

Fast quantitative ROS detection based on dual-color single rare-earth nanoparticle imaging reveals signaling pathway kinetics in living cells

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ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

Dual probe design

We discuss here the principles and requirements for the design of a quantitative and time-resolved probing method based on the simultaneous detection of two luminescent reporters.

Note first that H_2O_2 probing is an inverse problem in the sense that we measure photoluminescence (PL_i) signals from which we need to deduce the metabolite (M) concentration in the milieu. In the case of two luminescent reporters, we aim at writing the following relation:

$$[M] = F(\{PL_i\}),$$

where F is an arbitrary well defined function, *i.e.* every argument $\{PL_i\}$ has no more than one image M . In the general case, photoluminescence signals can be written as $PL_i(t)=f_i([M],t)$, where f_i is the probe response function. Only if each $\{f_i\}$ is monotonous with t , can we write $t=u_i([M], PL_i(t))$. In this case, we can define an implicit relation between the photoluminescence signals and $[M]$:

$$G([M], PL_1, PL_2) = 0, \text{ where } G \text{ is a function depending on } f_1 \text{ and } f_2.$$

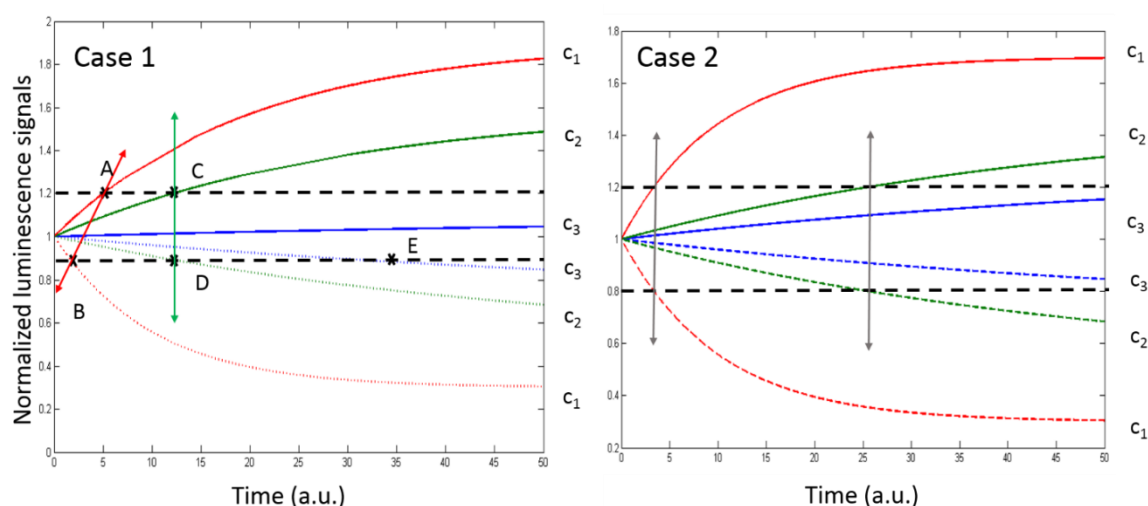
This condition has strong consequences on the required properties of $PL_1([M],t)$ and $PL_2([M],t)$. PL_1 and PL_2 must be monotonous functions of t , *i.e.* the sensor response has to increase (or decrease) with time for a given $[M]$. Each sensor has to be redox sensitive and must be used below the saturation range and above the detection limit, so that significant temporal variations may be detected.

This condition is necessary but not sufficient to ensure the existence of the function F : its existence depends on the relative shapes of the response of each sensor to M .

We exemplify this principle in the following scheme where we represent two sensor responses to three different metabolite concentrations (c_1 , c_2 and c_3). In the first case, the responses of each sensor are independent exponential functions - fulfilling the previously mentioned requirements – and, in the second case, the responses are also exponentials but with $PL_1 = -k \cdot PL_2$ with k a positive constant. We then examine the response to a constant concentration $[M] = c_2$ and address the question whether it is possible to determine the concentration based only on the simultaneous measurement of the two photoluminescence signals.

In the first case (Fig. S1 left), we consider the signal values of 1.2 and 0.9 measured by sensor 1 and 2, respectively. These signal values are observed in points A, B, C, D and E. However, E, and consequently concentration c_3 , is eliminated because E has no homologous observation in sensor 1. Moreover, A and B cannot be observed simultaneously (the [AB] segment is not vertical) which also excludes c_1 . Hence, the unique concentration that fulfills the simultaneity condition for the measured pair of PL signals (1.2, 0.9) is c_2 . This example shows how we can measure the metabolite concentration at the acquisition rate without taking into account, or being limited by, the sensor response kinetics.

In contrast, in the second case (Fig. S1 right), the pair of values (1.2, 0.8) measured by sensor 1 and 2, respectively, gives rise to several vertical segments, *i. e.* several concentrations for which these two values could have been observed simultaneously. There is therefore no unique concentration $[M]$ that can be attributed to these measurements and this pair of sensors is not adequate for the dual-probe approach, because their responses are not uncorrelated.



Scheme S1. Dual probe principle. Two sensor responses to 3 different metabolite concentrations. c_1 : red, c_2 : green, c_3 : blue. Sensor 1: solid lines, Sensor 2: dashed lines. The black dashed lines indicate the two measured signal values. Left (Case 1): The two sensor responses are independent. We examine the case of the measured signals being 1.2 and 0.9 for sensor 1 and 2, respectively. The pair of measured signals (1.2, 0.9) corresponds to

c_2 because it fulfills the simultaneity condition, i.e. the pair of measured signals can be realized at the same time only for this concentration. Right (Case 2): The two sensor responses are not independent with $PL_1 = -k \cdot PL_2$. For the pair (1.2, 0.8) of measured signals, multiple concentrations are possible (several vertical segments exist).

Characterization of YAG:Ce (4%) and $Gd_{0.6}Eu_{0.4}VO_4$ nanoparticles

We measured a zeta potential of 37 mV and -18 mV for YAG:Ce (4%) and $Gd_{0.6}Eu_{0.4}VO_4$ nanoparticles, respectively (Zetasizer, Malvern).

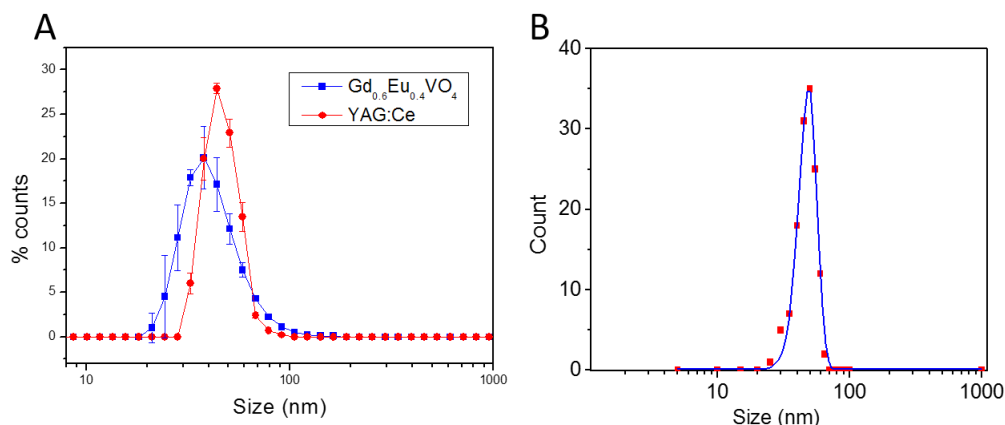


Figure S1. Characterization of YAG:Ce (4%) and $Gd_{0.6}Eu_{0.4}VO_4$ particles. (a) Dynamic light scattering measurements. (b) Size distribution obtained from transmission electron microscopy images of the YAG:Ce (4%) colloid. (shown in fig. 1, main text) for 136 single nanoparticles. The solid line is a Gaussian fit ($R^2=0.99$) centered at 48.6 ± 0.2 nm.

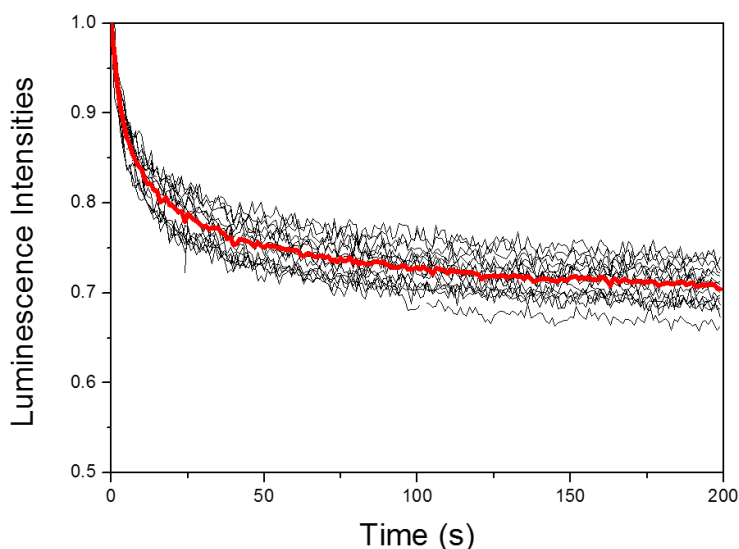


Figure S2. Luminescence decay is similar for all YAG:Ce nanoparticles. The black lines are the signals of individual nanoparticles upon addition of 500 μ M H_2O_2 at $t=0$ and the red line is the average signal ($N=15$). Excitation at 488 nm with an intensity of 0.1 kW/cm^2 ; the acquisition time equals 1 s.

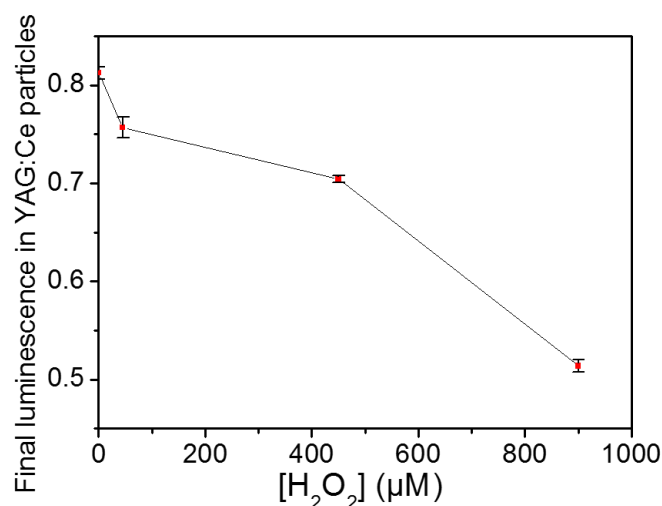


Figure S3. Steady-state YAG:Ce nanoparticle luminescence in function of H₂O₂ concentration. The final luminescence value reached at long times after addition of H₂O₂ (normalized to the initial t=0 signal) is a monotonously decaying function of the oxidant concentration. Final values from curves shown in Fig. 2c.

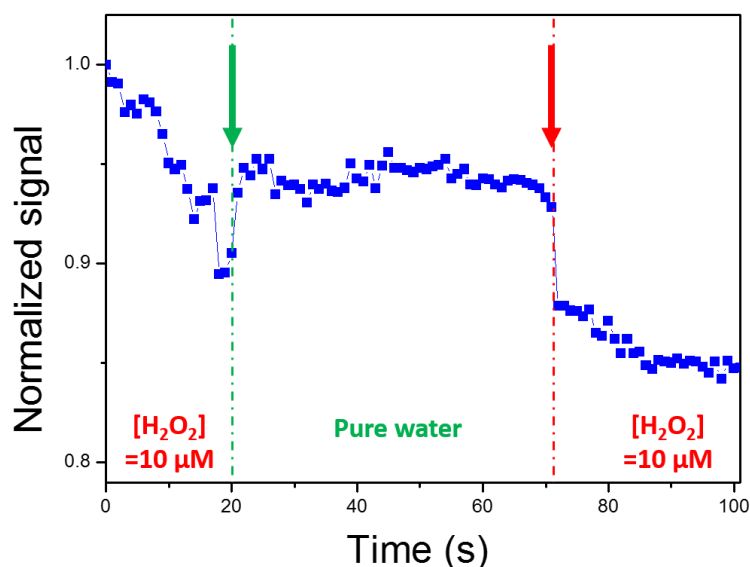


Figure S4. Associative oxidation of YAG:Ce (4%) nanoparticles. Excitation with 488 nm and 396 nm lasers both with $I = 0.1 \text{ kW/cm}^2$. Green arrow: washing with water, red arrow: addition of $10 \text{ } \mu\text{M}$ hydrogen peroxide. From t=0 to t=20 s, the luminescence signal decreases due to joint photoinduced and chemical oxidation in the presence of $10 \text{ } \mu\text{M}$ H₂O₂. The first rinsing at t=20 s induces a luminescence recovery which then stabilizes at the value expected for photo-oxidation in water. This recovery is due to partial Ce⁴⁺ reduction to Ce³⁺ indicating capacity of the Ce-based NPs to respond to changing hydrogen peroxide concentrations. The second H₂O₂ addition then leads to further oxidation.

Nanoparticle response to different oxidants

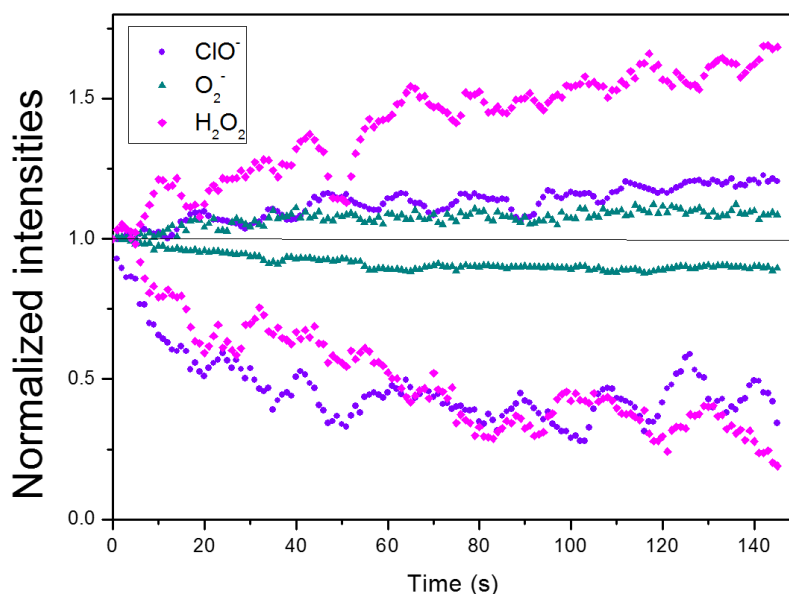


Figure S5. Nanoparticle response to various oxidants. Evolution of average luminescence signals in 10-20 individually detected $\text{Gd}_{0.6}\text{Eu}_{0.4}\text{VO}_4$ (above the black line) and YAG:Ce (4%) nanoparticles (below the black line) after addition of 10 μM of hydrogen peroxide (H_2O_2 , diamonds), 10 μM of hypochlorite ion (ClO^- , circles) and of approx. 250 μM of superoxide anion (O_2^- , triangles) obtained by the addition of 25 munits/mL of xanthine oxidase to 750 μM of hypoxanthine (O_2^- production rate is of approx. 50 $\mu\text{M}/\text{min}$), in the presence of 100 munits/mL of catalase. The illumination conditions are the same as in the calibration experiments: $I = 0.1 \text{ kW}/\text{cm}^2$, excitation wavelengths are 396 nm and 488 nm for Eu-based and Ce-based nanoparticles, respectively. Note that both Eu-based and Ce-based nanoparticles respond to all three oxidants with a luminescence increase and decrease, respectively. However, the amplitude of the response may differ depending on the oxidant. The superoxide anion elicits a small response for both types of nanoparticles, whereas the hypochlorite ion induces a weak Eu-based nanoparticle response and a Ce-based nanoparticle response similar to the one observed for the same H_2O_2 concentration.

Chemical reduction of $\text{Gd}_{0.6}\text{Eu}_{0.4}\text{VO}_4$ nanoparticles

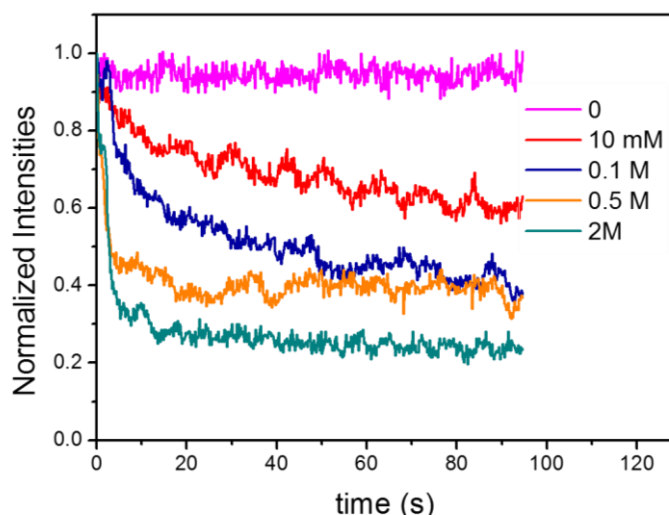


Figure S6. $\text{Gd}_{0.6}\text{Eu}_{0.4}\text{VO}_4$ nanoparticle reduction upon addition of NaBH_4 . Ensemble measurements of the luminescence decay of a nanoparticle solution in the presence of various concentrations of NaBH_4 . The reduction takes place faster for higher NaBH_4 concentrations. For concentrations above 0.5 M, the luminescence signal has already stabilized after 1 min.

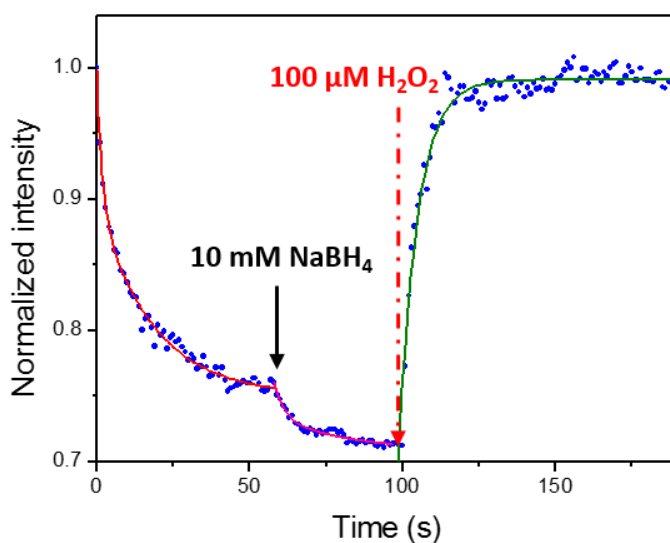


Figure S7. Photoinduced and chemical reduction of $\text{Gd}_{0.6}\text{Eu}_{0.4}\text{VO}_4$ nanoparticles followed by chemical reoxidation. Luminescence signal for individually detected $\text{Gd}_{0.6}\text{Eu}_{0.4}\text{VO}_4$ nanoparticles averaged for 8 nanoparticles. Between $t=0$ and $t=55$ s, photoinduced reduction revealed by luminescence loss takes place in distilled water. At $t=55$ s, 10 mM NaBH_4 is added in the solution and chemically induced reduction and luminescence loss is observed. At $t=100$ s, the solvent is replaced by distilled water containing 100 μM H_2O_2 leading to chemical reoxidation and luminescence recovery. Solid lines are guides to the eye.

Spectroscopic measurements and characterization of Eu ions in $\text{Gd}_{0.6}\text{Eu}_{0.4}\text{VO}_4$ particles.

We investigated the valence state of Eu ions in $\text{Gd}_{0.6}\text{Eu}_{0.4}\text{VO}_4$ nanoparticles by luminescence spectroscopy with a Varian Cary Eclipse spectrometer. We prepared $[\text{VO}_4^{3-}] = 10 \text{ mM}$ solutions of Eu-based colloids and measured their emission and excitation spectra before and after reduction with 1 M NaBH_4 for 2 minutes and 5 washing steps in water.

We show in Fig. S8 that the main emission peak of Eu^{3+} ions at 617 nm is considerably weaker after reduction. Moreover, we show that the absorption of Eu^{3+} ions in reduced and non-reduced $\text{Gd}_{0.6}\text{Eu}_{0.4}\text{VO}_4$ nanoparticles at their characteristic absorption peak at 396 nm is practically unchanged (Fig. S9). This demonstrates that Eu^{3+} ions are present in the same quantity in reduced and non-reduced $\text{Gd}_{0.6}\text{Eu}_{0.4}\text{VO}_4$ nanoparticles suggesting that the observed quenching of the particles is probably due to electron transfer in the reduced particles.

The losses and recoveries in luminescence that we observe are thus representative of the photochemical oxidations and reductions of the $\text{Gd}_{0.6}\text{Eu}_{0.4}\text{VO}_4$ nanoparticles which affect the luminescence properties of the emitting Eu^{3+} ions.

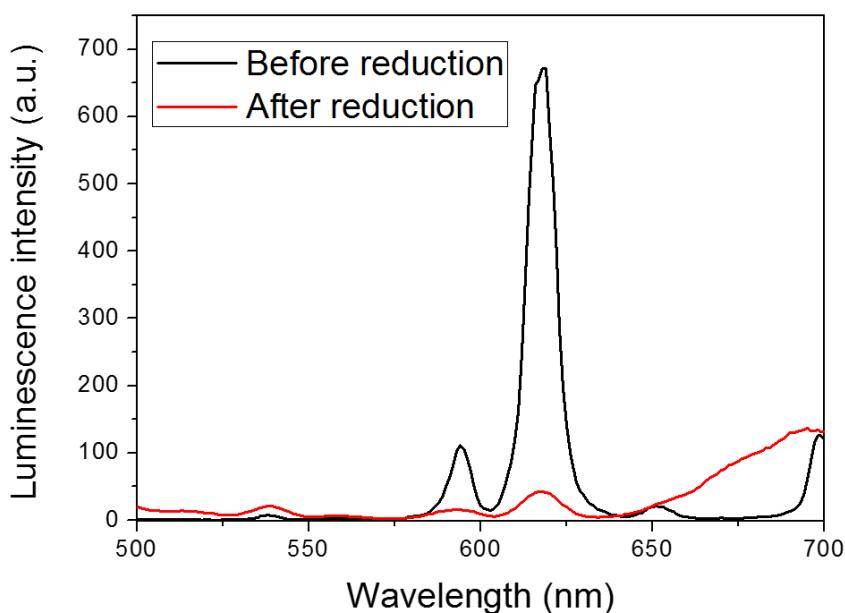


Figure S8. Reduction diminishes Eu^{3+} luminescence. Emission spectrum of $\text{Gd}_{0.6}\text{Eu}_{0.4}\text{VO}_4$ particles before and after reduction with 1 M NaBH_4 . The emission peaks at 590 nm and 617 nm are typical of radiative electronic transitions in Eu^{3+} . Both solutions were prepared at the same vanadate concentration.

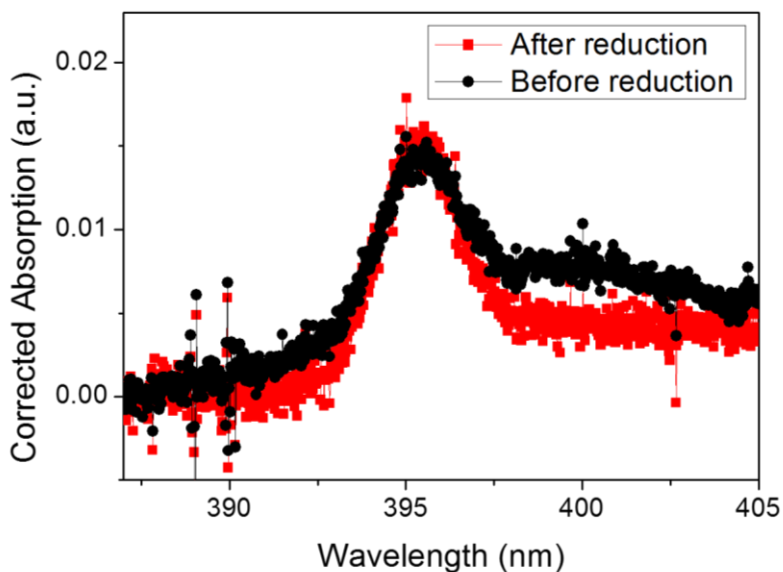


Figure S9. Reduction does not affect Eu^{3+} -ion absorption. Absorption spectra of $\text{Gd}_{0.6}\text{Eu}_{0.4}\text{VO}_4$ particles before and after reduction. Colloids were of similar rare-earth concentration. Spectra were corrected for the scattering background by subtraction of an affine function that approximates the scattered intensities in this narrow spectral range.

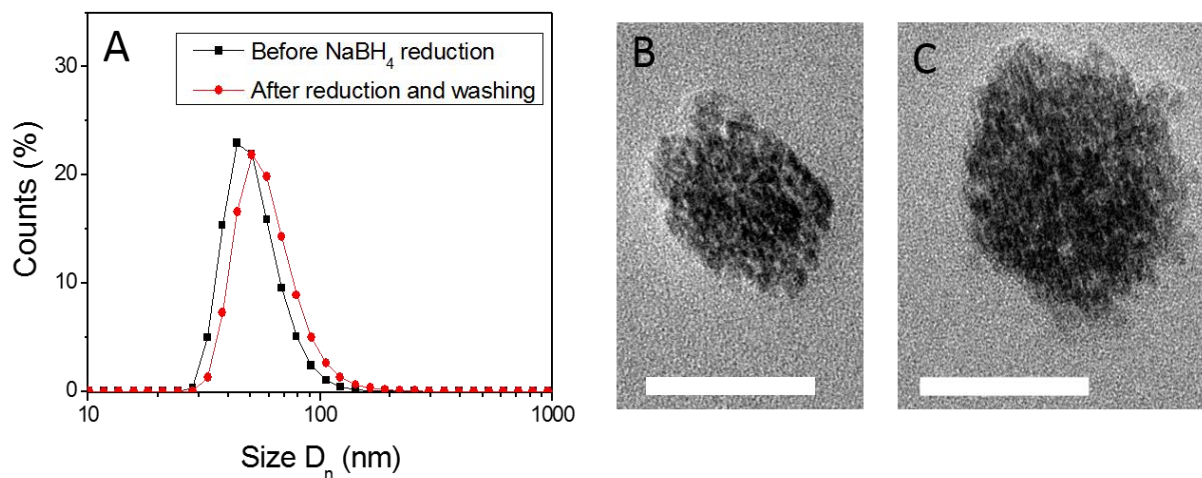


Figure S10. Chemical reduction preserves particle size and integrity. (A) Dynamic light scattering measurement of $\text{G}_{0.6}\text{Eu}_{0.4}\text{VO}_4$ particles (number average) before and after 1 M NaBH_4 reduction for 2 minutes and 5 washing steps in water. Transmission electron microscopy of two typical individual $\text{G}_{0.6}\text{Eu}_{0.4}\text{VO}_4$ particles before (B) and after (C) reduction with 1 M NaBH_4 for 2 minutes and 5 washing steps. The white halo around the nanoparticles is due to a polymer (polyacrylic acid) added to avoid aggregation on the electron microscopy grid. The size and microstructure of $\text{G}_{0.6}\text{Eu}_{0.4}\text{VO}_4$ particles are preserved after reduction. Scale bars in B and C correspond to 50 nm.

Optical setup

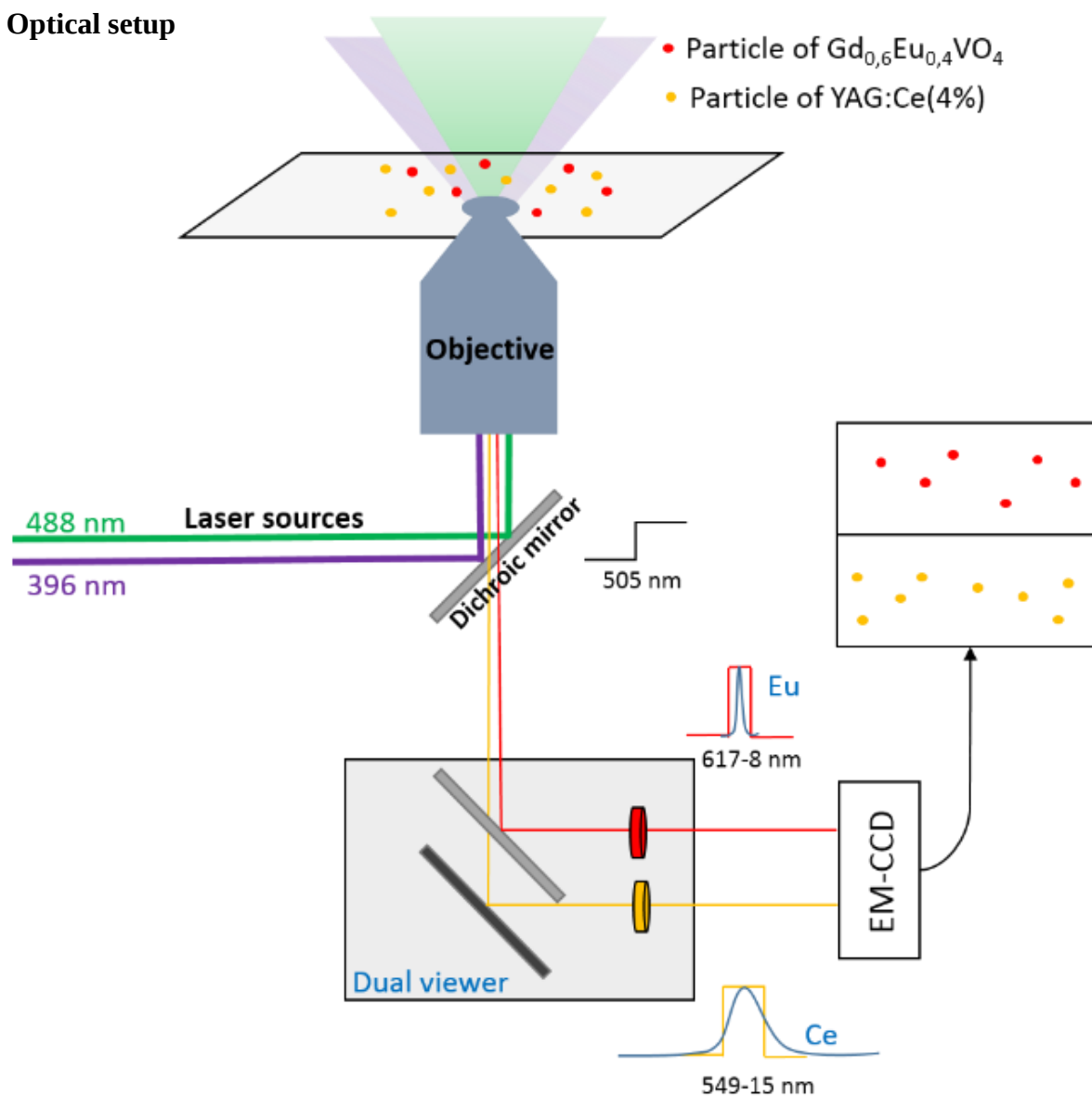


Figure S11. Dual-color imaging optical setup.

Sensor calibration and H₂O₂ concentration extraction method

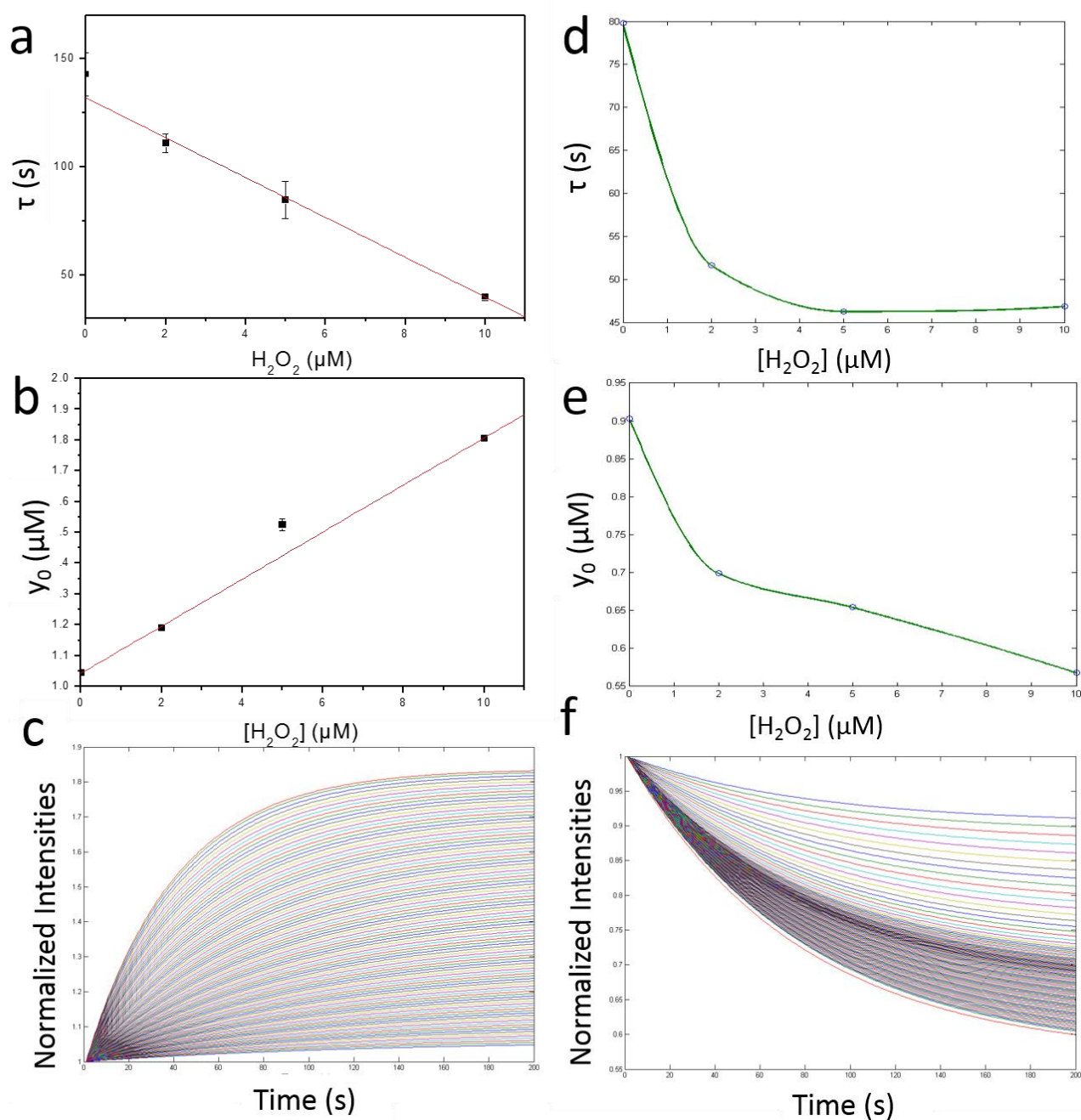


Figure S12. Calibration method. The experimental responses to controlled H₂O₂ concentrations shown in Fig. 3a were fitted to mono-exponential functions $PL(t) = y_0 + \exp(-t/\tau)$, where y_0 represents the amplitude of the probe response to a given concentration of hydrogen peroxide and τ is the characteristic response time. For Eu-based nanoparticles (a-c), the parameters τ and y_0 were linearly fitted in the range 0-10 μ M with correlation coefficients higher than 0.95 (a and b). The resulting interpolated responses are represented in (c). For Ce-based nanoparticles (d-f), the parameters τ and y_0 were interpolated using shape-preserving spline functions (d and e). The resulting interpolated responses are represented in (f). The range of response interpolation is chosen to account for H₂O₂ concentrations varying between 0 and 10 μ M every 0.1 μ M.

Single-probe calibration and comparison to the dual-probe one

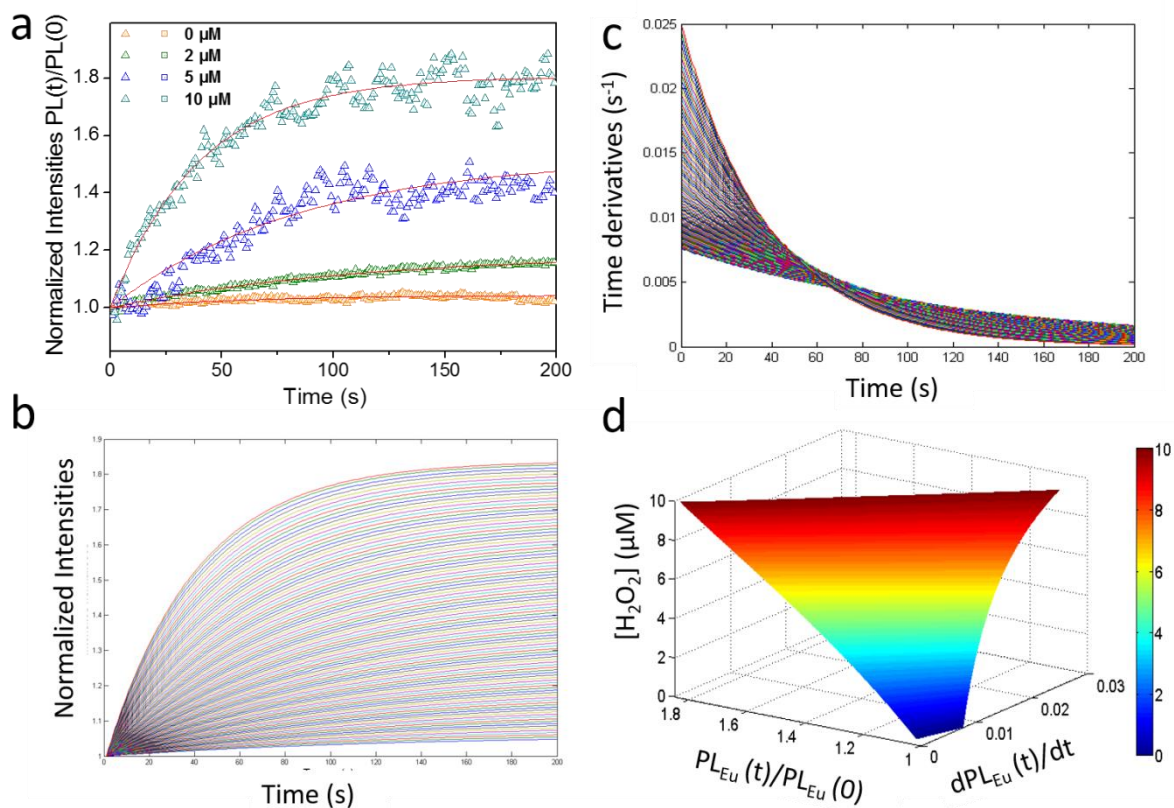


Figure S13. Calibration of single-probe based sensing. (a) Mean evolution of normalized luminescence signal for 10-20 individually detected nanoparticles of pre-reduced $Gd_{0.6}Eu_{0.4}VO_4$ after addition of different H_2O_2 concentrations. The solid lines are fits with exponential growth for the $Gd_{0.6}Eu_{0.4}VO_4$ nanoparticle emission signal. The calibration experiments were done in HBSS-10 mM HEPES medium pH7.4. (b) Interpolated normalized PL of Eu-based NPs in response to 0-10 μM range, generated similarly to Fig. S12. (c) Interpolated time derivatives of PL signals in Eu-based NPs. These curves are the time derivatives of the curves shown in b. (d) Calibration surface built from the curves in (a-c).

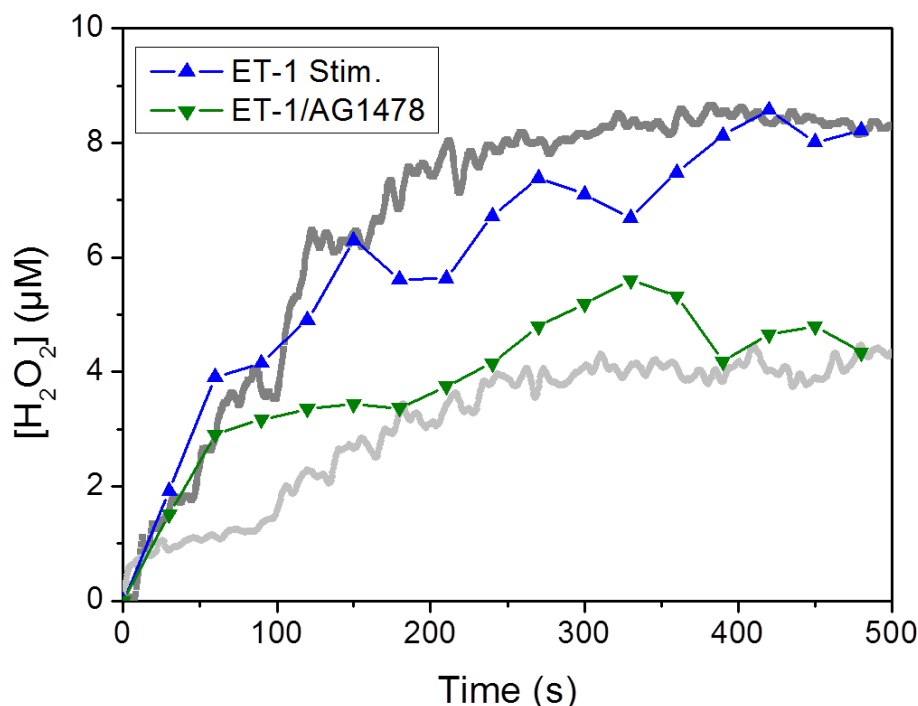


Figure S14. Comparison of dual- and single-probe methods for the extraction of hydrogen peroxide concentrations. The PL signals originating from Eu-based NPs internalized in ET-1 stimulated cells (data shown in Fig. 4b, main text) were processed to extract their time derivative. A time duration of 30 s is required to accurately extract the time derivative of the PL. These derivatives were obtained by a linear fit of PL values measured and smoothed on a 30 s range. The data couples (PL, dPL/dt) were used to extract the concentration of H_2O_2 with the 3D calibration surface shown in Fig. S13d in the case of ET-1 stimulation without (blue curve) and with inhibition of the EGFR with AG1478 (green curve). The dark and light grey curves are the time evolutions of H_2O_2 concentrations that were obtained with the dual-probe method (these are shown and modeled in Fig. 4c). Note that much fewer data points and a lower time resolution of 30 s, limited by the time required to determine the PL derivative, are obtained with the single-probe method, as opposed to the time resolution of 500 ms obtained with the dual-probe approach, limited only by the time required to accurately measure the PL signal.

Probe sensitivity and [H₂O₂] measurement accuracy. The sensitivity, i.e. the lowest value measureable, of our setup is 1 μM as shown in the calibration curves in Fig. 3. The accuracy of our *in vitro* measurements is limited by various sources of noise and variability in our detection system that can originate from the luminescence detection conditions (approx. 1%), the inter-particle variability ($\sim 10\%$) and the calibration process.

In the case of inter-particle variability together with the instrumental accuracy, we detect approx. 10 different nanoparticles which brings the typical error to $10\%/\sqrt{10} = 3\%$. In terms of concentration, this is equivalent to a typical error of 0.3-0.5 μM when we detect 10 μM of hydrogen peroxide.

We determine here more specifically the typical concentration error resulting from the interpolation process, inherent to the construction of the calibration surface shown in Fig. 3B, as follows: as explained previously, we extract the ROS concentrations from a calibration matrix M . We should hence deduce the effect of a 1% error in luminescence signals on the concentration we would extract from the calibration matrix M . We do this by comparing each value $M(i,j)$ to its eight closest neighbors ($M(i-1,j-1)$, $M(i,j-1)$, $M(i+1,j-1)$, $M(i-1,j)$, $M(i+1,j)$, $M(i-1,j+1)$, $M(i,j+1)$ and $M(i+1,j+1)$). The maximum difference between $M(i,j)$ and one of its closest neighbors is the maximum error we would make if we measure inaccurately luminescence signals from Eu-doped or Ce-doped particles. This results in determining the accuracy for each extracted concentration, i.e. each point position on the calibration curve, leading to a typical error of 0.5 μM in the relevant range for ROS detection in our experiments (a few μM).

The various limitations presented above yield to a typical accuracy of 0.5 μM . This value represents the accuracy of an *in vitro* measurement of hydrogen peroxide, which is significantly lower than the dispersion introduced in *in cellulo* measurements by the inter-cellular variability.

Nanoparticle consumption of oxidants. We count a maximum number of 30 nanoparticles of both types in a single cell (See Fig. 4). Based on the typical size of the nanoparticles, the number of unit cells per nanoparticle, and the number of ions per unit cell, this represents a total number of ions susceptible to oxidation of approx. 6×10^6 . A single VSM cell has a typical volume of $100 \times 100 \times 10 \text{ (}\mu\text{m)}^3$, which represents a total cellular volume of 10^{-10} L . This yields a typical intracellular ion concentration of approx. 100 nM which is negligible compared to the detected H_2O_2 concentrations of 1-10 μM .

Cell viability tests

We assessed cell mortality with MTT tests. The reduction of the tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) into the blue-colored formazan by the mitochondrial enzyme succinate dehydrogenase takes place only in living cells. Thus, the produced amount of formazan is proportional to the number of living cells. We grew VSMCs in six-well plates with three wells for each pinocytosis condition (N=3) with 2 mL of culture medium. We proceeded to pinocytosis with 10 μL of one or both nanoparticle solution (both with rare-earth concentration of approx. 0.1 mM), as described in the Methods. The sample “Pino Ctrl” corresponds to the cells with pinocytosis only, *i.e.* without colloid addition. After the incubation time (3 hours), we measured the sample absorbance $A_{570 \text{ nm}}$ - $A_{630 \text{ nm}}$. The N=3 measurements were averaged and normalized to the control sample (“Negative Ctrl”). As mentioned in the main text, the pinocytosis process induced a significant mortality, but no additional effect was observed for any of the nanoparticle treatments.

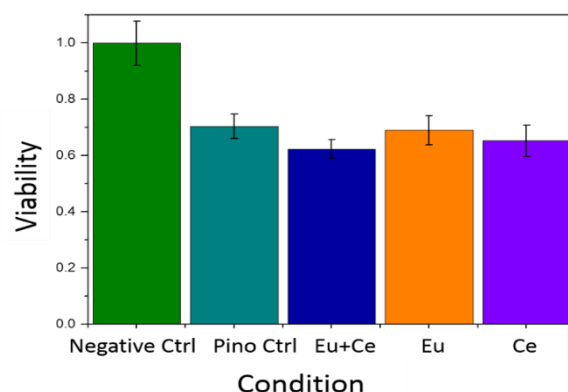


Figure S15. Cell viability MTT tests upon NP internalization. MTT tests on vascular smooth muscle cells after nanoparticle internalization through pinocytosis. Each condition was repeated for N= 3 independent cell cultures. The condition “Negative Ctrl” corresponds to incubation in the culture medium. The condition “Pino Ctrl” represents the pinocytosis effect only as no nanoparticles were added to the hypertonic medium. In the other conditions, the hypertonic medium was supplemented with 10 μ L of one of the two lanthanide-based colloids $Gd_{0.6}Eu_{0.4}VO_4$ (“Eu”) and YAG:Ce (4%) (“Ce”) (where rare-earth concentrations were of approximately 0.1 mM) or with 10 μ L of each colloid (“Eu+Ce”). The MTT tests were performed 3 hours after internalization through pinocytosis. The results show no significant viability decrease due to the presence of the nanoparticles in the pinocytosis process.

Response of individual nanoparticles internalized in a cell upon ET-1 stimulation

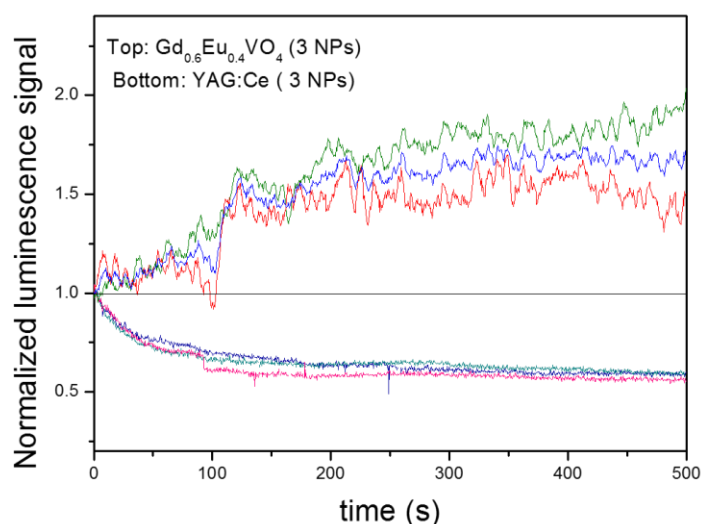


Figure S16. Luminescence evolution for YAG:Ce (4%) and $Gd_{0.6}Eu_{0.4}VO_4$ single nanoparticles in a single vascular smooth muscle cell (VSMC) after ET-1 stimulation. Luminescence evolution for 3 single $Gd_{0.6}Eu_{0.4}VO_4$ NPs (top curves) and YAG:Ce NPs (bottom curves) in a single VSMC cells under saturating ET-1 stimulation (270 nM). The response variability between individual nanoparticles remains small compared to

the average signal. The abrupt signal change for one nanoparticle (pink curve) around 100 s is probably due to the nanoparticle displacement or a mechanical perturbation.

Intercellular dispersion of H_2O_2 production upon ET-1 stimulation

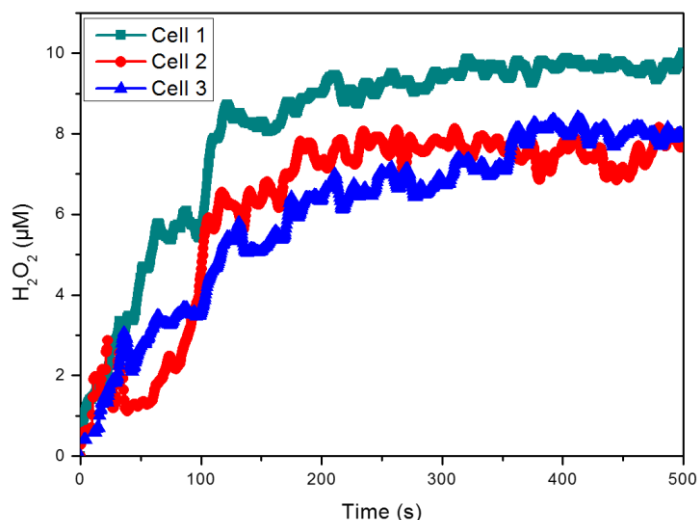


Figure S17. Cytosolic H_2O_2 produced by VSMCs after ET-1 stimulation. Evolution of the intracellular H_2O_2 concentrations extracted from the $\text{Gd}_{0.6}\text{Eu}_{0.4}\text{VO}_4/\text{YAG}:\text{Ce}$ (4%) nanoprobe signals, as explained in the main text, in 3 vascular smooth muscle cells after saturating ET-1 stimulation (270 nM) at $t=0$. The final H_2O_2 concentration ranges from 8 to 10 μM .

Control experiment without ET-1 stimulation

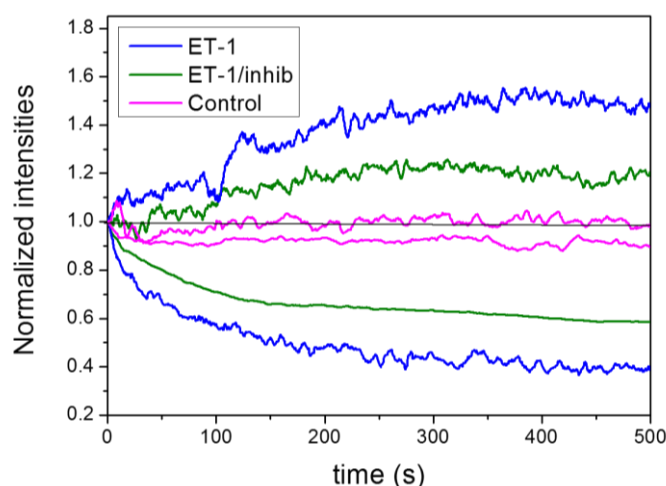


Figure S18. Luminescence signal evolution of $\text{Gd}_{0.6}\text{Eu}_{0.4}\text{VO}_4$ and $\text{YAG}:\text{Ce}$ (4%) nanoparticles internalized in VSMCs with versus without ET-1 stimulation. Averaged luminescence signal evolutions in VSMCs under saturating ET-1 stimulation with and without inhibition of EGF receptors with 500 nM AG1478 (data shown in main text, Fig. 4) compared to a control cell without ET-1 stimulation (pink signal, average of 5

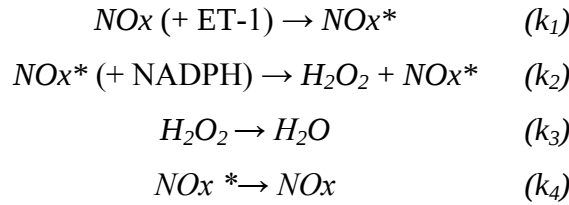
nanoparticles). The data showing a luminescence increase correspond to $Gd_{0.6}Eu_{0.4}VO_4$ particles; those showing a luminescence decrease correspond to YAG:Ce (4%) particles. No significant ROS production is detected in the absence of ET-1 stimulation. The YAG:Ce (4%) luminescence signal exhibits a small decrease in the absence of stimulation due to photooxidation induced by the excitation laser, similarly to what is observed in Fig. 3a.

ET-1 signaling cascade model

We base the following derivation on two main hypotheses: (i), there are no activated NOx before stimulation ($[NOx^*]_{t=0} = 0$), and (ii) the background, or homeostatic concentration of H_2O_2 , *i.e.* the concentration before stimulation, is negligible ($[H_2O_2]_{t=0} = 0$).

N_0 is the number of NOx complexes at the cell plasma membrane, which we consider constant in each cell during the experiment.

The four elementary steps of signaling are the following:



The variation in activated NOx concentration $[NOx^*]$ is due to the interplay between the activation and deactivation steps:

$$\frac{d}{dt} [NOx^*](t) = k_1[NOx] - k_4[NOx^*]$$

The time evolution of NOx activation is thus the solution of the previous equation:

$$[NOx^*](t) = \frac{k_1 N_0}{k_1 + k_4} (1 - e^{-(k_1 + k_4)t})$$

We then note $K = k_1 + k_4$.

Hydrogen peroxide concentrations are governed by the interplay between steps 2 and 3, *i.e.* production and degradation:

$$\frac{d}{dt} [H_2O_2](t) = k_2[NOx^*] - k_3[H_2O_2]$$

Knowing the time evolution of $[NOx^*]$ we can rewrite the previous equation as:

$$\frac{d}{dt} [H_2O_2](t) = k_2 \frac{k_1 N_0}{k_1 + k_4} (1 - e^{-(k_1 + k_4)t}) - k_3 [H_2O_2]$$

The time evolution of the hydrogen peroxide concentration is the solution of the previous differential equation:

$$[H_2O_2](t) = \frac{k_2 k_1 N_0}{K} \left(\frac{1}{k_3} (1 - e^{-k_3 t}) + \frac{1}{K - k_3} (e^{-Kt} - e^{-k_3 t}) \right).$$

Hence:

$$[H_2O_2](t) = A \left(\frac{1}{k_3} (1 - e^{-k_3 t}) + \frac{1}{K - k_3} (e^{-Kt} - e^{-k_3 t}) \right)$$

with

$$A = \frac{k_2 k_1 N_0}{K}.$$

This model predicts a final equilibrium concentration in hydrogen peroxide upon stimulation with ET-1 given by:

$$[H_2O_2](t \rightarrow \infty) = \frac{k_2 k_1 N_0}{K k_3} = \frac{A}{k_3}$$

Our model thus depends on three independent parameters A , K and k_3 that can be determined by fitting the $[H_2O_2](t)$ curves of Fig. 4c.

We evaluate the relevance of this model versus a simpler empirical model based on an exponential increase in the hydrogen peroxide concentration thanks to the Akaike Information Criterion (AIC)². In the case of the exponential model, we have ($n=2$) independent parameters, knowing the number of measurements ($N=997$) and the residual sum of squares (RSS), we obtain:

$$AIC_{exp}^{ET-1} = 2n + N \ln(RSS) = 5250$$

For the model presented above we obtain:

$$AIC_{model}^{ET-1} = 4431 < AIC_{exp}^{ET-1}$$

In the inhibition condition, we also obtain $AIC_{model}^{AG1478} < AIC_{exp}^{AG1478}$ which indicates that our model describes the signaling pathway more accurately than an exponential model.

Fitting Results. The time evolutions of hydrogen peroxide concentration are represented in Fig. 4c and were fitted with the three independent parameters A , K and k_3 . We provide the values and standard deviations of these parameters in the table below.

Condition	A (s^{-1})	k_3 (min^{-1})	K (s^{-1})
ET-1 Stimulation	0.105 ± 0.003	0.7 ± 0.2	0.033 ± 0.002
ET-1/AG1478	0.042 ± 0.002	0.6 ± 0.3	0.024 ± 0.002

Table S1. Fitting parameters of the time evolution of hydrogen peroxide concentrations in cells.

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