themselves, but also to the food chain.<sup>34</sup> Current knowledge indicates that heavy metal-based QDs accumulate in and have ecotoxic effects on microorganisms, aquatic invertebrates, and vertebrates in fresh and seawater; the risk to the environment, however, is highly dependent on the physicochemical properties, environmental conditions, concentration, and, above all, exposure time (dose).<sup>34</sup>

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## Conclusion

In everyday life, QDs are already used in products such as solar cell technologies<sup>35</sup> or TV screens<sup>36</sup> (known as “quantum dot solar cells” or “QLED TVs”). Nevertheless, a release of QDs into the environment and resulting negative effects cannot be ruled out over the product life cycle. At present, however, only limited data are available on possible environmental and health risks.

The future increased use of QDs in biomedicine or environmental analysis is a great opportunity, but can lead to greater exposure, therefore also posing a risk to human health and the environment. To date, little is known about the efficacy and interactions of QDs in humans and organisms, and their fate in the environment. With regard to environmental compatibility, it must be assessed to what extent existing study results from *in vitro* tests are transferable to *in vivo* scenarios, and if such simplified tests can actually represent real conditions and allow for an assessment of the possible environmental effects. Studies on the risk potential of QDs have shown that these nanoparticles can exhibit hazardous substance properties depending on their material composition (heavy metal or pure carbon-based), particle size, and specific surface properties, i.e. QDs may have bioaccumulative, toxic or persistent properties. In the EU, the use of QDs *in vivo* or for clinical use in humans is currently not permitted because of their heavy metal content. For this reason, intensive research is being conducted on pure carbon-based QDs. The intention of the “safe and sustainable by design” concept currently being pursued in the EU chemicals strategy is to ensure future safe and environmentally compatible development of substances, materials, and applications such as QDs.

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