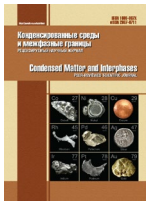

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TEM and XPS studies of bio-nanohybrid material based on bacterial ferritin-like protein Dps

E. V. Parinova[1] ✉, S. S. Antipov[1,2], E. A. Belikov[1], O. A. Chuvenkova[1], I. S. Kakuliia[1], D. A. Koyuda[1], S. Yu. Trebunskikh[1], M. S. Skorobogatov[1], R. G. Chumakov[3], A. M. Lebedev[3], A. A. Sinelnikov[1], V. G. Artyukhov[1], O. V. Ovchinnikov[1], M. S. Smirnov[1], S. Yu. Turishchev[1]

[1]Voronezh State University,
1 Universitetskaya pl., Voronezh 394018, Russian Federation

[2]Immanuel Kant Baltic Federal University,
2 Universitetskaya ul., Kaliningrad 236041, Russian Federation

[3]National Research Center "Kurchatov Institute"
1 Akademika Kurchatova pl., Moscow 123182, Russian Federation

Abstract

The work is related to the research of a biohybrid nanomaterial formed on the basis of protein molecules of bacterial origin recombinant ferritin Dps.

To obtain recombinant protein, *Escherichia coli* cells were used as producers, and purification was carried out chromatographically. The source of iron atoms for the formation of the biohybrid nanomaterial was the Mohr salt. The possibility of the hybrid particles formation, the shape and size of their inorganic core were studied experimentally by high-resolution transmission electron microscopy. The composition and specificity of hybrid particles inorganic core physico–chemical state were studied by X-ray photoelectron spectroscopy, including the use of focused ion etching.

It is shown that using the chosen method of nanomaterial formation, the internal cavities of protein molecules deposited inorganic nanoparticles. The sizes of these nanoparticles formed in hollow protein molecules averaged 2 nm. A complex composition of particles has been established, mainly including oxides of the iron-oxygen system. Inclusions of metallic iron are also possible.

The results obtained show the possibility of smooth properties control of the biohybrid nanomaterial through their composition. This makes it extremely attractive for the implementation of modern technologies tasks such as spintronics or targeted delivery of functional nanoparticles.

Keywords: Nanostructures, Biomolecules, Hybrid materials, Developed surface, Recombinant ferritin-like Dps protein, Transmission electron microscopy, Combination, X-ray photoelectron spectroscopy

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✉ Elena V. Parinova, e-mail: parinova@phys.vsu.ru

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1. Introduction

Synthesis and research of new functional materials is an extremely popular task in science, engineering and technology. Nature-like technologies for the formation of functional nanomaterials attract great scientific and practical interest. This is due to such promising directions as high efficiency and reproducibility in combination with insignificant economic costs when introducing these technologies based on biosynthesis into production, compared with physical or chemical methods of nanomaterials synthesis [1–2]. A great example of such a nature-like formation technology of the functional nanomaterials can be the synthesis of inorganic nanoparticles inside a natural protein molecule. Ferritins are natural complex structures consisting of a protein shell and an inorganic (metal-oxide) core, about ten nm in size [3–4].

Ferritin-like protein Dps (DNA-binding Protein from Starved cells) has a unique set of properties, including affinity for iron, small size and the ability to form strong complexes with DNA [5–7]. The composition and structure of the Dps core is strictly dependent on the method of its isolation and purification, storage conditions, as well as methods of further modification and use [8–9]. Previously, we have shown the possibility of forming two-dimensional structures by Dps ferritin molecules [10], but there is no clear understanding about the Dps inorganic core structure specificity yet. In this work, a combination of Transmission Electron Microscopy (TEM) and X-ray Photoelectron Spectroscopy (XPS) methods was used, which provides information about the composition, morphology and physico-chemical state of the object under study.

Thus, it is actual to study the formation of hybrid nanostructures with inorganic nanoparticles – cores of ferritin Dps protein molecules. This work is devoted to the study of inorganic cores in the structure of a hybrid material based on ferritin Dps, including the analysis of their sizes and composition under conditions of equilibrium formation of nanoparticles and stimulated one by the additional introduction of iron ions.

2. Experimental

Recombinant Dps protein was obtained using *Escherichia coli* BL21*(DE3) cells as producers. *E.coli* cells were transformed by the pGEM_ dps plasmid. In [11], detailed information is provided on the preparation of recombinant protein purified from inorganic components by step hydrolysis and dialysis, its subsequent isolation and purification. The protein solution had a concentration of 1.2 mg/ml in a buffer containing 10 mM NaCl, 50mM tris-HCl (pH 7.0) and 0.1 mM EDTA. A freshly prepared solution of Mohr salt $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ was used as a source of iron, which was added to the protein solution until an iron ion concentration of 0.25 mM was reached and incubated for 15 minutes, after which the same portion of Mohr salt was added and incubation was repeated, the resulting sample was used in studies.

The sizes of protein molecules were controlled by Dynamic Light Scattering according to the technique described in [12]. For the TEM experiments, prepared thin carbon replicas ~ 15 nm thick were used, on which a molecular culture was placed by immersion in a solution and subsequent evacuation in the loading chamber of a Zeiss LIBRA 120 microscope. The Image J software package was used to estimate the number and size of nanoparticles.

For XPS experiments, protein molecules were deposited on the surface of formed and pre-purified silicon substrates by layering 10 μl of solution. After that, the resulting structure was dried in laboratory conditions, washed with deionized water (by pulling) in order to remove residual salts and dried again under the same conditions.

XPS studies was carried out on the NANOFES beamline ESCA module of the Kurchatov synchrotron ultrahigh vacuum experimental end-station (National Research Center Kurchatov Institute, Moscow), equipped with an electron

energy analyzer SPECS Phoibos 150 [13]. Monochromatized Al Ka radiation of an X-ray tube (1486.61 eV) was used, the depth of the informative layer was ~ 2-3 nm [14]. Survey spectra in the binding energy range of 0-800 eV and data of Fe 2*p* states were recorded, for the measured data interpretation reference structures were used: iron foil covered by natural oxide, as well as commercially available powders of Fe_2O_3, Fe_3O_4 and FeO(OH) compounds produced by AlfaAesar. A standard approach to data normalization and calibration based on independent recording of the pure gold foil (Au 4f) signal was used. To register the spectral data of reference iron compounds, C1*s* calibration of the hydrocarbon contamination line was used [14]. To compare and analyze the main features of Fe 2*p* XPS spectra, well-known databases were used, from which the actual and most accurate (monochromatic) spectra were selected [14–16]. A focused source of surface etching with argon ions at an accelerating voltage of 1 kV with an etching duration of 30 minutes was used. The area of the etching site was selected with an excess of the surface area from which the XPS data were recorded.

3. Results and discussion

Fig. 1 presents high-resolution TEM data for a bio-nanohybrid material based on bacterial ferritin-like protein Dps and an estimate of the average sizes distribution of inorganic nanoparticles that make up the "core" of molecules and their number.

The data obtained by the Dynamic Light Scattering on the molecules sizes distribution coincide with the results [12], which indicates the successful formation of the molecular culture. The TEM data confirm the deposition of inorganic nanoparticles in the internal cavities of molecules as a result of the Mohr salt introduction into the protein culture solution. Inorganic particles have an almost identical shape, agglomeration is not observed, most likely due to the presence of protein walls of individual molecules that prevent them from sticking together. At the same time, the average particle size was about 2 nm, which is almost two times less than the data [11]. This effect can be observed for several reasons: due to the lack of iron ions in the solution to completely fill the molecules, insufficient incubation time, the use of stepwise saturation of protein molecules. According to the evaluation results, the number of particles in the field of view was ~ 280.

Fig. 2 shows the X-ray photoelectron spectra surveys for the initial surface of the prepared sample and after half an hour etching with argon ions at an accelerating voltage of 1 kV. Note that this value referred to the "softest" effect on the surface, with an estimated removal of 1.5 A per minute obtained for the silicon substrate.

According to the data of the initial sample photoelectron survey spectra, the main line is carbon, that is, a hybrid material. The presence

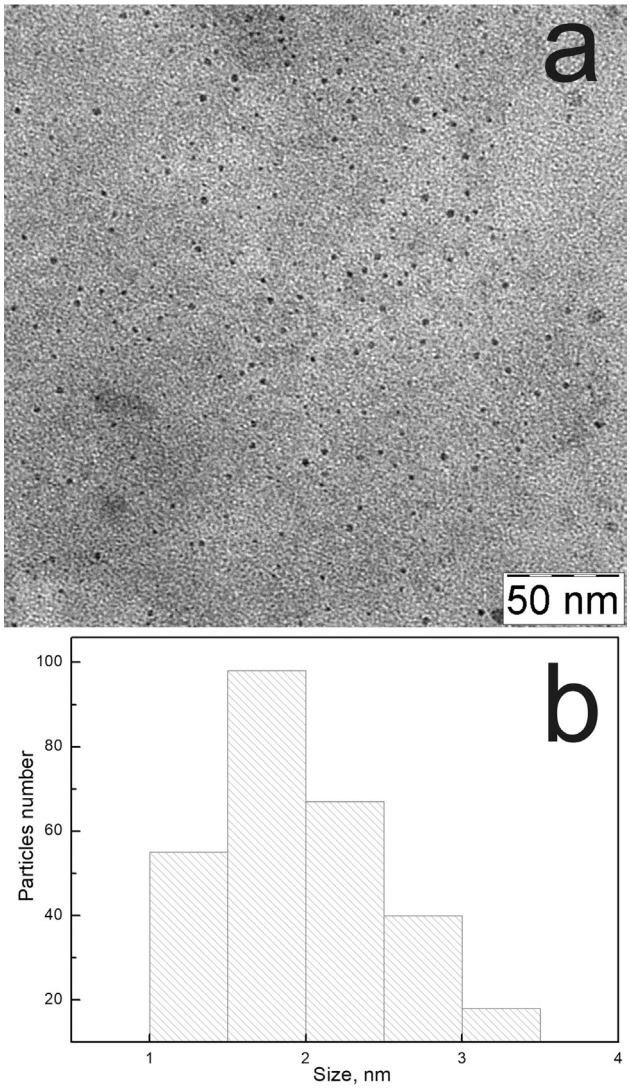

Fig. 1. TEM data of bio-nanohybrid material based on bacterial ferritin-like protein Dps (*a*). Estimation of inorganic nanoparticles average sizes distribution and their number (*b*)

of oxygen and nitrogen 1*s* lines noticeable in intensity confirms the enough amount of the sample itself. A relatively small amount of hybrid material makes it possible to reliably register a signal from a silicon substrate. Traces of salt of the buffer solution (lines of sodium and chlorine) are also noticeable. However, their intensity is noticeably low, which indicates a sufficient degree of the sample washing after layering. The presence of salt in the surface layers and the organic material of the sample as a whole did not lead to a variation of lines positions for the observed core levels associated with the charging of the sample surface. Finally, for the initial sample, the fact of deposited iron nanoparticles signal observation on the survey spectrum is not obvious. Nevertheless, in order to register the iron 2*p* core level data with a high resolution, we performed a long signal collection, which will be discussed below.

The situation changes slightly after half an hour surface treatment (etching) with argon ions. After prolonged etching, the position and relative intensity of the carbon line practically did not change, which indicates a sufficient amount of the biohybrid sample remaining after removal. The silicon substrate lines (approximately 100 and 150 eV) began to be observed more intensively, confirming the fact of ion beam exposure and partial removal of the etched sample. At the same time, an argon line is observed, which confirms the assumption that the bio-coating of the substrates is saturated with ions during processing and is characteristic of this class of materials.

The fact that there are no core level lines of the sulfur on the survey spectra (Fig. 2) (S 2*p* and S 2*s* states at ~ 163 eV and ~ 228 eV respectively) allows us to conclude that there are no residual traces of Mohr salt. Thus, the source of iron atoms was completely used up for the molecular culture deposition. The effect of washing can be excluded, since after ion etching (in the deep part of the bio-coating), no signal from sulfur atoms was observed.

Finally, in the region of iron atoms binding energy (about 710 eV), a low-intensity feature

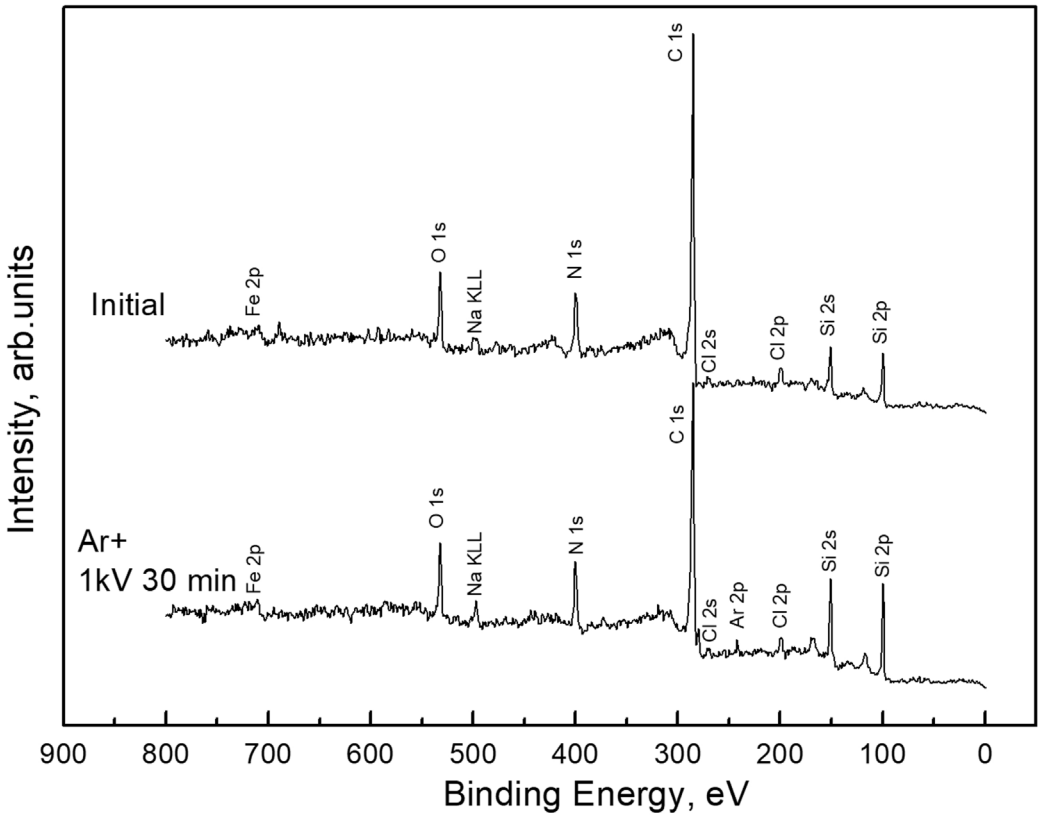

Fig. 2. XPS survey spectra of a bio-nanohybrid material sample based on bacterial ferritin-like protein Dps: the initial state of the surface (initial) and after half an hour etching with an ion beam (Ar+ 1 kV 30 min). The elements that make up the studied surface of the sample are designated

is observed. This fact confirmed the need for an increased collection time to record a signal from iron atoms, which we used for the initial sample.

Fig. 3 shows the Fe 2*p* data of X-ray photoelectron spectra from the prepared and processed sample together with the data of reference samples registered under the same methodological conditions (only with a significantly smaller, no more than 20, spectra scans collection number). The binding energy values for the reference structures are in full agreement with the known literature data [14-16]. The most significant uncertainty is observed in these data for FeO oxide unstable under normal conditions, for which Fig. 3 shows the range of values of the Fe 2*p* binding energy level according to the data of the sources used [14–16]. Thus, we emphasized the range of binding energies in which it is possible to observe this compound in the composition of the experimental sample surface. Earlier [12] we obtained data by the synchrotron XANES technique (X-ray Absorption Near Edge Structure), which showed the expected complex structure and composition of inorganic nanoparticles of bio-nanohybrid material.

For this reason and due to the close values of the observed components binding energies with a generally high amount of signal collection time (several hours), we carried out a qualitative assessment of the prepared sample studied surface composition. For this purpose, the fine structure of the Fe 2*p* spectra (Fig. 3) and the energy position of its features were considered.

Analysis of the XPS Fe 2*p* spectra fine structure for reference samples when compared with the data of a hybrid nano-biomaterial made it possible to carry out a qualitative assessment of inorganic nanoparticles cores composition. The position of the XPS Fe 2*p* spectra main maximum of the studied sample is almost unchanged after ion etching. Thus, we received a signal from inorganic nanoparticles of the iron-oxygen system, including from the "bulk" part of the sample (after ion etching). This signal is less noisy. In our opinion, this is due to significantly different rates of ion etching of the bio-environment and the inorganic nanoparticles themselves. It means that the increase of the etching time leads to an increase in the number of particles available for probing by the XPS technique. The most interesting is the observation of a binding energies feature of ~ 706.7 eV corresponding to metallic iron (Fig. 3). There are two possible reasons for such an observation. Firstly, the "bulk" part of the nanoparticles may contain iron atoms unbound with oxygen. However, the weak signal of the initial spectrum at binding energies of ~ 706.7 eV does not allow us to confirm or deny this statement. Secondly, partial reduction of iron can occur as a result of prolonged exposure to argon ions. Stability of the survey spectra data (see Fig. 2) does not confirm such an assumption. We plan to further investigate this issue in further

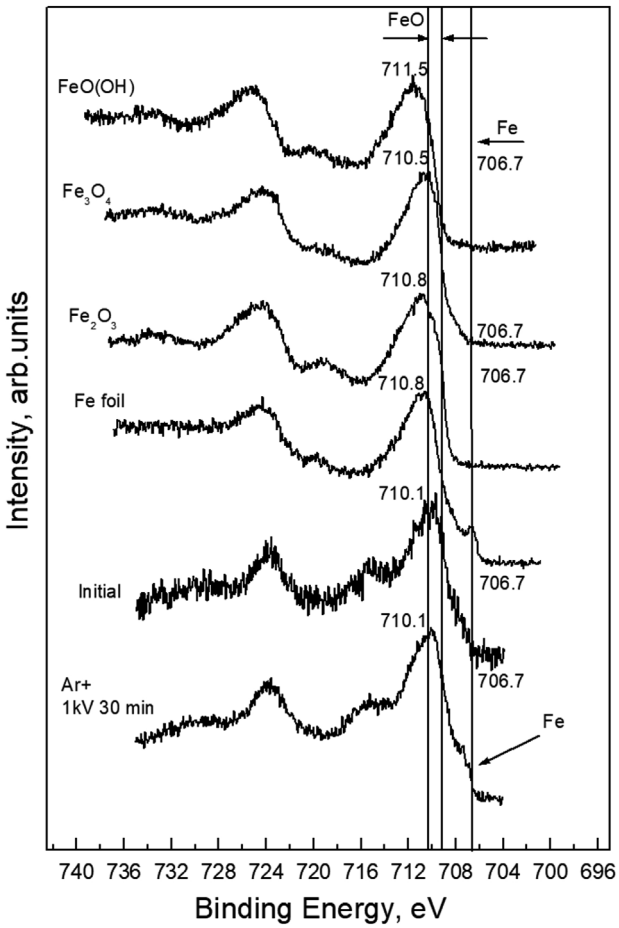

Fig. 3. Fe 2*p* high-resolution XPS spectra of a bio-nanohybrid material prepared sample based on bacterial ferritin-like protein Dps: the initial state of the surface (initial) and after half an hour etching with an ion beam (Ar+ 1 kV 30 min). The data of reference structures are given: iron foil covered with natural oxide (Fe foil), as well as Fe_2O_3, Fe_3O_4 and FeO(OH) powders. The positions of the observed spectral curves main maxima are indicated. The range of binding energies for FeO and the metallic iron line are indicated as well

experiments, while effectively using a stepwise general etching mode.

Of all the analyzed binding energies values [14–16] for reference objects, the closest values of the main maximum position for studied hybrid nano- biomaterial sample relate to Fe_3O_4, confirming the conclusions made earlier [12]. At the same time, the observed signal in the region of binding energies ~ 709–710 eV indicates the possible presence of FeO in the composition of nanoparticles, which is also in agreement with [12]. Nevertheless, since the XPS has a greater sensitivity to the surface (compared to XANES), the observation of a sufficiently wide main maximum of the initial experimental sample and one after ion etching does not exclude the presence of Fe_2O_3 and FeO(OH) in the nanoparticles composition. This indicates a complex, composition of iron-oxygen system inorganic particles in the composition of a bio-nanohybrid material based on bacterial ferritin-like protein Dps. The studied sample was reproduced by a series of control samples, all data were obtained at the same time and under identical conditions, including about three weeks of laboratory conditions storage before the XPS measurements. At the same time, a signal different from the expected Fe_2O_3 oxide (the uppermost layers of the surface, within the method probing depth) is reliably detected, which is in good agreement with synchrotron data [12] and confirms the complex, composite nature of inorganic nanoparticles of the hybrid material.

Finally, the above results of high-resolution TEM and XPS data analysis allow us to state that by variation of the samples incubation time and the concentration of iron source salts, it is possible to control the size and composition of inorganic nanoparticles of the studied bio-nanohybrid material based on bacterial ferritin-like protein Dps.

4. Conclusions

For the first time, a joint study of a bio-nanohybrid material based on bacterial ferritin-like protein Dps was carried out using TEM and XPS techniques. In molecular culture, the possibility of small inorganic particles formation of identical shape and an average size of about 2 nm has been shown. Agglomeration is not observed. The results obtained demonstrate a complex composite nature of inorganic particles, including Fe^{2+} and Fe^{3+} oxides of the iron-oxygen system, mainly close to Fe_3O_4. Inclusions of metallic iron have been established. Thus, it is possible to adjust the properties of nanomaterials by varying the composition and modes of formation. This makes it promising to use bio-nanohybrid structures based on bacterial ferritin-like protein Dps for targeted delivery of nanoparticles, as well as in modern technologies for surface functionalization, for example, in spintronics.

Author contributions

All authors made an equivalent contribution to the preparation of the publication.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Information about the authors

Elena V. Parinova, Cand. Sci. (Phys.–Math.), Researcher of the Joint Laboratory "Atomic and Electronic Structure of Functional Materials", Voronezh State University (Voronezh, Russian Federation).
https://orcid.org/0000-0003-2817-3547
parinova@phys.vsu.ru

Sergey S. Antipov, Dr. Sci. (Biology), Associate Professor, Biophysics and Biotechnology Department, Voronezh State University (Voronezh, Russian Federation).
https://orcid.org/0000-0003-3244-1428
ss.antipov@gmail.com

Evgeny A. Belikov, Engineer of Joint Laboratory "Atomic and Electronic Structure of Functional Materials", Voronezh State University Voronezh (Voronezh, Russian Federation).
https://orcid.org/0000-0001-8336-2231
belikov@phys.vsu.ru

Olga A. Chuvenkova, Cand. Sci. (Phys.–Math.), Researcher of the Joint Laboratory "Atomic and Electronic Structure of Functional Materials", Voronezh State University (Voronezh, Russian Federation).
https://orcid.org/0000-0001-5701-6909
chuvenkova@phys.vsu.ru

Iuliia S. Kakuliia, Leading Engineer of General Physics Department, Voronezh State University (Voronezh, Russian Federation).
https://orcid.org/0000-0002-0953-9024
kakuliia@phys.vsu.ru

Dmitry A. Koyuda, Junior Researcher of the Joint Laboratory "Atomic and Electronic Structure of Functional Materials", Voronezh State University (Voronezh, Russian Federation).
https://orcid.org/0000-0001-5635-0788
koyuda@phys.vsu.ru

Sergey Yu. Trebunskikh, Cand. Sci. (Phys.–Math.), Researcher of the Joint Laboratory "Atomic and Electronic Structure of Functional Materials", Voronezh State University (Voronezh, Russian Federation).
https://orcid.org/0000-0002-2481-2797
tsu@phys.vsu.ru

Matvey S. Skorobogatov, bachelor student of the Department of Biophysics and Biotechnology, Voronezh State University (Voronezh, Russian Federation).
https://orcid.org/0000-0002-9051-8174
mataska7111@gmail.com

Ratibor G. Chumakov, Cand. Sci. (Phys.–Math.), Senior Researcher of the National Research Center "Kurchatov Institute" (Moscow, Russian Federation).
https://orcid.org/0000-0002-3737-5012
ratibor.chumakov@gmail.com

Alexei M. Lebedev, Cand. Sci. (Phys.–Math.), Senior Researcher of the National Research Center "Kurchatov Institute" (Moscow, Russian Federation).
https://orcid.org/0000-0001-9998-8941
lebedev.alex.m@gmail.com

Alexander A. Sinelnikov, Cand. Sci. (Phys.–Math.), Director of the Collective use Center of Scientific Equipment, Voronezh State University (Voronezh, Russian Federation).
https://orcid.org/0000-0002-0549-4615
tsu@phys.vsu.ru

Valerii G Artyukhov., Dr. Sci. (Biology), Professor, Head of the Biophysics and Biotechnology Department, Voronezh State University (Voronezh, Russian Federation).
https://orcid.org/0000-0002-5872-8382

Oleg V. Ovchinnikov, Dr. Sci. (Phys.-Math.), Professor, Head of the Optics and Spectroscopy Department, Voronezh State University (Voronezh, Russian Federation).
https://orcid.org/0000-0001-6032-9295
ovchinnikov_o_v@rambler.ru

Mikhail S. Smirnov, Dr. Sci. (Phys.-Math.), Associate Professor, Associate Professor of the Optics and Spectroscopy Department, Voronezh State University (Voronezh, Russian Federation).
https://orcid.org/0000-0001-8765-0986
smirnov_m_s@mail.ru

Sergey Yu. Turishchev, Dr. Sci. (Phys.-Math.), Associate Professor, Head of the General Physics Department, Voronezh State University (Voronezh, Russian Federation).
https://orcid.org/0000-0003-3320-1979
tsu@phys.vsu.ru

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