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Review

Hydrogen peroxide luminescent sensors based on rare-earth doped nanocrystals: sensing mechanisms and biological applications

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Understanding of the ubiquitous role of reactive oxygen species (ROS) in living cells provided by modern biological studies determined the need for their precise quantification and control. In this review we discuss the new field of luminescent sensors for detection of ROS in water solutions and biological samples. The main attention we put on the hydrogen peroxide (HP) luminescent sensors as HP besides being most common type of ROS is the most stable of all of them. Luminescent HP sensors based on rare-earth doped nanoparticles (NPs) are highly sensitive, photo- and temperature stable, and reversible, and both sensitivity and reversibility of these sensors can be further improved. Within the list of rare-earth doped nanocrystals for HP sensing, the special place we reserved for the nanoparticles with ROS scavenging activity which can decrease the concentration of ROS in living cells visualizing the change of ROS content in the process. Finally, we enlist the existing biological applications of these newly made HP sensors, and discuss the ways of improving their applicability in this field.

Keywords: hydrogen peroxide, nanoparticles, sensors, luminescence, redox activity, reactive oxygen species.

Люмінесцентні сенсори перекису водню на основі нанокристалів, активованих рідкоземельними іонами: механізми детектування та біологічне застосування. *В.В. Семінько, Є. І. Неугодов, П. О. Максимчук, Н. С. Кавок, С.Л. Єфімова*

Розуміння повсюдної ролі активних форм кисню (АФК) у живих клітинах, отримане завдяки сучасним біологічним дослідженням, зумовило необхідність їх точної кількісної оцінки та контролю. У цьому огляді ми обговорюємо нову область люмінесцентних сенсорів для виявлення АФК у водних розчинах і біологічних зразках. Основну увагу ми приділяємо люмінесцентним сенсорам перекису водню, оскільки перекис водню є не тільки найпоширенішим типом АФК, але й найстабільнішим з усіх них. Люмінесцентні сенсори перекису водню на основі наночастинок, активованих рідкоземельними іонами, є високочутливими, фото- та температурно стабільними та оборотними, і як чутливість, так і оборотність цих сенсорів можна додатково покращувати. Серед нанокристалів, активованих рідкісноземельними іонами, для вимірювання концентрації перекису водню, ми окремо розглядаємо наночастинки, здатні до нейтралізації АФК, які можуть зменшувати концентрацію АФК у живих клітинах, візуалізуючи зміну вмісту АФК у процесі нейтралізації. Також у роботі розглянуто існуючі біологічні застосування зазначених сенсорів перекису водню та обговорюються шляхи покращення їх властивостей у цій галузі.

1. Reactive oxygen species and the needs for their sensing

Reactive oxygen species such as hydrogen peroxide (HP), singlet oxygen, hydroxyl radicals, superoxide anions, and nitric oxide are irreplaceable in biological systems as cell signaling molecules and immune agents [1-4]. Free radicals (ROS with unpaired electron such as singlet oxygen, hydroxyl radicals, or superoxide anions) are highly reactive and very short-lived making the measurement of their levels possible only by indirect methods, i.e. by assessing the products of ROS-sensor interaction. The sensors applied for detection of free radicals vary from spin traps [5, 6] to fluorescent dyes [7] and devote a separate review.

In this mini-review we have focused on the sensors of hydrogen peroxide (H_2O_2), which is the most stable type of ROS. The methods of HP detection include titrimetry, electrochemistry, chromatography and spectroscopy (photometry, chemiluminescence, fluorescence and phosphorescence) [8-10]. The most widely used are electrochemical methods (which can be sufficiently improved by use of immobilized enzymes and/or NPs [11, 12]) and fluorescent sensors of different kinds (mostly based on organic molecules [13, 14]). Recent progress in obtaining NPs of various kinds allowed proposing them as an alternative to organic fluorescent sensors. The list of NPs successfully applied for HP sensing include gold nanoparticles [15], polymeric nanoparticles with embedded enzymes [16], quantum dots [17], carbon-based nanomaterials [18], and rare-earth doped nanocrystals. These NPs have an advantage of higher stability and reversibility, so, in contrast to most organic HP sensors they are able for multi-use. Another advantage of HP sensors based on doped NPs compared to sensors based on luminescence of organic molecules (such as dichlorodihydrofluorescein diacetate, dihydrorhodamine and Amplex Red (Invitrogen)) is the ability for time-resolved measurements, as the organic sensors are non-reversible and therefore can detect the total amount of HP generated, but not the instantaneous HP concentration.

In this review we limited ourselves to rare-earth doped nanomaterials (including polymeric nanoparticles, metal-organic frameworks (MOFs), with main attention to rare-earth doped inorganic NPs). Detailed analysis of current literature data, and our own experience in the field allowed us to focus on the main challenges and pitfalls of HP sensing using rare-earth doped nanomaterials, and propose the ways to improve the sensing properties of these materials.

2. Rare-earth ions used for luminescent HP sensors

Eu^{3+}

Eu^{3+} ions are most widely used as part of luminescent MOFs or organic - inorganic complexes and dopants for inorganic NPs due to high absorption value at Eu^{3+}-O^{2-} transition, and narrow luminescence bands from 560 nm to 700 nm corresponding to electrical dipole and magnetic dipole $^5D_0 \rightarrow {}^7F_J$ (J= 0, 1, 2, 3, 4) transitions of Eu^{3+} ions (Fig. 1). Also red emission of europium ions has lower overlap with autofluorescence of biological tissues that determine wide use of Eu^{3+}-based luminescence sensors for biological applications.

Eu^{3+}-based organic - inorganic complexes were proposed as HP sensors in 2002 [19]. The authors have revealed that Eu^{3+}-tetracycline complex binds HP to form a strongly fluorescent complex ([Eu(hp)(tc)]) with 15-fold increase in $^5D_0 \rightarrow {}^7F_2$ luminescence intensity after HP addition. These sensors were able to detect HP with limit of detection (LOD) of 1.8 μM. Another approach is the use of metal-organic frameworks (MOFs) with RE ions for HP sensors. MOFs are porous structures consisting of metal ions and organic linkers providing high number of sites for HP-sensor interaction. As an example of Eu^{3+}-based MOFs for HP sensing we can mention boric-acid-functional Eu-MOFs proposed in [20] as sensors with high selectivity to HP due to unique ambiphilic reactivity of HP to boric group (LOD = 33.5 nM).

Eu^{3+}-doped NPs are also widely used as HP sensors since 2009 when YVO_4:Eu^{3+} NPs was proposed as HP sensors based on reversible $Eu^{2+} \leftrightarrow Eu^{3+}$ reaction [21]. In [22] the HP sensors based on YVO_4:Eu^{3+} NPs were obtained in the form of films that can be favorable for some applications. The CeO_2:Eu^{3+} NPs with both catalase-like activity and high HP sensing ability (LOD = 0.15 μM) and also indirect ability to ethanol sensing (by HP level formed when coupled with alcohol oxidase) was proposed by [23].

Tb^{3+}

Tb^{3+}-based HP sensors are also widely studied in literature.Tb^{3+}ions have a number of $^5D_4 \rightarrow {}^7F_J$ narrow luminescence lines in the green

part of the spectra which intensity changes during interaction with HP (Fig. 1).

In [24] the authors have obtained functionalized coordination polymer nanoparticles (Phe/Tb-CPBA CPNPs) based on the chemical coordination of 4-carboxyphenylboronic acid (CPBA) with phenylalanine molecules acting as bridge ligands and terbium ions. Selective interaction of HP with boronic acid groups of CPBA provided formation of 4-oxo anions and UV-induced intramolecular charge transfer from the formed4-oxo anion to terbium ion led to quenching of Tb^{3+}luminescence. More complex coordination polymer nanoparticles consisting of guanine diphosphate (GDP), terephthalic acid (TA), Tb^{3+} and Cu^{2+} were recently proposed by [25]. In the presence of HP Cu^{2+} ions catalyzed the oxidation of TA generating a new blue fluorescent product, while fluorescence of Tb^{3+} was used as an additional channel. Luminescent metal-organic frameworks (MOFs) with Tb^{3+} ions were also proposed as HP sensors. The Tb-GMP@MIL-53(Fe) sensor was obtained in [26] by post-synthetic modification of MIL-53(Fe) and provided effective HP sensing with LOD = 0.18 μM.

The use of Tb^{3+}-doped NPs as HP probes was discussed in [27, 28]. In [28] luminescent $CePO_4$:Tb^{3+} NPs with LOD = 1.03 μM with turn-off luminescent HP sensing were obtained. In [27] CeO_2:Tb^{3+},Ce^{3+} nanoparticles self-doped by Ce^{3+} ions and co-doped by Tb^{3+} ions were proposed as HP sensors. Ce^{3+} ions played the role of sensitizers of Tb^{3+} luminescence, and addition of HP leading to $Ce^{3+}\rightarrow Ce^{4+}$ oxidation decreased the luminescence intensity of Tb^{3+} ions that was applied as a mechanism of sensing HP concentration. The decrease of energy transfer between Ce^{3+} and Tb^{3+} ions was proposed as a cause of HP-induced quenching of Tb^{3+} luminescence in Ce^{3+}/Tb^{3+}-doped $NaYF_4$ microrods as well [29], but not due to $Ce^{3+}\rightarrow Ce^{4+}$ oxidation, but as a result of strong absorption of the Ce^{3+} emission by the product of capping agent (para-phenylenediamine) oxidation.

Ce^{3+}

Ce^{3+}ions can be used not only as sensitizers of Tb^{3+} luminescence in HP sensing nanomaterials as in [27], but also as luminescent probes by themselves. Intraconfigurational 5d-4f luminescence of rare-earth ions differs from interconfigurational 4f-4f luminescence providing wide intense bands with short decay times, and Ce^{3+} is the only rare-earth ion with 5d-4f luminescence in the visible range (Fig. 1).

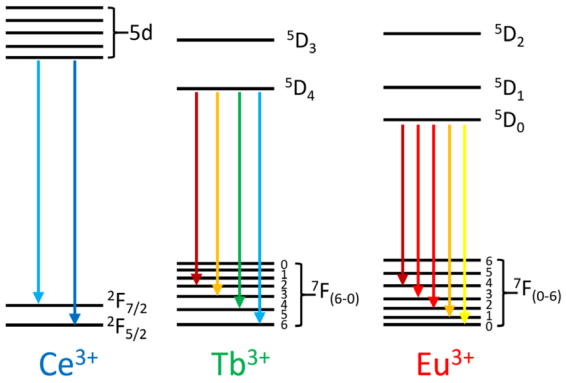

Fig.1. Energy levels of rare-earth ions commonly used for HP sensing (Eu^{3+}, Tb^{3+}, Ce^{3+}).

Use of Ce^{3+} ions for luminescent HP sensing is based on HP-induced $Ce^{3+}\rightarrow Ce^{4+}$ oxidation. The addition of HP to fluorescent ATP-Ce(III)-Tris coordination polymer nanoparticles turned them to non-fluorescent ones enabling their application for HP sensing with a detection limit down to 0.6 nM [30]. In our own studies we have revealed the presence of 5d-4f luminescence of Ce^{3+} ions in 2 nm and 10 nm CeO_2 NPs, which intensity depended on the HP concentration in water solutions [31]. This fact allowed us to propose CeO_2 NPs as HP sensors in water solutions, and, possibly, in biological objects.

Other rare-earth ions (Nd^{3+}, Sm^{3+}, Er^{3+}, Ho^{3+})

The publications on the HP sensors with other rare-earth ions (besides Eu^{3+}, Tb^{3+}, and Ce^{3+}) are rather scarce. Quenching of $^4G_{5/2} \rightarrow {}^6H_{7/2}$ luminescence of Sm^{3+} ions in $CePO_4$:Sm^{3+}nanorods was used for HP sensing in [32] providing linear sensor response from 0 to150 μMand LOD of 3.17 μM. In [33] (Nd^{3+}, Yb^{3+}, Er^{3+})-doped $NaYF_4$NPs covered byDCM–H_2O_2 were used as HP sensors.Nd^{3+}played the role of energy donor transferring energy to DCM–OH providing ratiometric up-conversion luminescence (540 nm/660 nm) signal that was used to visualize the H_2O_2 level (LOD was 0.168 μM). In [34] core–shell $NaErF_4$:Ho@$NaYF_4$ were obtained, where the ratio between luminescence bands of Ho^{3+} ions (1180 nm, up-conversion luminescence) and Er^{3+} ions (980 nm) depended directly on the HP concentration.

3. Methods of luminescent HP sensing by rare-earth doped NPs

Turn-off and turn-on luminescent sensing

The principles of luminescent HP sensing in most sensors listed in the previous section are related to the change of luminescence intensity of rare-earth ions after HP addition. More common is the quenching of luminescence bands of RE ions by either HP itself or products of its reaction with the sensor; however, in some cases a rise in the luminescence intensity can be observed. These two types of sensors are known as turn-off and turn-on sensors. The examples of turn-off sensors are colloidal CeO_{2-x} [31] and $GdVO_4:Eu^{3+}$ [35] NPs, which luminescence spectra after addition of different HP concentrations are shown in the Fig. 2a and 2b, respectively. The turn-off HP sensing is based on the fact that addition of HP leads to quenching of luminescence of the characteristic bands of rare-earth ions (Ce^{3+} and Eu^{3+}, respectively) with value of decrease of luminescence intensity directly determined by HP concentration.

Turn-on luminescent HP sensors (such as Eu^{3+}-tetracycline complexes with 15-fold increase in $^5D_0 \rightarrow {}^7F_2$ luminescence intensity as a result HP binding [19]) are much less studied, and the mechanisms of HP sensing in this case are more complex.

Ratio of luminescence bands (ratiometry)

Precise measurements of luminescence intensities of HP sensors in real tasks can be difficult with requirement of complex optical equipment and sample preparation procedures. Ratiometric measurements (i.e. measurements of ratio of two luminescence bands) can be a way simple, and can give more reliable data on the HP concentrations in water solutions and biological samples. This method can be used when intensity of one of the luminescence bands of luminescent sensor decreases/increases with HP addition, while other one remains unchanged. For these needs, the authors of [36] have obtained mixed $CeO_2:Eu^{3+}/Y_2O_3:Tb^{3+}$ samples, where the luminescence of Eu^{3+} ions in CeO_2 was HP-sensitive, and luminescence of Tb^{3+} ions in Y_2O_3 was insensitive to HP.

Other approach involves the measurement of the ratio between $^5D_0 \rightarrow {}^7F_1$ and $^5D_0 \rightarrow {}^7F_2$ luminescence bands of Eu^{3+} ion as these bands can be differently affected by HP addition.

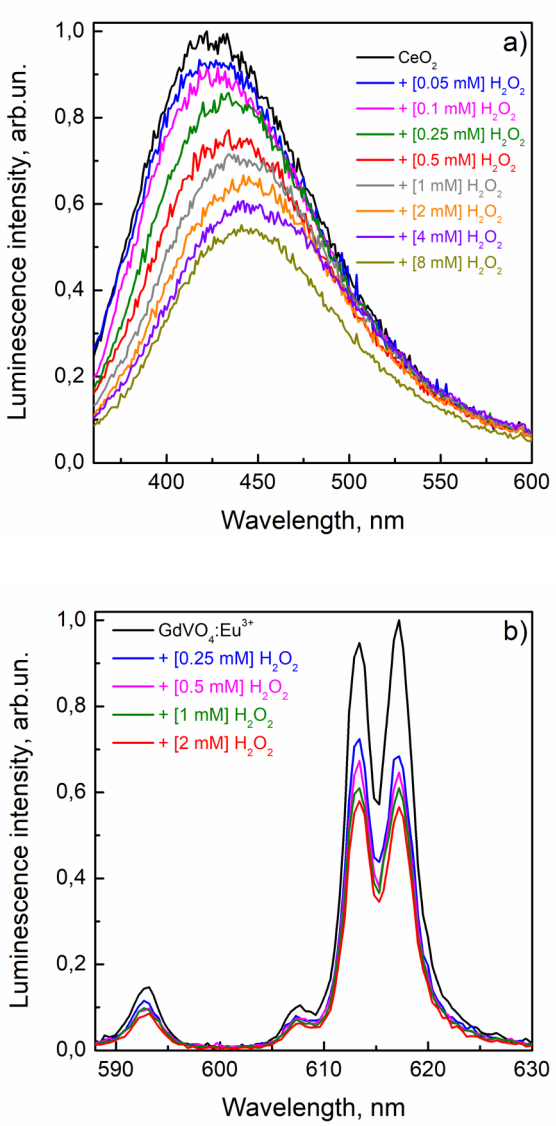

Fig.2. Turn-off luminescent sensing of HP concentration in water solutions by Ce^{3+} (a) and Eu^{3+} (b) doped ions (in CeO_{2-x} [31] and $GdVO_4$ [35] NPs, respectively).

$^5D_0 \rightarrow {}^7F_1$ transition is symmetry insensitive (magnetodipole), while $^5D_0 \rightarrow {}^7F_2$ transition is highly sensitive to symmetry (electrodipole), so if an interaction of NP with HP involves the modification of the symmetry of luminescent center, the intensity of $^5D_0 \rightarrow {}^7F_2$ band can change significantly relative to intensity of $^5D_0 \rightarrow {}^7F_1$ one. The combined approach (use of ratio between these two Eu^{3+} transitions in $REPO_4$-$REVO_4$ heterostructures) was proposed in [37]. The ability for ratiometric measurements in this study was determined by sensitivity of $^5D_0 \rightarrow {}^7F_1/ {}^5D_0 \rightarrow {}^7F_2$ ratio to HP concentration in vanadates, and insensitivity of this ratio to HP concentration in phosphates.

4. The mechanisms of luminescence quenching providing turn-off HP sensing

Dynamic quenching

One of approaches to description of the processes of turn-off HP sensing by quenching of luminescence of rare-earth doped nanocrystals by HP is the description in the framework of theory of dynamic quenching. Dynamic quenching, also called collisional quenching, according to definition, is a diffusion-mediated process that depends on the diffusion of an emitter or a quencher through a medium. In the case of dynamic quenching, the concentration dependence of emitter intensity is described by the Stern–Volmer relationship [38]:

$$I_0/I = 1 + K_{SV}[Q],$$

where I_0 is the initial luminescence intensity before analyte (i.e. HP) addition, I is the luminescence of the solution for specific analyte concentration [Q] and K_{SV} is the Stern–Volmer constant. Providing the plot of $I_0/I_{vs}[Q]$ is linear, K_{SV} can be determined. This approach was successfully used for $CePO_4:Tb^{3+}$NPs [28], Eu^{3+}- doped $REPO_4$-$REVO_4$ heterostructures [37], Ce^{3+}/Tb^{3+}-doped $NaYF_4$ microrods [29], and $CePO_4:Sm^{3+}$ nanorods [32], for which the linear dependence of I_0/I ratio on HP concentration was shown (Fig. 3a).

Besides the change of luminescence intensity, the luminescence decay times of doped ions also decrease as a result of interaction with analyte. The concentration dependence of decay time takes the form:

$$\tau_0/\tau = 1 + K_{SV}[Q] = k_q\tau_0[Q],$$

where τ_0 is the decay time of doped ions in the absence of quencher (analyte), and k_q is bimolecular quenching constant. So, the change in the decay time of doped ions can be measured instead of the change of luminescence intensity.

Redox processes

Recently a number of luminescent ROS sensors based on the redox reactions involving either oxidation or reduction of doped ions were proposed. HP can play the roles of both oxidant and reductant providing the change of the oxidation state of doped ion. For instance, $Ce^{3+} \rightarrow Ce^{4+}$ [31] and $Eu^{2+} \rightarrow Eu^{3+}$ [21] oxidation reactions accompanied by corresponding modification of luminescence spectra were used for sensing HP concentration in water solutions. The effect of decrease of luminescence intensity with increase of HP concentration is common for both dynamic quenching and quenching induced by redox processes, but the concentration dependences differ (Fig. 3). In Fig. 3 the concentration dependences for different mechanisms of HP-induced luminescence quenching are shown, namely, dynamic quenching shown for $CePO_4:Tb^{3+}$ NPs [28], and quenching induced by $Ce^{3+}\rightarrow Ce^{4+}$ oxidation in CeO_{2-x} NPs [31]. In the latter case, luminescence intensity was directly proportional to lg[Q] (where [Q] is HP concentration in mM) in contrast to dynamic quenching, where I_0/I was directly

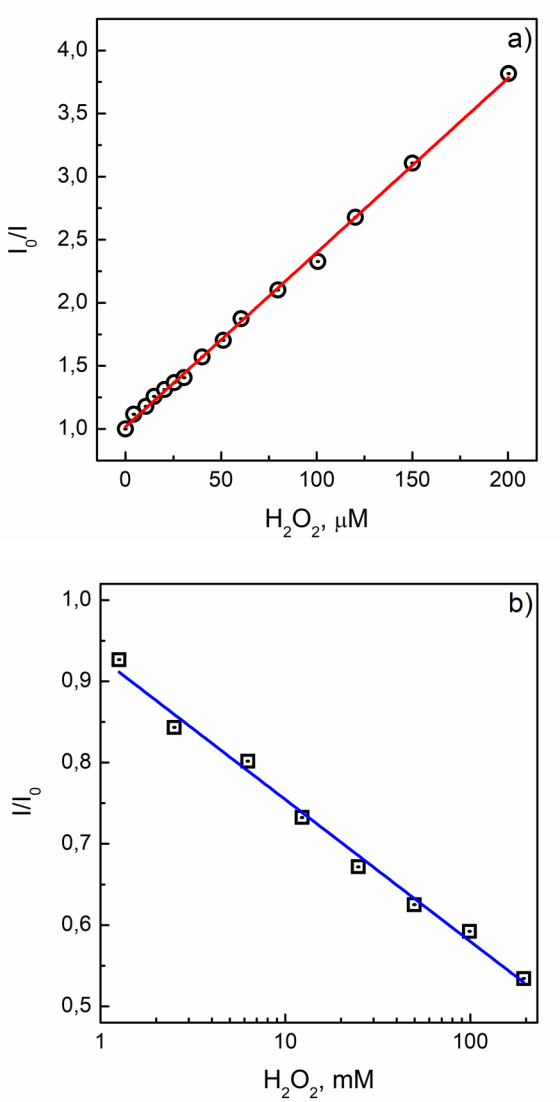

Fig.3. Luminescence intensity - HP concentration dependences for different mechanisms of HP-induced luminescence quenching: a) Dynamic quenching in $CePO_4:Tb^{3+}$ NPs [28], b) Quenching induced by $Ce^{3+}\rightarrow Ce^{4+}$ oxidation in CeO_{2-x} NPs [31].

proportional to [Q]. Clear distinction between these two mechanisms of HP sensing can be made by measurement of decay times, as oxidation/reduction of part of doped ions changes only the number of emitting centers, but not the rate of emission.

Products of ROS decomposition as quenchers

It is noticeable that some nanoparticles are not just sensors of HP, but also can take an active part in the processes of HP decomposition. The catalase-like (decomposition of HP to water and oxygen without formation of intermediate oxygen radicals) and SOD-like (turning of superoxide molecules to HP and oxygen) activities of cerium oxide NPs was a subject of wide discussions since mid-2000s [39, 40]. So, the idea of combining enzyme-like and sensing properties of such NPs proposed at first by [23] was absolutely natural. The CeO_2:Eu^{3+} NPs with low Eu^{3+} concentrations (less than 5 at. %) obtained by [23] preserved catalase-like activity providing at the same time an efficient HP sensing via the quenching of Eu^{3+} luminescence. At the same time, our own results on the same NPs (CeO_2:Eu^{3+}) with Eu^{3+} concentration of 10 at. % have shown that these NP preserve HP sensing properties, but their catalase-like activity decreased sufficiently turning into Fenton-like.

NPs with Fenton-like activity decompose HP with formation of hydroxyl radicals. As a result, hydroxylation of the surface NPs during the process of HP decomposition via Fenton-like mechanism leads to quenching of luminescence of doped ions due to energy transfer between these ions and hydroxyl groups.Eu^{3+} luminescence quenching by hydroxyl groups was initially shown for SnO_2:Eu^{3+} NPs [41]. This mechanism of HP-induced Eu^{3+}luminescence quenching was also confirmed in our studies of HP sensing by CeO_2:Eu^{3+} [42] and $GdVO_4$:Eu^{3+} [35] nanoparticles.

5. Improvement of sensing and reversibility of NPs-based luminescent sensors

Antenna effect

One of the main drawbacks of rare-earth ions for luminescent applications is the low value of molar absorption coefficient because 4f intraconfigurational transitions are forbidden by the Laporte rule. Low probability of these transitions leads to low luminescence intensity, and so, HP sensitivity. The intensity of luminescence of doped rare-earth ions can be increased by sensitizing effect of co-dopants (ions with higher absorption coefficients), or organic molecules. The sensitization of luminescence of doped rare-earth ions by organic ligands is also known as antenna effect, and is widely used in sensors based on lanthanide chelates/MOFs. For instance, authors of [20] obtained Eu-metal organic framework (Eu-MOF) HP probe, in which 5-boronobenzene-1,3-dicarboxylic acid (BBDC) effectively absorbed photons to produce its triplet state through intersystem crossing procedure. As the triplet state matched the energy levels of Eu^{3+} and Tb^{3+}, the luminescent signal of these ions increased providing more efficient HP detection. The sensitization can be achieved not only by using organic ligands, but also by co-doping with different doped ions with higher absorption value. Tb^{3+}-doped and Ce^{3+}-co-doped CeO_2 [27] and $NaYF_4$ [29] HP sensors involve the use of sensitizing effect of Ce^{3+} ions, which effectively absorb UV radiation and transfer excitation energy to Tb^{3+} ions, which intensity, in turn, changes with HP content.

Reversibility of NPs-based luminescent sensors

In contrast to sensors based on luminescence of organic molecules (such as dichlorodihydrofluorescein diacetate, dihydrorhodamine and Amplex Red (Invitrogen)), NPs-based HP sensors are able for multi-use. The reversibility (i.e. the ability to recover their luminescence properties after interaction with HP) is one of the most important parameters of NPs-based HP sensors. The recovery of initial luminescent signal of HP-sensing NPs can be attained by different ways: by addition of reducing agents [43], photoreduction [21], or high temperature [22]. In [21] the authors have obtained $Y_{0.6}Eu_{0.4}VO_4$ nanoparticles, which underwent $Eu^{3+}\rightarrow Eu^{2+}$ photoreduction under laser irradiation before HP addition leading to $Eu^{2+}\rightarrow Eu^{3+}$ oxidation. Both these processes were accompanied by corresponding changes of the luminescence spectra allowing to sense HP concentrations in the range of 1–45 μM. An alternative approach was proposed in [43], where the reversibility of CeO_2:Eu^{3+} luminescent sensors was achieved by subsequent addition of oxidant (HP) and reductant (ascorbic acid) leading to recovery of Ce^{3+}/Ce^{4+} balance and, accordingly, Eu^{3+} luminescent signal. As a result, these materials were potent as both HP (with recovery by ascorbic acid) and ascorbic acid (with recovery by HP) sensors [43]. Instead, in [22] the authors heated the YVO_4:

Eu^{3+} NPs-based films to 200 °C to provide recovery of the sensor after interaction with HP.

Some NPs are able to decompose HP and recover their initial state after completion of HP decomposition, so, generally, they do not need special techniques to get into initial state. Cerium oxide nanocrystals (nanoceria) are ones of the most known NPs with ability to self-regeneration determined by reversible Ce^{3+}/Ce^{4+} switching [44, 45]. However, the times of recovery of initial luminescence intensity of doped ions after HP addition in these nanocrystals can be too long for practical application of these sensors. So, increasing the rate of recovery of luminescent signal of NPs-based sensors can be of high importance for a number of technical and biomedical applications. For instance, our studies have shown that the process of recovery of initial Ce^{3+} content in 2 nm nanoceria can last for more than 120 hours at room temperature. The duration of this process, however, decreases sufficiently at higher temperatures (from 120 hours at 22 °C to 22 hours at 37 °C, and to 4.5 hours at 52 °C) (Fig. 4a). The recovery rate can be increased even more by UV irradiation with λ = 325 nm (down to tens of minutes). The irradiation with the wavelengths corresponding to absorption band of nanoceria leads to $O^{2-} \rightarrow Ce^{4+}$ electron transfer stimulating $Ce^{4+} \rightarrow Ce^{3+}$ reduction, and so, favors the processes of recovery of Ce^{3+} luminescence intensity [42] (Fig. 4b).The same effect of temperature and UV on the recovery rate was observed for CeO_2:Eu^{3+} HP luminescent sensors [42].

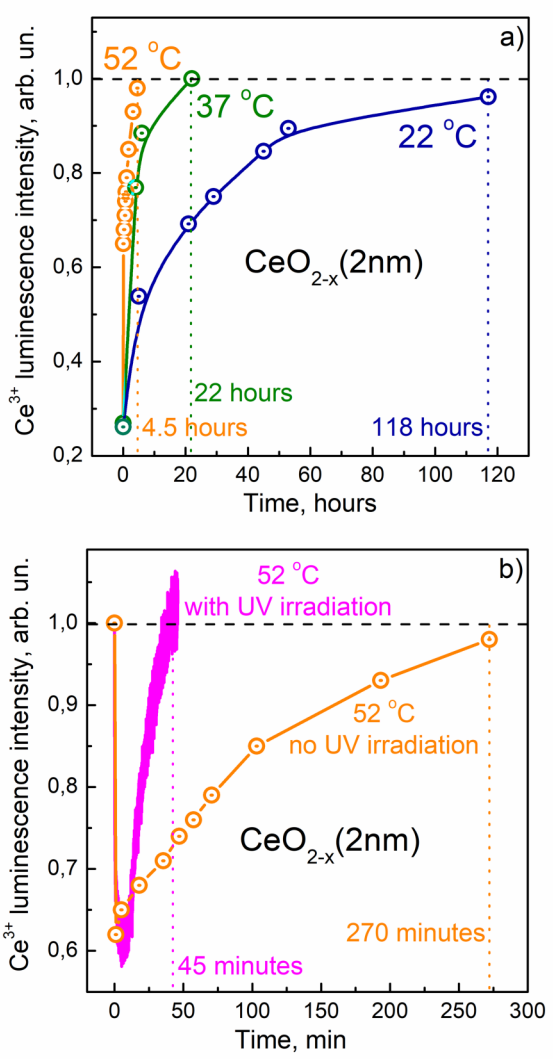

Fig. 4. Possible mechanisms of improvement of recovery rates of sensors on the example of CeO_{2-x} NPs [42]: a) high temperature, b) UV irradiation.

6. Biomedical applications of HP sensors based on luminescence of rare-earth ions

The most perspective field of application of materials described in the above sections is HP sensing in biological objects of various kinds. HP is a byproduct of various enzymes and one of the most widespread cell signaling molecules. In this way, assessing the content of HP in the living cell provide reliable information on the various biological processes inside the cell. Biomedical applications of luminescent HP sensors based on rare-earth ions up to now are limited by relatively high LOD levels for most sensors, however, some publications already report the successful use of rare-earth-based sensors in the biological cells which produce high HP concentrations (of μM range).

The authors [36] have studied the response of CeO_2:Eu^{3+} luminescent sensors in bacterial cell cultures using Streptococcus pneumoniae (the pneumococcus) clinical isolates that generate high amounts of HP (BHN31 and BHN32). The concentrations of HP determined using CeO_2:Eu^{3+}sensors (~250 μM for BHN31 and (~250 μM for BHN32) were close to the ones obtained previously using alternative methods confirming the reliability of the sensors.

Even less HP concentrations were detected by $Y_{0.6}Eu_{0.4}VO_4$NPs, which were used for sensing HP formed in the signaling processes [21]. Invascular cells ET-1 and PDGF receptors regulate HP level produced by NOx enzyme, and corresponding values of HP concentration turned out to be in the range measurable by $Y_{0.6}Eu_{0.4}VO_4$ luminescent sensors. The measurement of sensor response gives 7 μM HP at PDGF stimulation and 13 μM HP at ET-1receptor stimulation.

Besides HP production in cells, luminescent sensors were used for sensing HP level in biological fluids. In this way, Phe/Tb-CPBA CPNPs was used as a fluorescent probe for the measurement of the level of HP in urine sample revealing HP concentration of ~15 μM [24].

HP concentration determined by the change of fluorescence signal of nanoparticle itself or organic molecules attached to the surface of this NP, can be used as an indirect measure of the concentration of other biologically important molecules. The level of HP can be used as a measure of the content of antioxidant enzymes (catalase and superoxide dismutase). Catalase is the enzyme responsible for decomposition of hydrogen peroxide to water and oxygen, while superoxide dismutase turns superoxide anion (O_2^-) into oxygen and hydrogen peroxide. The action of both these enzymes changes the content of HP either decreasing (catalase) or increasing it (superoxide dismutase). In [46] the HP-dependent luminescence of Eu^{3+}-tetracycline complex was used for measuring the content of catalase in water solutions. High sensitivity to HP of these complexes (LOD = 1.8 μM) opens the way for precise measurements of catalase content (LOD = 0.046 units/ml). In the same way, in [47] Eu^{3+}-tetracycline complexes were used for measurement of the content of superoxide dismutase in water solutions.

HP is a trace product of action of glucose oxidase (GOx) turning glucose into gluconic acid and HP in the presence of molecular oxygen, so besides determination of HP content, the content of glucose in water solutions of biological media can be determined using NPs-based sensors. HP detection in concentrations down to 130 nM and glucose detection in concentrations down to 8.9 μM by ceria NPs covered by fluorescent-labeled DNA probes was reported in [48]. Displacement of DNA from the surface induced by HP formed in GOx-catalyzed reaction led to 20-fold fluorescence enhancement of the probes [48]. In [49] peroxidase activity of Fe-doped ceria nanorods was used for indirect measurement of glucose concentration by the change of absorption spectra during TMB peroxidation by HP formed by GOx-catalyzed reaction. In the similar way [50], the level of HP produced by cholesterol oxidase (ChOx) from cholesterol was determined by O-phenylenediamine (OPD) peroxidation catalyzed by carbon dots-doped CeO_2 nanoparticles enabling the indirect measurement of cholesterol concentration.

Conclusions

The luminescence of rare-earth ions in inorganic NPs, as well as in polymer NPs and MOFs is sensitive to the content of reactive oxygen species (such as HP) in water solutions and biological objects. The dependence of luminescence intensity/spectral composition on the HP concentration allowed proposing these materials as a new type of HP sensors with improved sensibility, stability, and reversibility compared to traditional HP sensors based on organic molecules. The new trend is related to NPs combining enzyme-like and sensing properties which are able to decrease the level of oxidative stress in living cells allowing controlling the change of ROS content during ROS scavenging process. The possible biological applications of NPs-based HP sensors include, but not limited by, the assessment of the signaling processes, level of oxidative stress, and content of other important biological molecules that can be indirectly measured by the HP content in living cells.

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