Colloidal quantum dots for fluorescent labels of proteins

P Gladyshev1, V Kouznetsov2, C Martinez Bonilla2, S Dezhurov3, D Krlsky3, A Vasiliev1,4, O Morenkov5, V Vrublevskaya5, P Tsygankov6, S Ibragimova1 and A Rybakova1

1State University "Dubna", Universitetskaya st. 19, 141982 Dubna, Moscow region, Russian Federation
2Universidad Industrial de Santander, Carrera 27, Calle 9, 68001000 Bucaramanga, Santander, Colombia
3Research Institute of Applied Acoustics, 9 Maya st. 7A, 141980 Dubna, Moscow region, Russian Federation
4National Research Center “Kurchatov Institute”, Akademika Kurchatova pl. 1, 123182 Moscow, Russian Federation
5Institute of Cell Biophysics, Institutskaya st. 3, 142290 Pushchino, Moscow region, Russian Federation
6Bauman Moscow State Technical University, 2-ya Baumanskaya st. 5/1, 105005 Moscow, Russian Federation

Corresponding author e-mail address: pglad@yandex.ru

Abstract
The work is devoted to the synthesis of colloidal quantum dots (QDs) and their bioconjugates with proteins. Various QDs were obtained as well with synthesis method in an organic solvent followed by hydrophilization and functionalization or synthesis in aqueous phase provides obtaining hydrophilic QDs directly. Particular attention is paid to the synthesis of QDs as fluorescent tags in the near infrared where minimum absorption occurs and the fluorescence of biological tissue and synthetic materials used in analytical systems. A method for the QDs synthesis of type fluorescent core/shell CdTeSe/CdS/CdZnS-PolyT with mixed telluride, selenide cadmium core with a high quantum yield and high resistance to photoaging. It is shown that these quantum dots may be effectively used in the immunoassay.

1. Introduction
Quantum dots (QDs) - semiconductor nanocrystals, due to their small size, exhibit extraordinary optical and electronic properties, which are due to quantum effects are physically limited in space of the electron cloud. It is important that these properties are determined not only by the type of crystalline material of but their size. This makes it possible to synthesize the quantum dots with desired optical and other physical properties. Rapid progress has been associated with the use of QDs nanotechnology as fluorescent labeling of proteins and other biomolecules fluorescent labels instead of organic bioconjugates selectively binding to biomarkers led to their widespread use in a variety of biological and biomedical research [1]. Various aspects of the synthesis and use of QDs are discussed in the review [2]. However, the compounds that allow providing long-term stability and a simultaneous detection of several biomarkers. The unique optical properties of quantum dots (QDs), the possibility of obtaining water-soluble QDs and their potential use of QDs in medical diagnostics has not been fully disclosed and must be a lot to do to use it to the fullest. In this regard, it is important to continue the work on the synthesis and surface modification of QDs and development on their basis of new
bioconjugates for immunochemical analysis. The problem of potential toxicity and high stability of the QDs can be solved by the use of new non-toxic materials [3,4] and the formation on QDs the additional encapsulating layers [5]. QDs have a wide spectral range of emitted light that can be tuned to NIR where low background occurs. The development of increasingly sophisticated methods to create NIR-emitting QDs is one of the directions of the colloidal synthesis of materials with unique optical properties [6,7]. New developed NIR-emitting QDs should provide a high brightness and photostability for sustainable high-sensitivity detection of analytes in complex biological systems. The use of quantum dots (QDs) fluorescent labels in the NIR spectral region is promising in areas such as molecular biology, diagnostics, and visualization. In this spectral region, there is a minimal absorption and fluorescence of biological tissue and synthetic materials used in analytical systems and thus occurs the maximum ratio of the analytical signal/noise ratio. One of the key limitations of most NIR phosphors is their limited stability, in particular, instability to photooxidation. The method of synthesis of fluorescent core/shell based on a mixed cadmium telluride selenide core with high quantum yield and high resistance to photodegradation was developed. It is shown that these QDs can be effectively used in immunoassay [6,7]. Despite the achievements, there is still much to be done in a reliable and reproducible functionalizing the QDs surface and the development of effective QDs bioconjugates. This work is devoted to the synthesis colloidal QD and their bioconjugates with proteins.

QDs have a wide spectral range of emitted light that can be tuned to NIR where low background occurs. The development of increasingly sophisticated methods to create NIR-emitting QDs is one of the directions of the colloidal synthesis of materials with unique optical properties [1,2]. New developed NIR-emitting QDs should provide a high brightness and photostability for sustainable high-sensitivity detection of analytes in complex biological systems.

2. The synthesis and properties of colloidal quantum dots for proteins labeling

In most cases, the synthesis of colloidal QDs performed in organic solvents in the presence of surface-active compounds and leads to the formation of water-insoluble hydrophobic QDs [2]. QDs for labeling proteins and protein conjugates must be hydrophilic and soluble in water. Therefore were developed methods for the synthesis CdTe QDs in water, which leads directly to the water-soluble QDs [8]. It is believed that the aqueous synthesis in comparison with the organic phase synthesis is more reproducible, cheaper and QDs are more soluble in water and biocompatible.

For strong bond QDs with proteins necessary to form covalent bonds between them. This suggests the presence of reactive groups capable of binding with the carboxyl or amino groups on the surface of the protein globules. As such groups on the surface of QDs can act primarily respectively amino and carboxyl groups. In both cases, the peptide bonds are formed between the protein and QDs. Various methods of hydrophilization, functionalization QDs and an introduction to their reactive groups were described [9,10].

The composition and structure of synthesized in this work of colloidal quantum dots are presented in Table 1. Colloidal QDs QD1 - QD6 were synthesized in the aqueous phase by the procedure similar to that described in [8], and QDs QD7 - QD12 were synthesized according to previously described methods [7,11,12] with subsequent functionalization.

Synthesized QDs properties are shown in Table 2. The method [13] was used to determine the average QDs size. The quantum yield QDs was measured by method [14]. As can be seen from Table. 2 fluorescence of Ds cover a wide spectral range, and these QDs can be used for labeling many objects, including biomolecules. For some QDs (QD1 - QD5), having the same structure, the difference between the spectral characteristics changes achieved synthesis time 1 to 10 hours, respectively QDs size. All other QDs have one or two additional shells, that increases the stability of QDs and provide a high quantum yield.

3. Conjugates of NIR-emitting QDs with proteins for Lateral Flow Assay

In connection with the development of new analytical detection platform especially dangerous infections IDORI [15] of particular interest to the author presents obtain conjugates of antibodies with NIR-emitting QDs. IDORI is based on the immunochromatographic analysis (ICH) – Lateral Flow Assay (LFA) and is using QDs as fluorescent labels. For this purpose were synthesized very bright
and photostability NIR-radiating CdTeSe / CdS / CdZnS QDs on which were obtained by QDs QD10 - QD12 with COOH-functional groups.

**Table 1.** Composition and structure of the synthesized colloidal quantum dots.

| Code | Composition     | Structure | Code   | Composition     | Structure |
|------|----------------|-----------|--------|----------------|-----------|
| QD1  | CdTe-TGA       |           | QD7    | CdSe/CdS/ ZnS-PolyT |           |
| QD2  | CdTe-TGA       |           | QD8    | CdSe/CdS/ ZnS-PolyT-APS |           |
| QD3  | CdTe-TGA       |           | QD9    | CdSe/CdS/ ZnS-PTVP |           |
| QD4  | CdTe-TGA       |           | QD10   | CdTeSe/CdS/CdZnS-PolyT |           |
| QD5  | CdTe-TGA       |           | QD11   | CdTeSe/CdS/CdZnS-PolyT-APS |           |
| QD6  | CdTe/ZnS-TGA   |           | QD12   | CdTeSe/CdS/CdZnS-PTVP |           |
Table 2. Characteristics of the synthesized colloidal quantum dots.

| Code | Composition                  | Particle size, nm | Excitation, nm | Max. fluorescence, nm | Quantum yield, % |
|------|------------------------------|------------------|----------------|-----------------------|-----------------|
| QD1  | CdTe-TGA                     | 2-3              | 490-650        | 530                   | 24              |
| QD2  | CdTe-TGA                     | 3-4              | 490-650        | 570                   | 37              |
| QD3  | CdTe-TGA                     | 4-6              | 490-650        | 610                   | 43              |
| QD4  | CdTe-TGA                     | 6-7              | 490-650        | 630                   | 40              |
| QD5  | CdTe-TGA                     | 6-7              | 490-650        | 680                   | 38              |
| QD6  | CdTe/ZnS-TGA                 | 5-6              | 320-650        | 534                   | 68              |
| QD7  | CdSe/CdS/ZnS-PolyT           | 10-20            | 300-590        | 600                   | 40-50           |
| QD8  | CdSe/CdS/ZnS-PolyT-APS       | 15-40            | 300-600        | 610                   | 30-50           |
| QD9  | CdSe/CdS/ZnS-PTVP            | 15-35            | 300-630        | 640                   | 70-80           |
| QD10 | CdTeSe/CdS/CdZnS-PolyT       | 10-20            | 300-690        | 700                   | 40-50           |
| QD11 | CdTeSe/CdS/CdZnS-PolyT-APS   | 15-40            | 300-710        | 720                   | 30-50           |
| QD12 | CdTeSe/CdS/CdZnS-PTVP        | 15-35            | 300-730        | 740                   | 70-80           |

The basis of the developed technique was taken from the paper [16]. At the same time, the thickness of the shell of wide bandgap semiconductors (CdS, ZnS) was increased to 7 or more monolayers. Building CdS shell not only significantly increases quantum yield, but also passivates considerably QDs. It was also proposed to use Te additives (3-7 mol. % with respect to Se) in the synthesis of nuclei. This increased the bathochromic shift of the QDs cores up to 70 nm relative to the results of [16]. The additional approach was to build the finishing shell of ZnS to passivate QDs in water. Additionally, ZnS is a more bio-compatible material compared to cadmium chalcogenides. Along with additional bathochromic shift (10-30 nm), the application of additional semiconductor shells leads to more symmetric emission peaks while retaining the quantum yield. The synthesis of CdTeSe / CdS / CdZnS / ZnS QDs involves several steps. First colloidal synthesis of CdTeSe was implemented. Then, these nuclei were coated sequentially with CdS, CdZnS and ZnS shells. Interjacent CdTeSe, CdTeSe / CdS, CdTeSe / CdS / CdZnS and end CdTeSe / CdS / CdZnS / ZnS QDs were purified by reprecipitation from toluene with methanol-butanol. The size of nanocrystals after the formation of shells increased from 4-5 to 10-11 nm. These QDs were functionalized with the COOH-group using thiol-modified PVP. Further QDs were covalently conjugated to a monoclonal antibody directed to glycoprotein B, pseudorabies virus (PRV-gB) using carbodiimide chemistry methods. The best system PRV-gB LFA was based on QDs with an emission maximum at 700 nm (QDs QD10), which showed a detection limit of 0.5-1 ng / ml PRV-gB in buffers and tissue lysates with a very high specificity. This COOH functionalized CdTeSe / CdS / CdZnS core-shell QDs can be successfully used to prepare conjugates with various proteins.

4. Conclusions
It was synthesized colloidal quantum dots, which can be used as labels for various proteins. It was demonstrated that fluorescent labels based on QDs are of great interest for diagnosis of diseases and immunoassay. Maximum fluorescence QDs can be realized in a wide spectral range. This makes it possible to realize the visible labels and hidden from the eyes NIR-labels. Highly bright and photostable NIR-emitting CdTe / QdS / CdZnS core / shell colloidal QDs with fluorescence maximum at about 700 nm were synthesized. QDs has a half-width of the fluorescence peaks of 60-70 nm, the quantum yield of 80% and a high photostability. QDs were functionalized with the COOH-group. It is shown that these functionalized QDs can be successfully used for the preparation of active conjugates with proteins for LFA applications.
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