Supporting Information

Direct monitoring of the ROS-cerium dioxide nanoparticles interaction in living cells

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Materials and methods

The following solutions were used as starting materials: 0.01 M $CeCl_3$ solution in water, 0.01 M $Ce(SO_4)_2$ solution in 0,5 M H_2SO_4, 0.001 M calcein (CLC) solution. 0.01 M non-stabilized aqueous cerium dioxide nanoparticles (CDN) sol was synthesized according to previously reported protocol [1].

Fluorescence measurements were carried out on a Varian Cary Eclipse spectrofluorimeter equipped with a xenon lamp (150 V). Spectrophotometric measurements were performed on a Shimadzu UV-2401PC spectrophotometer. pH value of the media was adjusted to 7.2, using a Tris-buffer solution (Sigma-Aldrich).

UV-vis spectroscopy of starting solutions

UV-vis absorption spectra of CLC, cerium(III) chloride, cerium(IV) sulfate) aqueous solutions and CDN aqueous sol are shown in Figure S1.

Calcein is a fluorescent dye with excitation and emission wavelengths of 495/515 nm, respectively (see Figure S2).

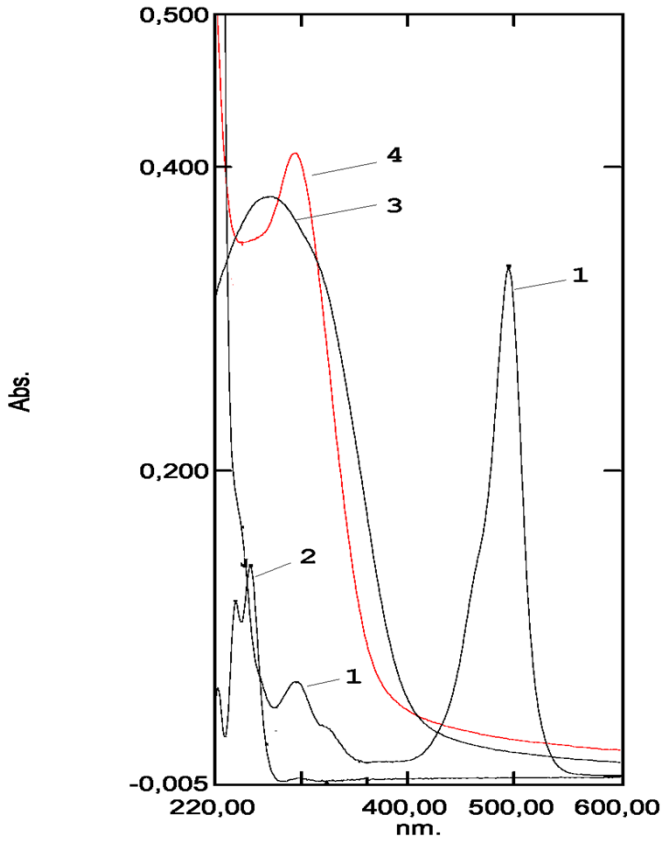

Figure S1. UV-Vis absorption spectra: 1 - 1×10^{-5} M CLC, 2 - 1×10^{-4} M $CeCl_3$, 3 - 1×10^{-4} M $Ce(SO_4)_2$, 4 – 1×10^{-4} M non-stabilized CDN sol.

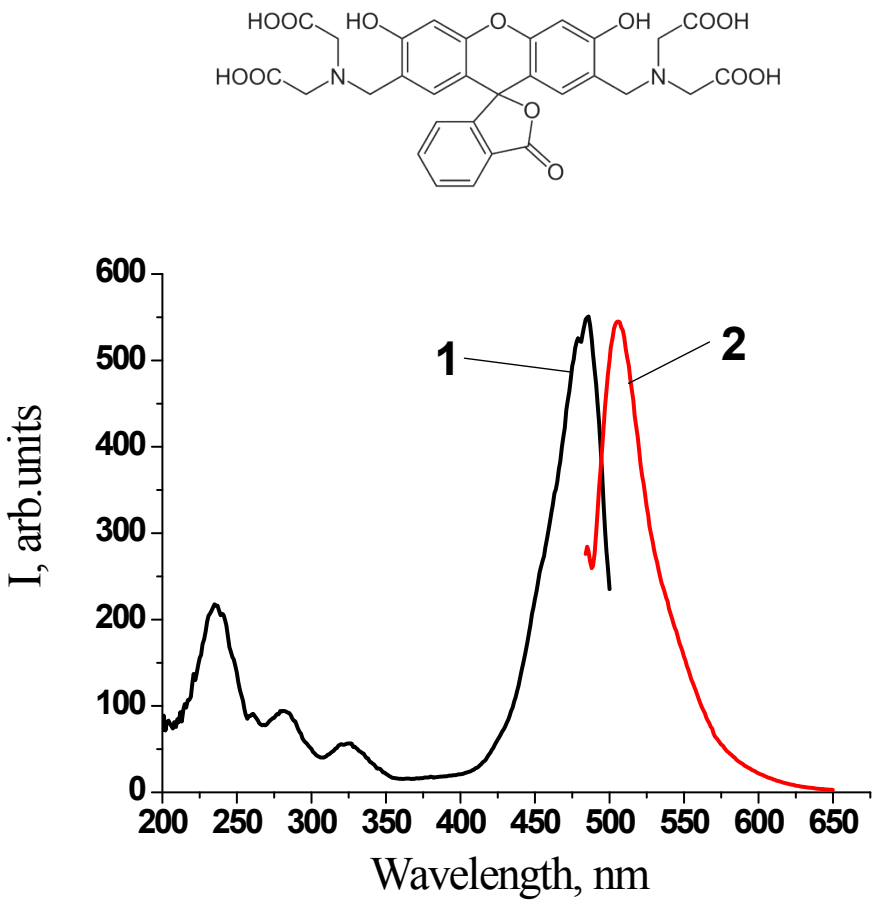

Figure S2. CLC molecule and its excitation (1) and emission (2) spectra ($C_{CLC} = 1 \times 10^{-5}$ M).

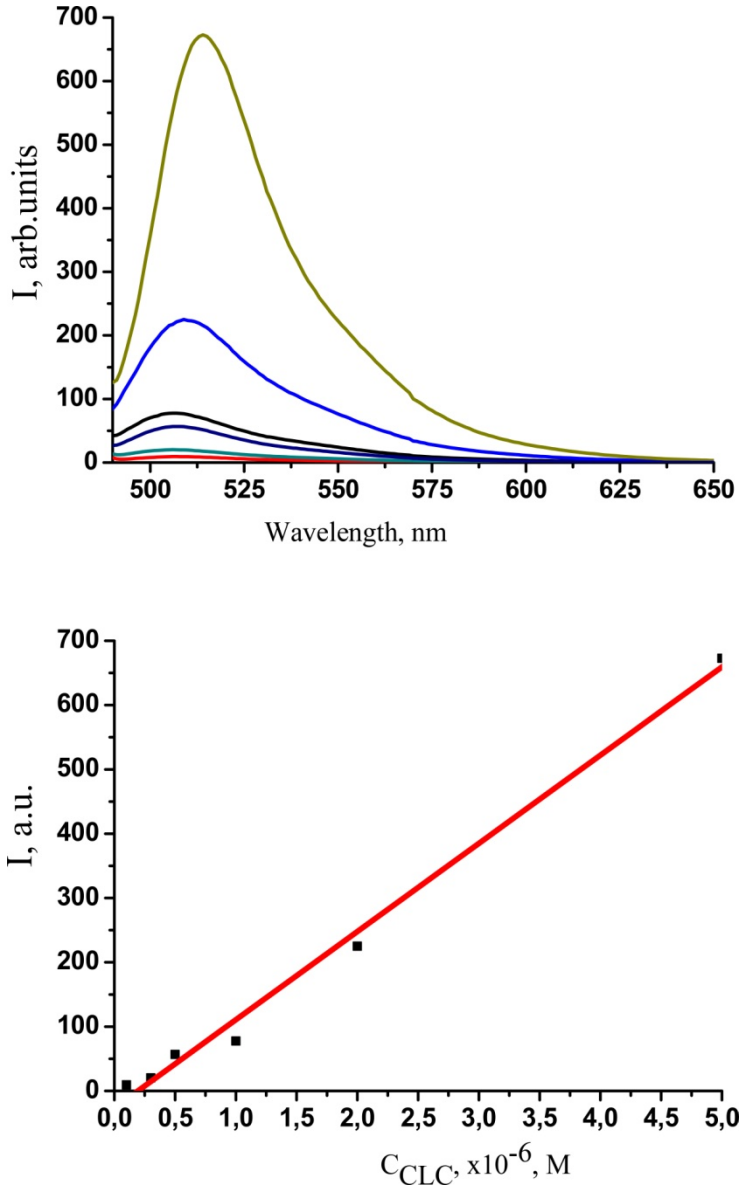

Figure S3. Fluorescence spectra of CLC (above) and corresponding calibration curve (below), pH=7.2, λ_{emiss} = 508 nm (λ_{excit} = 485 nm).

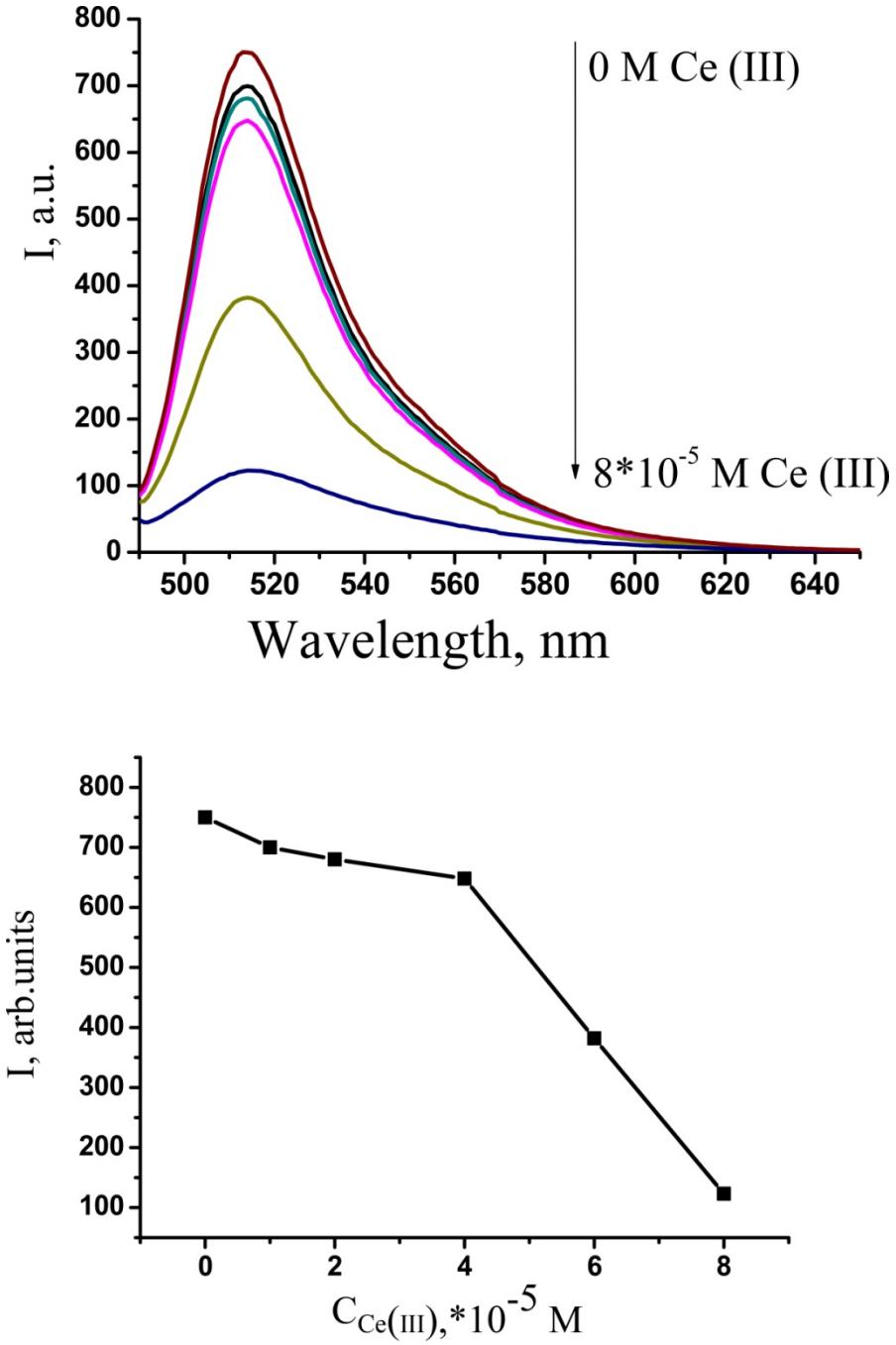

Figure S4. Intensity of CLC solutions fluorescence (C_{CLC} = 1×10^{-6} M, pH=7.2) in the presence of various concentrations of cerium(III) ions.

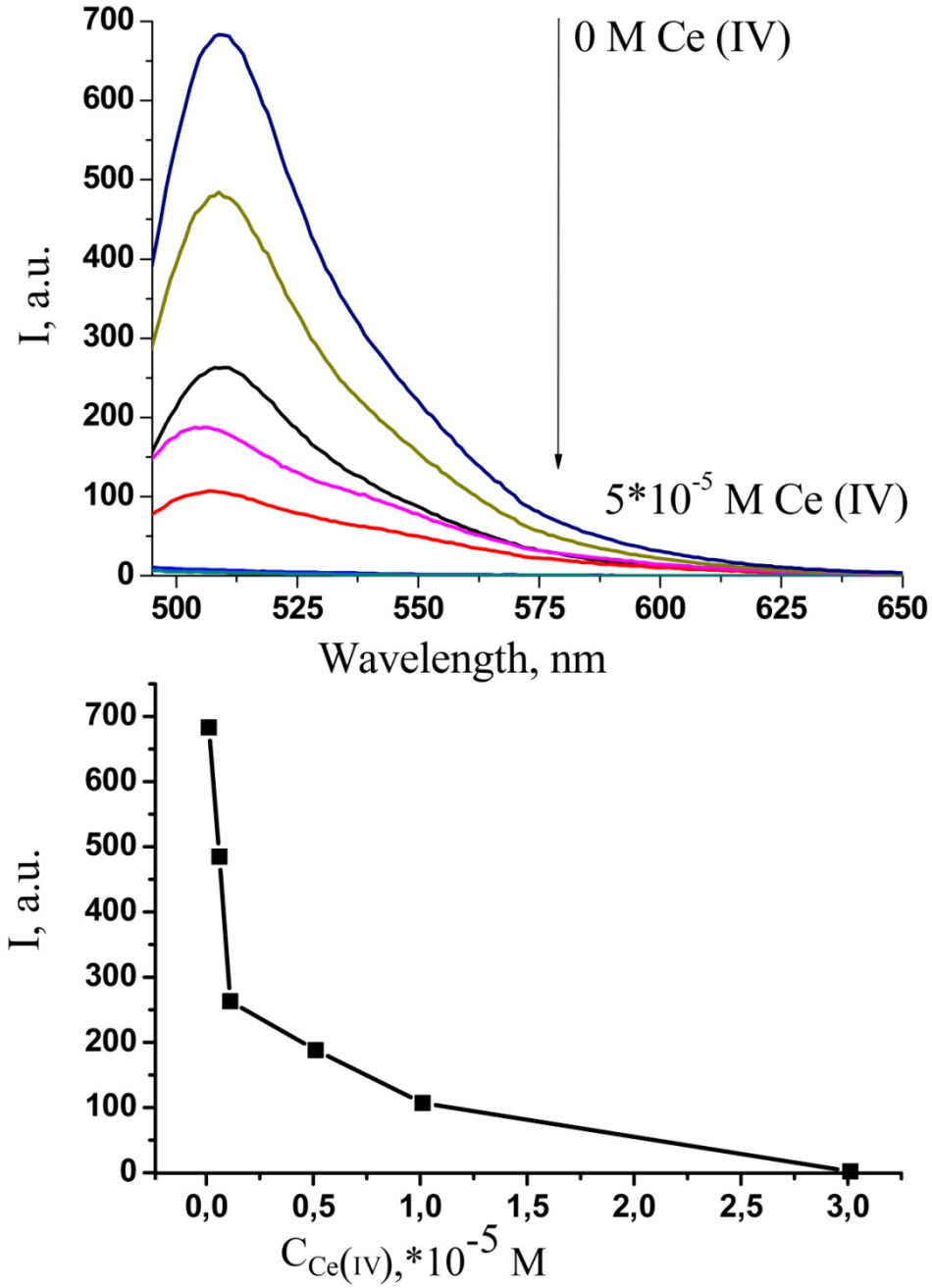

Figure S5. Intensity of the CLC solutions (C_{CLC} = 1×10^{-6} M, pH=7.2) fluorescence in the presence of various concentration of cerium (IV) ions.

Grafting density of CLC on the surface of CDN

When CLC molecule is in planar orientation on the CDN surface, it occupies a site containing ca. 20-25 atoms of cerium (see Figure S6).

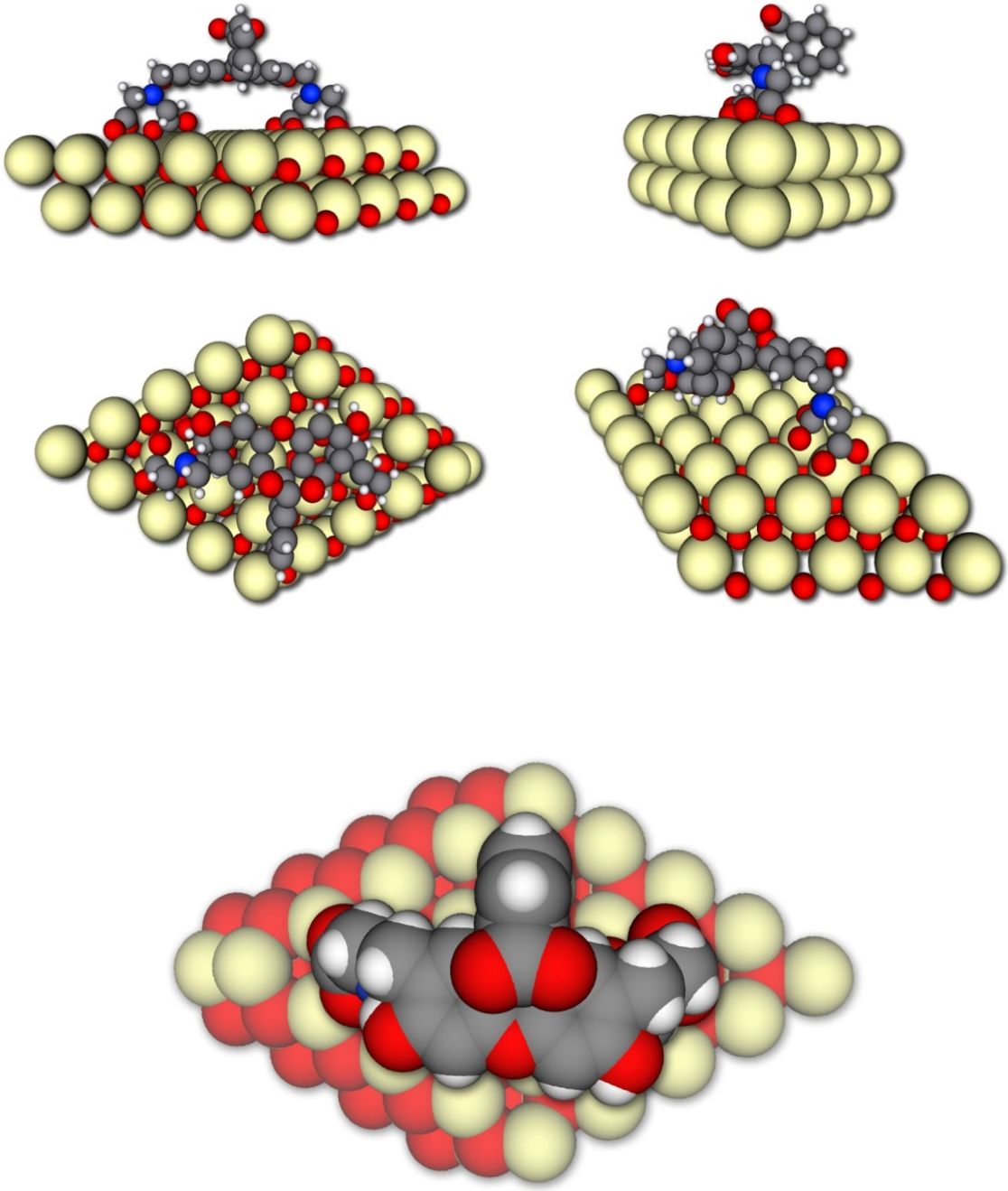

Figure S6. Top – Cerium dioxide nanocluster with a grafted CLC molecule (covalent radii). Bottom – the same structure plotted using van der Waals radii.

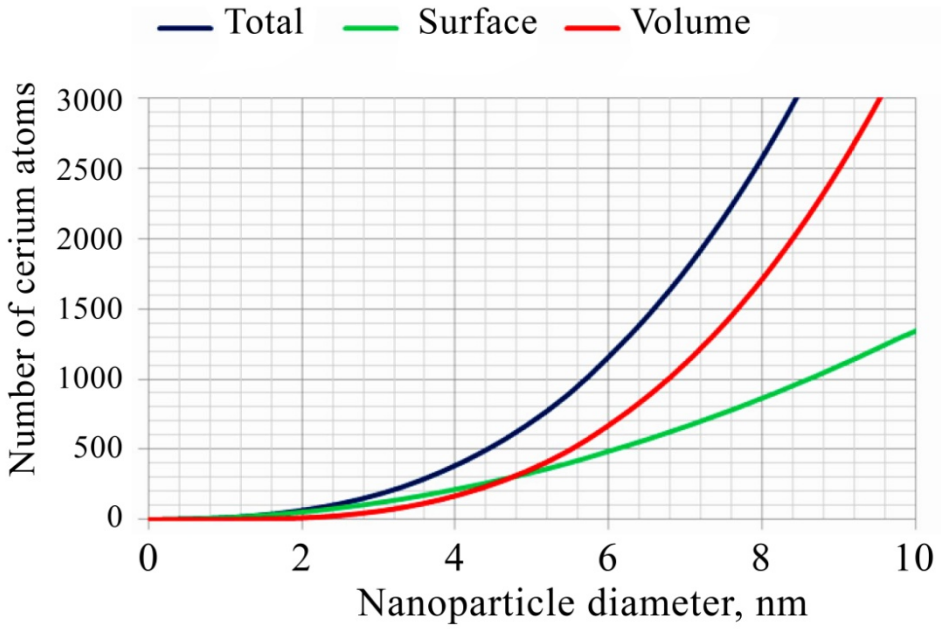

Figure S7. The calculated number of surface and bulk cerium atoms in CeO_2 nanoparticles, as a function of the particle size.

The quantity of atoms on the surface and in the bulk of the CDN depends on its size, as shown in Figure S7 (data for calculations taken from [2]). For the particle of ~6 nm size there are approximately (500/1200 *100) ≈ 40% of cerium atoms on the surface. For the particle of ~8 nm size there are approximately (850/2600 *100) ≈ 30% of cerium atoms on the surface. In case of dense packing, all CLC molecules are bound to the CeO_2 surface when the molar ratio of CLC to cerium is 1: (60…80). Taking into account that the surface of the particles is non-ideal, this molar ratio can be estimated as 1:50...100.

In Stern-Folmer coordinates, the linear dependence between the intensity of the fluorescence (I_0/I) and the concentration of cerium is observed in a narrow interval of concentrations ($5 \cdot 10^{-7}$ - $1 \cdot 10^{-4}$ M) (see Figure S8).

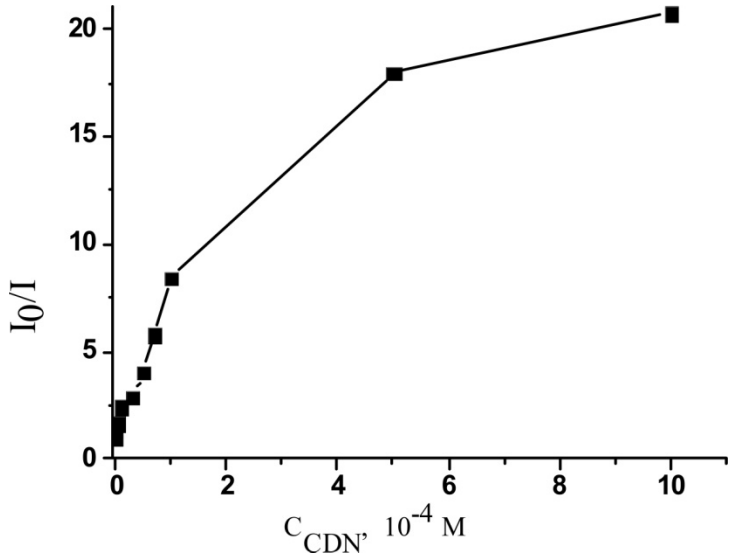

Figure S8. Dependence of the intensity of CLC fluorescence on CDN concentration in Stern-Folmer coordinates.

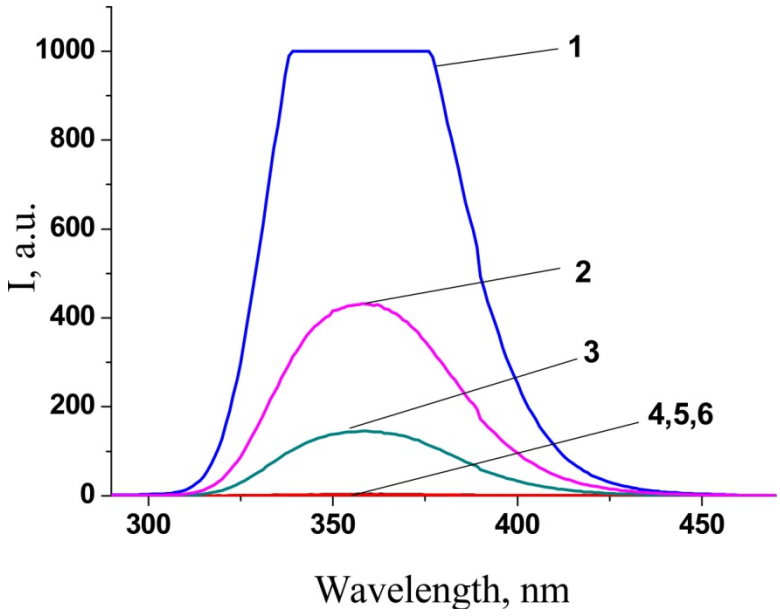

Figure S9. Fluorescence spectra of cerium(III) solutions (1 - $C_{Ce(III)} = 1 \times 10^{-4}$ M; 2 - $C_{Ce(III)} = 1 \times 10^{-5}$ M; 3 - $C_{Ce(III)} = 1 \times 10^{-6}$ M) and solutions of CDN (4 - $C_{CDN} = 1 \times 10^{-4}$ M; 5 – $C_{CDN} = 1 \times 10^{-5}$ M, 6 – $C_{CDN} = 1 \times 10^{-6}$ M). Excitation and emission wavelengths are 250/358 nm, respectively.

Influence of hydrogen peroxide (H_2O_2) on the intensity of fluorescence of CLC and complexes of CLC with CDN, cerium(III) ions, or cerium(IV) ions.

Hydrogen peroxide, in a wide range of concentrations (5×10^{-7} – 1×10^{-3} M), causes only insignificant suppression of the fluorescence of the CLC (not more than ~20 %) (Figure S10).

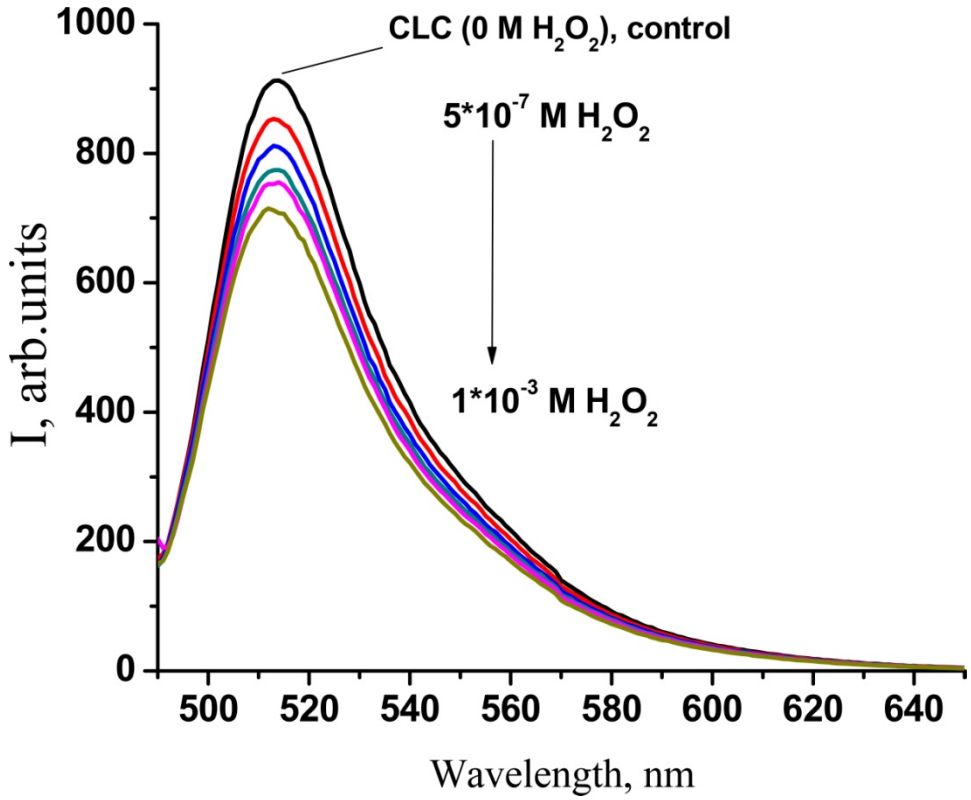

Figure S10. Fluorescence spectra of CLC solutions ($C_{CLC} = 1\times10^{-6}$ M; pH 7.2) in the presence of various concentrations of H_2O_2.

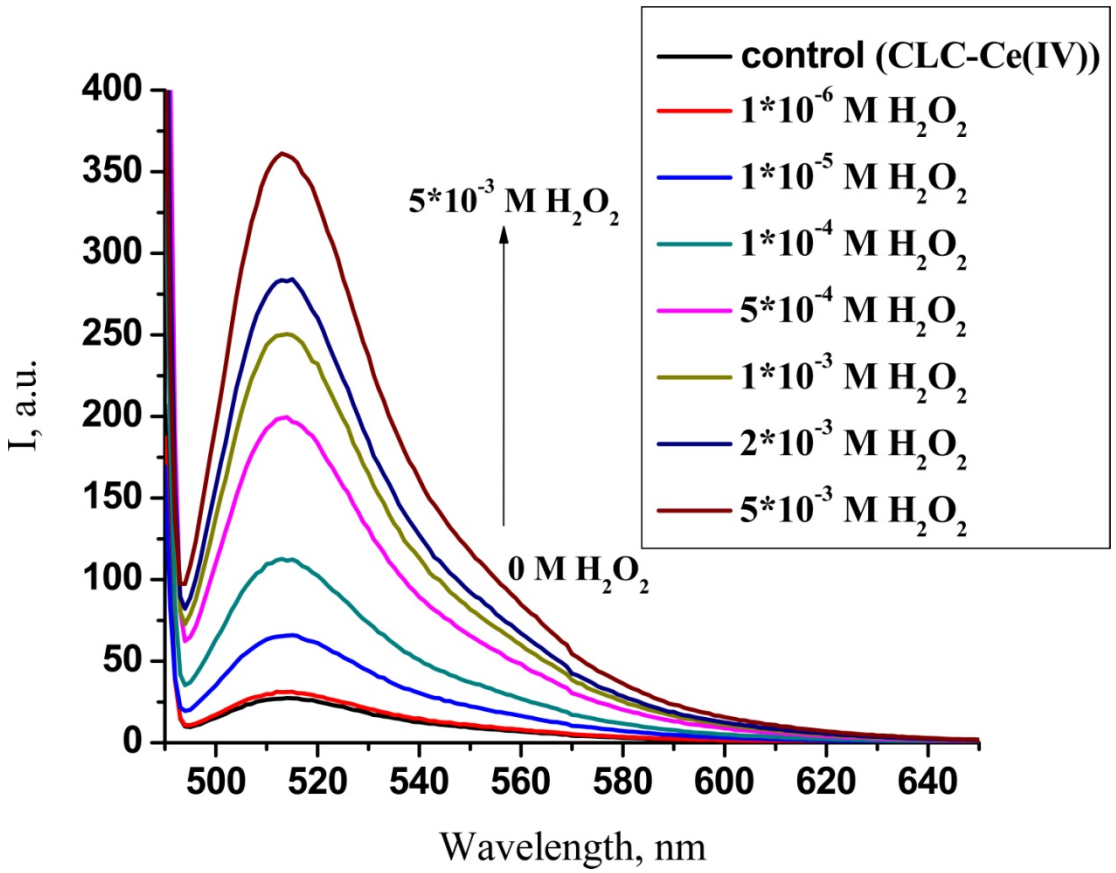

Figure S11. Fluorescence spectra of CLC solutions in the presence of cerium (IV) ions and various concentrations of hydrogen peroxide (1×10^{-6} - 5×10^{-3}). $C_{CLC} = 1\times10^{-6}$ M; $C_{Ce(IV)} = 5\times10^{-5}$ M; pH 7.2.

Introduction of hydrogen peroxide into a solution of CLC-Ce (III) complex gives a completely different result, namely, additional quenching of the CLC's fluorescence (Figs. S12, S13).

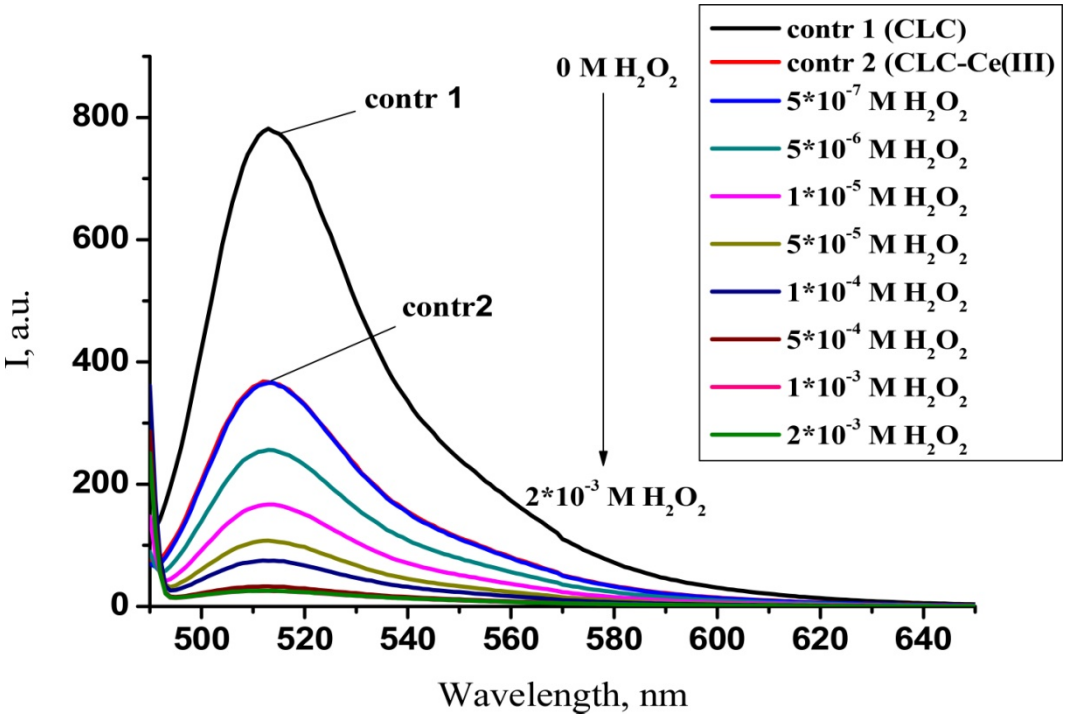

Figure S12. Fluorescence spectra of CLC in the presence of cerium(III) ions and various concentrations of hydrogen peroxide (1×10^{-6} - 5×10^{-3}). C_{CLC} = 1×10^{-6} M; $C_{Ce(III)}$ = 1×10^{-4} M; pH=7.2.

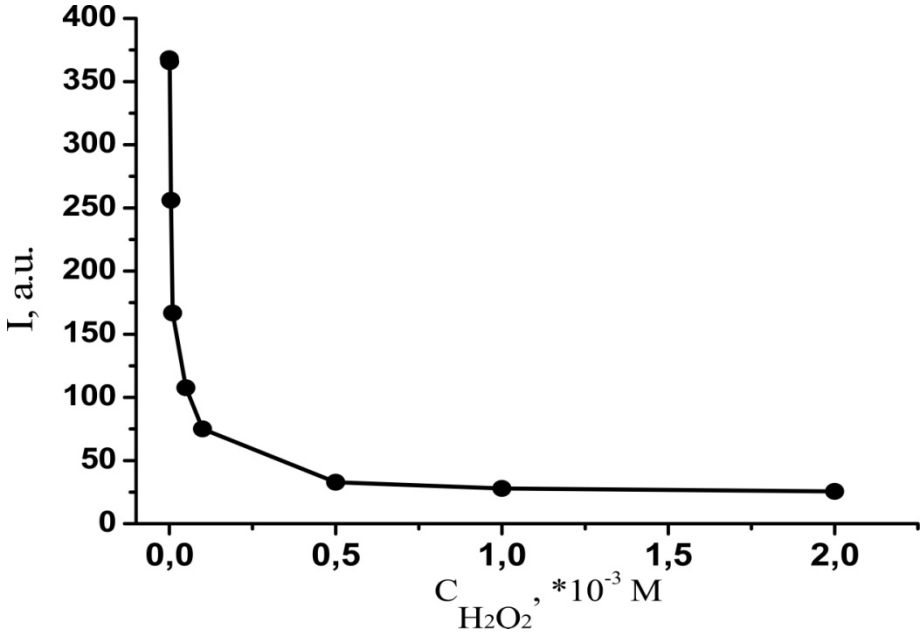

Figure S13. Dependence of the intensity of CLC fluorescence in the presence of Ce(III), upon increase in hydrogen peroxide concentration from 1×10^{-6} M to 2×10^{-3} M.

The fluorescence of cerium(III) ions in the presence of CLC is quenched, and introduction of hydrogen peroxide leads to additional suppression of the Ce(III) fluorescence (Fig. S15, S16).

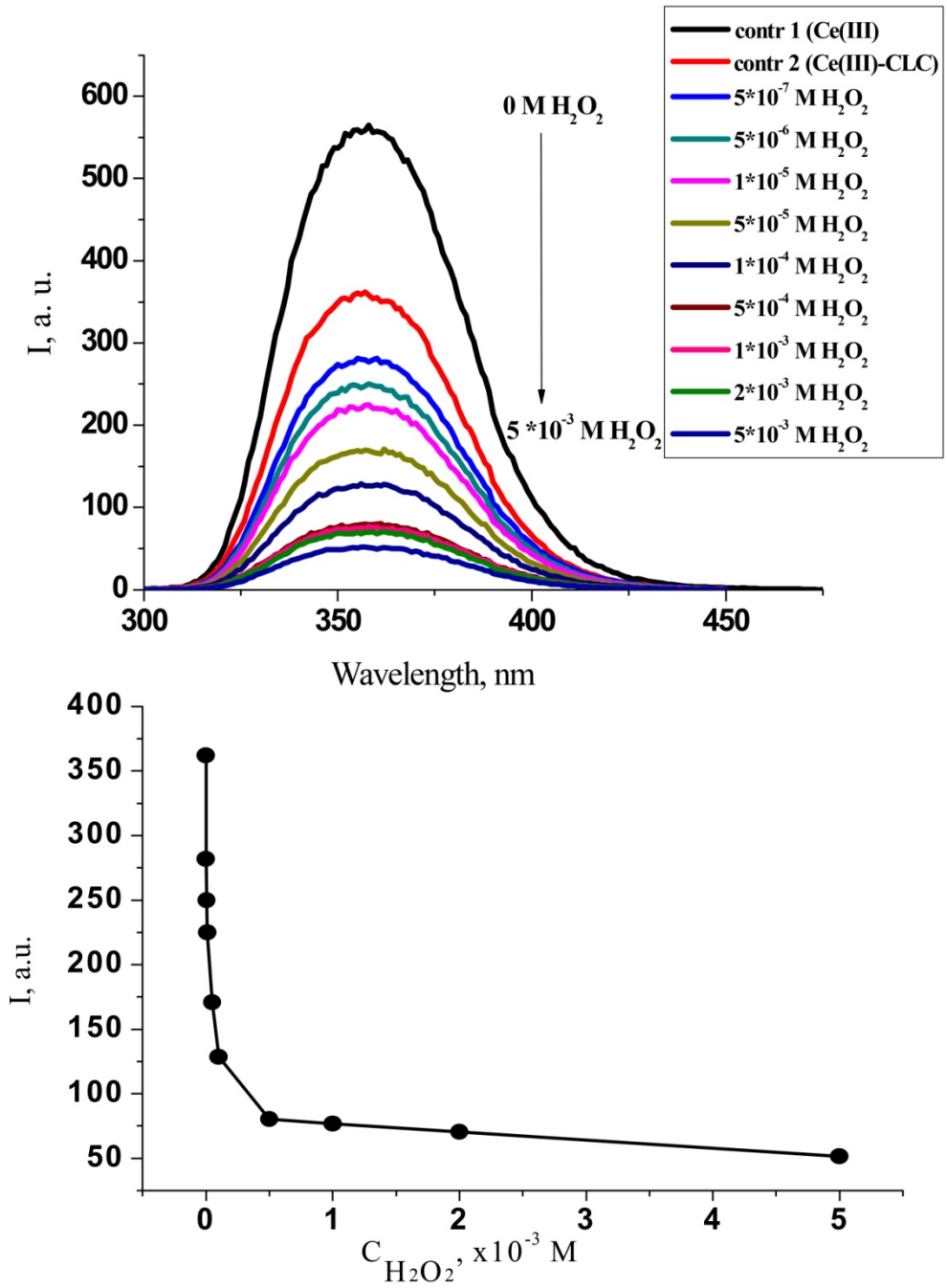

Figure S14. Fluorescence spectra of cerium(III) ions in the presence of CLC and various concentrations of hydrogen peroxide (1×10^{-6} - 5×10^{-3}). $C_{CLC} = 1\times10^{-6}$ M; $C_{Ce(III)} = 1\times10^{-4}$ M; pH=7.2; $\lambda_{ex} = 250$ nm.

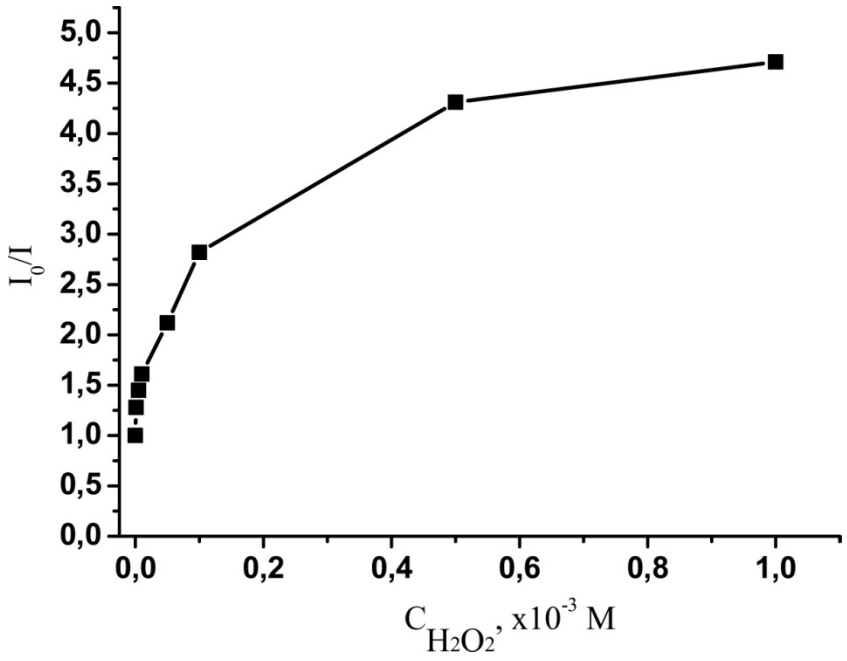

Figure S15. Intensity of cerium(III) ions fluorescence in the presence of CLC and various hydrogen peroxide concentrations (1×10^{-6} - 2×10^{-3}) in Stern-Folmer coordinates. $C_{CLC} = 1\times10^{-6}$ M; $C_{Ce(III)} = 1\times10^{-4}$ M; pH=7.2; $\lambda_{ex} = 250$ nm.

It is well known that the intensity of Ce(III) fluorescence decreases upon complexation. Ce(III) ions fluorescence quenching by CLC and H_2O_2 could be possibly explained by the formation of CLC and peroxide complexes. However, it is more likely that CLC is decomposed by peroxide radicals, which are formed at Fenton's reaction in the presence of cerium (III) ions and hydrogen peroxide [3], similarly to fluorescein decomposition [4].

$Ce^{3+} + H_2O_2 \rightarrow Ce^{4+} + HO^{\bullet} + OH^-$

CLC + $HO^{\bullet} \rightarrow$ oxidized CLC (non-fluorescent)

1. V.K. Ivanov, O.S. Polezhaeva, A.B. Shcherbakov and Y.D. Tret'yakov, *Russian Journal of Inorganic Chemistry,* 2010, **55**, 1.
2. C. Sun and D. Xue, *Physical Chemistry Chemical Physics,* 2013, **15**, 14414.
3. E. G. Heckert, S. Seal and W. T. Self, *Environmental science & technology,* 2008, **42**, 5014.
4. B. Ou, M. Hampsch-Woodill, J. Flanagan, E. K. Deemer, R. L. Prior and D. Huang, *Journal of Agricultural and Food Chemistry,* 2002, **50**, 2772.