

Peer Review File

Manuscript Title: Indefinite and Bidirectional Near Infrared Nanocrystal Photoswitching

Reviewer Comments & Author Rebuttals

Reviewer Reports on the Initial Version:

Referees' comments:

Referee #1 (Remarks to the Author):

The authors of this study report that high power irradiation at 1064 nm can turn off upconversion luminescence of Tm-activated photo avalanching nanoparticles. When illuminated with a broad spectrum of light ranging from 500 to 800 nm, the luminescence can also be entirely restored. Based on this, the authors demonstrate sub-Å localization superresolution imaging and its ability to distinguish nanoparticles that are closely packed together. I must say that this manuscript is a systematic and comprehensive investigation into photoswitching of a single colloidal inorganic nanoparticle without measurable photodegradation. However, I am of the opinion that this work is not suitable for Nature because the fundamental phenomenon (or science) behind this is well known and the method described in the study has very few potential applications. After some of the technical issues are resolved, Nature Communications might be the appropriate venue for this submission.

1. The literature provides ample evidence of the high power-induced photodarkening of Tm³⁺-doped materials. The authors didn't present any convincing evidence to back up their theory regarding the process behind photodarkening and recovery.
2. Why should Gd be included in the shell layer? Does the phenomenon still occur when a NaYF₄ shell is used? The authors should investigate the role of Gd at different concentrations. For example, NaYF₄:Tm@NaGdF₄:Eu core-shell nanocrystals can be prepared in order to study the intensity of Eu emission as a function of the power of a 1064 nm laser.
3. The authors reported that the luminescence can be recovered under 500-800 nm irradiation. Is it possible for the luminescence to be recovered by heating? If the electrons are being held in place by defects, luminescence should be recovered after heating since thermal stimulation can clear the traps. Thermoluminescence measurements can be performed to probe the trap depth present in nanocrystals.
4. What exactly happened to the valance state of Tm before, during, and after the photodarkening process (using EXAFS)? A change in valence should be observed If Tm ionization can be triggered by high-power illumination from a 1064 nm laser.
5. Metal oxides usually contain much more defects than fluorides and have a much smaller band gap (<4 eV). If photoionization is the true cause of photodarkening, it should be considerably simpler to produce in metal oxides.
6. For 2D and 3D microscale optical writing, erasing, and rewriting of stable near-infrared light-switchable ANPs, the pixel size for writing is limited by the beam focal spot size (hundreds of nanometers). What are the writing and erasing efficiencies at different depths? I do not see any advantages associated with the use of nanoparticles as a medium for information storage compared to other optical writing methods such as photonic crystal-based methods that have superior writing speed.
7. The authors demonstrate the application of a single nanoparticle photoswitching for PALM super-resolution imaging and demonstrate that ANP-based imaging has higher localization accuracy due to the high number of collected photons (averaging nearly 330,000 per frame). This comparison is not fair; the author should specify the parameter of the frame rate. It might take several hours to construct a single image since the switching processes take seconds, which is several orders of magnitude longer than those employed in conventional PALM. Also, a very small field of view can be achieved as a high power must be applied.

8. What is the error of the positioning accuracy of 0.098 nm and 0.058 nm, and what is the accuracy of the algorithm that removes the influence of sample movement on positioning? Because the authors did not give a full discussion of localization accuracy, the data do not provide sufficient evidence to support their claims.
9. The time trace of photoblinking of just one nanoparticle is shown in Figure 2f, with a large separation between emission peaks. Under the same excitation conditions, do the random emission combinations of all nanoparticles conform to the statistical distribution required for PAML imaging, or are they all concentrated within the same shorter time period? This determines the effectiveness and imaging speed of PALM imaging. Although the authors show that the temporal distribution of photoblinking is intensity-dependent, it is practically difficult to control the excitation intensity of each nanoparticle during imaging.
10. The influence of the shape and luminous efficiency of nanoparticles on the positioning accuracy needs to be discussed. According to the information presented in Fig. S3 and S4, an increase in switching speed results in a decrease in switching efficiency.

Referee #2 (Remarks to the Author):

The manuscript written by Lee et al. describes the photoswitching effect of Tm-doped avalanching nanoparticles. First of all, I would like to congratulate all the authors for such magnificent work and the detailed study of the photoluminescent properties of these fashionable nanoparticles. The authors have demonstrated an unknown behavior of ANPs such as the reversible (up to 1158 irradiation cycles) and controllable photodarkening/photo brightening process. Nevertheless, there are some uncertainties in the current version that I encourage the authors to address before publication.

- 1- The representative scheme in Figure 1a shows the photoswitchable ANPs. The ANPs are composed of a core based on Tm-doped NaYF₄ NPs and a NaGdF₄ inert shell whose dopant amount and layer thickness was tuned. In this scheme, it is highlighted and activated quencher, however, the authors don't use these words along the manuscript in the main text. There are indeed some references about photodarkening in Tm-doped bulk materials but the understanding of the manuscript would be aided by the inclusion of a short explanation about why 1064 nm light quenches the emission and 510-800 nm induce a brightening.
- 2- The authors claimed at the beginning of the MS that photobrightening occurs when using wavelengths between 510 and 800 nm, nevertheless, they use 700 nm for the experiments. What is the reason? From figure 3b one can say that the best recovery is produced at ca. 600 nm. Why did you not use that one?
- 3- Figure 2 caption says: "blue markers are 50% Na/50% Y, Y, F, and Na ions", I guess the authors would say 50% Na/50% Y, Gd, F.
- 4- Some abbreviations are not explained the first time used in the main text, for example, PA (line 55), AFM (Fig 2 caption), DRE (Fig 1 caption)...Please check carefully this issue throughout the MS.
- 5- Figure S1 and S2, table S2 and S3 are not mentioned/discussed in the main text.
- 6- Concerning the reproducibility of the experiments, in some plots, the error bars are missed e.g., Fig 3a, Fig S7, Fig S12, or Fig S13. Could the authors include that information? In some figure legends, it is written that the error bars are calculated as the standard deviation of 4 different measurements. These 4 measurements are made from 4 different films or are the same ANP film sample repeated four times? And if it is the first option, are all of them from the same batch of NPs? Otherwise, I recommend the authors revise all the figure legends to include how the error bars were calculated since in some of them the information is missed.
- 7- Supporting information: I might suggest carefully reviewing the caption of all tables and figures and be sure that there is no missed information: sample composition, excitation/emission wavelengths, or error bars. E.g., the excitation wavelength is missed Figure S3.
- 8- Line 77: surface quenching does not play a major role. Could the authors include a Figure showing this statement or a reference?
- 9- The authors repeatedly say that the process is reversible (e.g., line 95) but the experiments about reversibility and stability over photodarkening/photo switching cycles are shown in the final

part of the MS, and it is quite confusing because one is expecting to find this experiment. Probably, it is better to erase such a claim and leave it just when the experiment appears.

Referee #3 (Remarks to the Author):

Referee report 'Indefinite and Bidirectional Near Infrared Nanocrystal Photoswitching' by Lee et al.

The manuscript by Lee et al. describes experiments demonstrating reversible photoswitching in Tm³⁺-doped photo-avalanching NaYF₄ nanoparticles. Convincing experiments are reported and explained showing that at high 1064 nm excitation densities darkening occurs (ascribed to filling of defects) which induces non-radiative decay channels that shifts the onset for photo-avalanching to an order of magnitude higher excitation densities. In addition to reporting a highly robust photoswitchable nanoparticle, potential applications in high resolution imaging and patterning are demonstrated. The results are very interesting and the interpretation is generally sound and supported by experiments. The novelty is high and warrants publication in Nature. Still, there are points that are not clear (to me) and at some places a more thorough evaluation and discussion of the fidelity of the switching with these nanoparticles would be helpful.

In the comparison of photoswitchable probes the disadvantages of existing (bio-organic) probes are stressed vs. advantages of the present nanoparticles. A more balanced discussion would be appreciated. How harmful is 'the phototoxic dose' of UV or visible light compared to the NIR doses used for switching the NPs? What is the difference in time resolution, complexity of the imaging system and reproducibility/fidelity of the switching of the different probes? The organic probes, albeit not very stable, are successfully applied to realize high quality superresolution imaging. It may be good to also mention that reversible photoswitching in inorganic spin-crossover systems (including nanoparticles) has been demonstrated. Often in publications a one-sided view is presented on the disadvantages of existing methods to stress the importance of results without indicating potential drawbacks of the reported new system/method. This is not necessary, especially for highly innovative findings as reported in this manuscript.

The final line of the abstract section reports sub-Angstrom localization. I had a hard time understanding this claim – also later in the manuscripts where the results are presented that would show sub-Angstrom localization. It is not my field of expertise and it will be good if an expert with experience in this area comments on this claim and the experiments. A better description and explanation for non-experts of what sub-Angstrom localization means and how this follows from the experiments will be helpful. A first response is that at room temperature optical experiments are not capable of sub-Angstrom localization as this corresponds to a fraction of the size of a single atom/is less than half bond length between atoms.

An important aspect of photoswitching is that it is similar and reproducible for all (nano)probes. When looking at Figures 2c and 2d it seems that the threshold for bleaching varies between particles. In 2d we see a first partial drop in intensity already for the lowest excitation power (~80 kW/cm² – bleaching of 1 of the 4 nanocrystals?) and complete bleaching of all four at 110 kW/cm². In Figure 2c for a single nanoparticle the intensity continues to increase up to the highest power (164 kW/cm²) and then quenches completely. For the ensemble of four the particles the intensity switches on and off at the highest power (similar to what is also observed in Figure 2f). This aspect (explanation and implications) is not discussed clearly. Why do the particles behave different and how does this affect the fidelity in applications? For applications of reversible photoswitching the uniformity of switching thresholds and bleaching behavior is important and also turning off and on in the bleaching regime seems undesired.

The concern raised above is related to a point that is not addressed but is crucial for future applications: the switching relies on filling of defects. Defects are poorly understood in luminescent

materials and it will be very difficult to reproducibly produce nanoparticles that all have the desired defect(s). The variation in behavior is probably caused by variations in the number, maybe type, and localization of defects, even for these well-controlled synthesized batches of ANPs. To develop a reliable product/technique for high resolution imaging based on the action and presence of defects seems difficult. For a future direction, it may be an option to intentionally introduce trapping centers for charges that have the same effect as a defect/colour centre (quench NIR Tm³⁺ emission). Very similar to afterglow materials where photo-ionization leads to controlled trapping of charge carriers (e.g. by Eu²⁺/Dy³⁺ or Eu²⁺/Nd³⁺ pairs) that can be thermally (or photo!) stimulated, a reversible and better controlled switching of ANPs can possibly be realized if trapping centers can be intentionally incorporated in known (low) concentrations. In Figure 3 convincing evidence is presented for the on and off of switching of the photo-avalanching. Still, the figure raises some (minor) questions.

- The error bars in Figure 3b are very small and this would suggest that the structure observed (e.g. dip at 685 nm and 710 nm in the grey curve) is real. I assume this is not the case – a structureless broad band is expected for the colour centre to conduction band absorption. The error bars should be reconsidered.
- Comparing Figure 3b with Figure S14 shows a very different wavelength onset for the photobrightening efficiency (onset at 800 nm vs. 600 nm). In the caption of S14 the lower photobrightening efficiency is mentioned/explained but the difference in spectral shape/onset is not discussed.
- In Figure 3c the recovery seems almost immediate – already after 1s 50% (for 107 kW/cm²) or 90% (for 213 kW/cm²). It would be interesting to follow the recovery dynamics on a shorter sub-s time scale. Has this been attempted?

The 4% Tm-doped NCs do not show the same photodarkening (Figure S10). It is interesting to see that the intensity does drop for higher excitation densities – for 70 kW/cm² a two times higher intensity is observed than for 2.3 MW/cm². It would be good to discuss this gradual decrease of emission intensity upon increasing the laser power. In relation with this, the manuscript often makes a parallel of the present mechanism for photodarkening with what is observed in Tm-doped glasses/fibers. At the same time it is inferred that photo-avalanching is crucial for photodarkening 'When doping is increased to 8% Tm³⁺, the NPs exhibit pronounced PA and noticeable photodarkening...' while it not observed for the 4% Tm³⁺ NPs (without PA) (lines 69-75). Darkening/quenching is observed in glasses/fibers but I do not think that in these glasses/fibers PA occurs or does it? It would help if the authors clearly discuss if and why PA is required for photodarkening or if it can also occur without PA.

The mechanism for photodarkening, although not completely proven, is plausible. It would be interesting to record the luminescence decay curves of the 3F₄ emission directly after darkening to confirm the higher W2NR (faster and non-exponential decay expected, converting to longer and more single exponential upon photobrightening). This would also provide independent quantitative information on W2NR. In addition, it would be a relatively simple experiment to see if the colour centres disappear upon heating. Optical stimulation (emptying) of traps around 800 nm is observed and based on this onset wavelength it can be expected thermal emptying at temperatures below 100oC is possible (note that the thermal trap depth is always much smaller than the optical trap depth). Recovering the PA at a low power threshold after a heat treatment would provide further support for trapped charge/colour centre model presented in Figure 3d.

The filling of traps via the 1I₆ level is suggested. This is a valid mechanism. Instead of showing emission lines around 490 nm and 510 as evidence for 1I₆ emission, it would be more convincing to show shorter wavelength UV emission. The spectra do show clear evidence for 1D₂ emission around 360 nm. Evidence for 1I₆ emission would be obtained from emission around 290 nm (to 3H₆) and 350 nm (to 3F₄). The reason for looking for UV emission is that the weak lines around 490 and 510 nm could also originate from 1G₄ emission to higher crystal field components of the 3H₆ ground state. The power dependence of the intensities (Figure S17) seems to be the same for

the 490 nm and 510 nm emission as for the 450-470 nm 1G4 emission lines while a stronger power dependence would be expected for 1I6 emission lines (higher order upconversion process). In the inset of Figure S16 one can observe a weak 350 nm emission that I would assign to 1I6-3F4 emission.

In figure 3d the position of the 5d1 level is too high in energy. The lowest 5d states for Tm³⁺ in fluorides are around 160 nm corresponding to 7.75 eV above the 3H6 ground state. In the diagrams in Figure 3d this would place the 5d1 state around 9.5 eV above the valence band edge (not 11.3 eV). The lower energy 5d1 state would also allow for 1D2-1I6 energy transfer to populate the 5d1 state. One also has to be careful to assume that photoionization results in expelling an electron from Tm³⁺ to give Tm⁴⁺ + a conduction band electron as suggested here. Although harder to depict in a band diagram as Figure 3d, conversion to Tm²⁺ + hole in the valence band that can be trapped to form a colour centre is also possible. The two processes are completely analogous. It is good to realize that Tm⁴⁺ is notoriously unstable while Tm²⁺ is a well-known valence state for Tm. Overall, for the manuscript this is not an important point as the nature of the trap/defect/colour centre is not known.

In conclusion, this is another highly interesting manuscript from the Schuck-group and the team of collaborating groups showing the potential of photon avalanching in nanoparticles. I strongly support publication in Nature after the authors have considered my comments and questions.

Author Rebuttals to Initial Comments:

Referee #1 (Remarks to the Author):

The authors of this study report that high power irradiation at 1064 nm can turn off upconversion luminescence of Tm-activated photo avalanching nanoparticles. When illuminated with a broad spectrum of light ranging from 500 to 800 nm, the luminescence can also be entirely restored. Based on this, the authors demonstrate sub-Å localization superresolution imaging and its ability to distinguish nanoparticles that are closely packed together. I must say that this manuscript is a systematic and comprehensive investigation into photoswitching of a single colloidal inorganic nanoparticle without measurable photodegradation. However, I am of the opinion that this work is not suitable for Nature because the fundamental phenomenon (or science) behind this is well known and the method described in the study has very few potential applications. After some of the technical issues are resolved, Nature Communications might be the appropriate venue for this submission.

Our reply: We are grateful to the Referee for their careful reading, for their constructive comments and criticisms, and for noting that our work is “systematic and comprehensive”.

1. The literature provides ample evidence of the high power-induced photodarkening of Tm³⁺-doped materials. The authors didn't present any convincing evidence to back up their theory regarding the process behind photodarkening and recovery.

Our reply: We agree about the existing literature on photodarkening (PD) in Tm-doped bulk materials. In fact, an important point that we now make more clearly in the revised manuscript is that the photodarkening we observe is consistent with this literature; see, e.g., Sun, et al, “Progress and Summary of Photodarkening in Rare Earth Doped Fiber,” *Appl. Sci.* 11, 10386 (2021); now added to our references), which had included 6 other references to Tm photodarkening.

We should emphasize that there are *multiple unique features* in Tm-doped core/shell avalanching nanoparticles compared to Tm-doped bulk materials, arising from their nanostructured design and their specific – and tunable – photon avalanching properties, reported for the first time with this study. Further, we now have unique mechanistic insights into Tm-based photoswitching well beyond what is found in the literature.

Prompted by the referees' comments, we have now probed the mechanistic processes more deeply within the photoswitchable ANPs, performing **new experiments comprising 12 new figures and added discussions**, described below. These include measurements of Tm excited-state lifetimes, photobrightening (PB) dependencies on core size and Tm concentration, UV emission spectroscopy, as well as expanded and improved wavelength-dependent PB behaviors.

Our findings reveal several new insights for *all* Tm-doped materials, as well as those specific to nanoparticles. These enable us to explain why photoswitching and PD have not been observed in Ln-doped nanoparticles until now.

Specifically:

1) PD is caused by a shift to higher photon avalanching thresholds for all of our ANPs.

This sets a stringent requirement for any activated colour centres that play a role in PD within ANPs: namely, they should reduce the lifetime of the Tm 3F_4 excited state in the system, since previous kinetic rate analyses showed that this lifetime predominantly determines the avalanching threshold in our ANPs [Nature 589, 230 (2021)]. Thus, our measurements of the PA threshold intensity shift and 3F_4 lifetime reduction prove that these activated colour centres include an optical transition that overlaps energetically with the Tm 3F_4 -to-ground-state transition.

2) Photobrightening (PB) is strongly dependent on core size but not shell thickness. These new findings, along with materials synthesis considerations, provides evidence that activated colour centres reside at or within the core/shell interface, which is required to explain the key innovation of indefinite NIR photoswitching: both bright and dark states must be distinct from any possible surface quenchers. This is a fundamental difference with bulk Tm³-doped fibers, which have no heterostructure interface.

3) Wavelength-dependent measurements of PB as well as thermally activated luminescence recovery measurements suggest the trap depths of the colour centre(s) active in ANP PD is >2 eV. This explains the stability of the darkened state under ambient conditions, which is critical for bidirectional optical control without unwanted thermal relaxation.

Finally, as we expect the reviewer appreciates, the characterization of atomic colour centres and their direct correlation with specific optical functionalities can be exceptionally challenging [1991, Journal of the Ceramic Society of Japan, 99(1154), pp.923-942.], particularly for a system as complex as avalanching nanoparticles. Directly imaging a low-scattering element (e.g., F) point defect at the buried heterostructure interface of a dose-sensitive 25 nm core-shell nanoparticle is not possible by current methods in electron or x-ray microscopy, and has only been achieved in simpler systems [Acta Mater. 118721 (2023); Nat. Mater. 14, 1099 (2015); Chem. Soc. Rev. 47, 6764–6794 (2018)], so there will necessarily be gaps in our understanding of the photoswitching mechanism. We note in the manuscript that additional study will be required.

Changes to the manuscript:

1) We have updated Figure 3b,c with improved photobrightening (PB) studies, which provide a clearer picture on the wavelength and intensity dependencies of the PB process in ANPs.

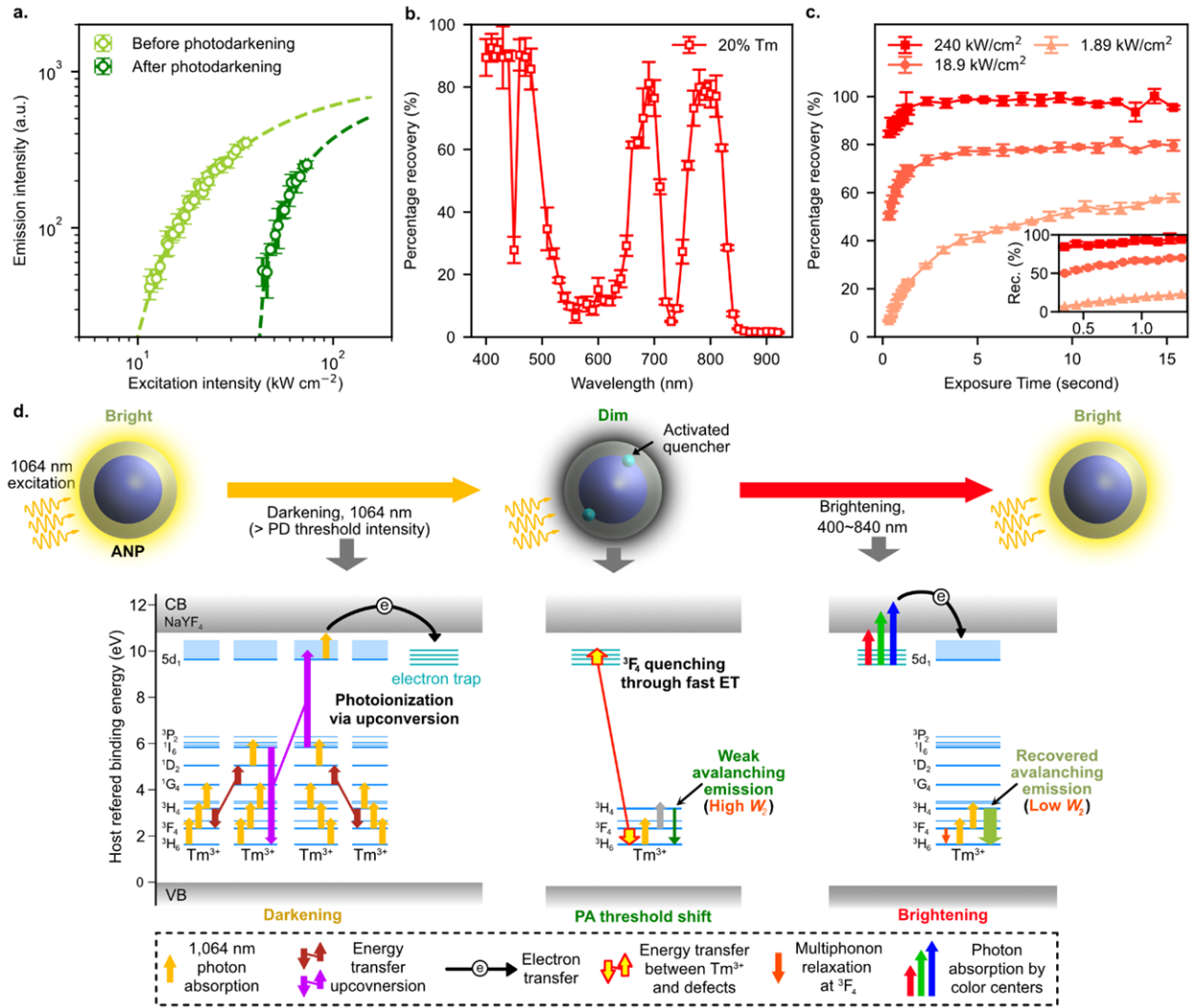


Fig. 3 | ANP Photobrightening and photodarkening. **a**, Emission intensity (800 nm) versus excitation intensity (1064 nm) of a single 8% Tm^{3+} 17.3/5.6 nm (core diameter/shell thickness) ANP before and after photodarkening. Switching between bright and dim states in ANPs is induced by shifting of the PA threshold, using 1064 nm light to darken and wavelengths ≤ 840 nm to brighten. **b,c**, Photobrightening recovery of a photodarkened 20% Tm^{3+} 10.4/2.7 nm core/shell ANP ensemble film sample versus **(b)** irradiation wavelength or **(c)** exposure time. The photobrightening laser power at the sample is set to 600 μW for all wavelengths in **b**, which corresponds to 90 kW cm^{-2} at 700 nm. Photobrightening exposure times are 1.3 sec in **b**. Photodarkening exposure times are 1.2 sec in **b** and **c**. 1064-nm laser intensities for luminescence pumping and photodarkening are 69 and 345 kW cm^{-2} , respectively, in **b** and **c**. 700-nm irradiation intensities for photobrightening are shown in the legend in **c**. The inset in **c** is a magnified plot in the exposure-time range from 340 ms to 1340 ms. Error bars are standard deviations of data points measured at the 4 different spots in the same ensemble sample. **d**, Potential mechanistic pathways for photodarkening and photobrightening in ANPs. CB: conduction band, VB: valence band, W_2 : $^3\text{F}_4$ relaxation rate.

Motivated by comments from all three reviewers, we have extended the PB activity measurements to include wavelengths spanning 400 nm to 920 nm. As part of this more in-depth study, we carefully reviewed our PB activation studies and have taken additional steps to reduce pump bleed-through in our filters and chromatic aberration of the dielectric objective, which had adversely affected our previously measured activity spectrum.

We have added **Figure S17**, which provides additional mechanistic insight, explaining the sharp dip in the PB activity spectrum at 450 nm.

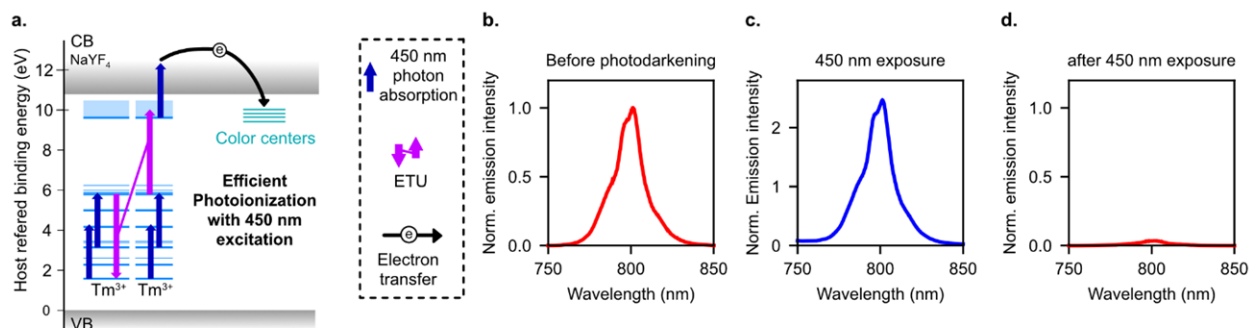


Fig. S17 | ANP Photodarkening with 450 nm excitation. **a**, Potential mechanistic pathways for photodarkening in ANPs under 450 nm pumping. Emission spectra from an ensemble film of 20% 10.4/2.7 nm core diameter/shell thickness ANPs before photodarkening (**b**), during 450 nm exposure (**c**), and after photodarkening with 450 nm exposure (**d**). 1064 nm illumination is used for pumping the luminescence in panels (**b**) and (**d**). The 1064 nm intensity is 75 kW cm⁻², and the 450 nm excitation intensity is 222 kW cm⁻². The emission intensities (panels **b,c,d**) are normalized to the maximum intensity in plot **b**.

We have updated the **Methods section**: “For measuring the photobrightening activity spectra, we used a reflective objective lens (Thorlabs, LMM40X-P01), which does not show significant chromatic aberration, and selected filters (Thorlabs, FESH700 and FESH750) to block residual light from the 800 nm pump source.”

We have added to the **discussion**: “The PB activity spectra (Fig. 3b, S16) include broad features at wavelengths <530 nm attributable to colour centres, as well as peaks at both 800 nm and 700 nm, which correspond directly to Tm³⁺ ³H₄ and ³F_{2,3} transitions. There is a sharp dip in the activity spectrum at 450 nm, corresponding to higher-energy ¹G₄ and ¹D₂ Tm³⁺ transitions, which may enhance upconversion to UV manifolds to induce further photodarkening rather than luminescence recovery (Fig. S17). Increasing the irradiation intensity or the exposure time also enhances PB (Fig. 3c), so that nearly full luminescence recovery can be achieved in a few seconds with 700 nm light (Fig. 3c).”

2) We have performed **new PB experiments** revealing that both ANP core size and Tm concentration affect PB recovery (**new SI Figs. S18 and S19**). These results show that PB recovery increases as ANP core diameters decrease from 27 to 10 nm. In addition, PB recovery increases as Tm³⁺ content increases from 8% to 30% when brightened with 700 or 800 nm irradiation.

The inverse relationship between core size and PB recovery percentage suggests that the number of colour centres actively participating in PD/PB switching is determined by the core size (if more active colour centers are present, more light must be absorbed in order to fully brighten the ANP), likely the core-shell interface area.

The strong Tm-concentration dependence of PB for 700 or 800 nm excitation, which correspond to Tm³⁺ transitions, suggests these wavelengths may induce PB by undergoing efficient upconversion to visible wavelengths (higher Tm content leads to shorter interionic distances, which increases the probability of Tm-based upconversion), which then releases carriers trapped in the colour centres.

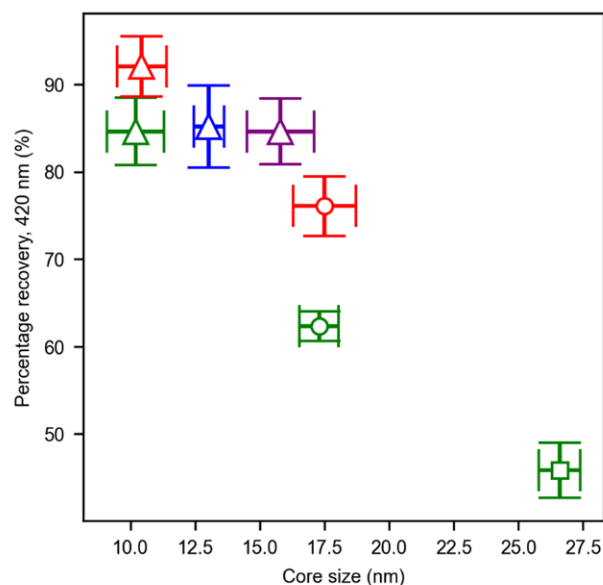


Fig. S18 | Photobrightening recovery as a function of ANP core size. Excitation (at 1064 nm) and photobrightening (at 420 nm) of ensemble film samples with intensities of 54 kW cm^{-2} and $277.87 \text{ kW cm}^{-2}$, respectively, for materials marked by the triangle symbols (see caption in Fig. S16). Excitation and photobrightening intensities are 33 kW cm^{-2} and 167 kW cm^{-2} , respectively, for the samples marked by the circle and square symbols. Exposure times for photodarkening and photobrightening are 1.2 and 1.3 sec, respectively. The 420-nm laser power at the sample is 0.6 mW for photobrightening.

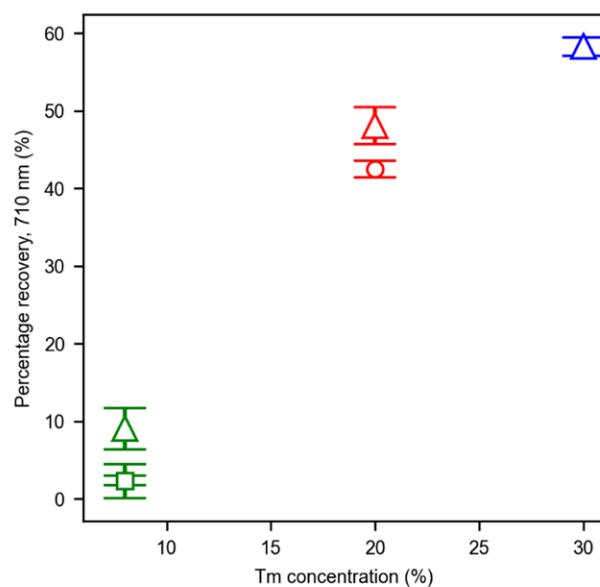


Fig. S19 | Photobrightening recovery percentage as a function of ANP Tm^{3+} concentration. Excitation (at 1064 nm) of ensemble film samples with intensities of 54 kW cm^{-2} for materials marked by the triangle symbols (see caption in Fig. S16). For photobrightening, the 710-nm laser power at the sample is 0.6 mW. Excitation intensities

for the samples marked by the circle and square symbols are 33 kW cm^{-2} . Exposure times for photodarkening and photobrightening are 1.2 and 1.3 sec, respectively.

We have added to the discussion: “Further, we observe that PB efficiency increases as ANP core diameters decrease. In addition, for 700 or 800 nm irradiation, PB becomes more efficient as Tm^{3+} content increases from 8% to 30% (Figs. S18, S19). The dependence on core size suggests that the number of active colour centres is determined by the core size. The strong Tm -content dependence of PB for 700 or 800 nm excitation, which correspond to Tm^{3+} transitions, suggests these wavelengths may induce PB by undergoing efficient upconversion to visible wavelengths (higher Tm content leads to shorter interionic distances, which increases the probability of Tm -based upconversion), which then releases carriers trapped in the colour centres.”

3) We have now directly measured the lifetime of the $\text{Tm}^{3+} {}^3\text{F}_4$ state (1740 nm) in ANPs before and after photodarkening (new Fig. S24). We observe a reduction in lifetime by more than a factor of 5 for photodarkened ANPs, providing direct evidence of nonradiative quenching of the ${}^3\text{F}_4$ state after photodarkening, and fully consistent with our rate equation analysis in Fig. 3. This is important in showing the PB/PD mechanisms hinge on the kinetics of this short-wave infrared transition by altering the threshold of photon avalanching.

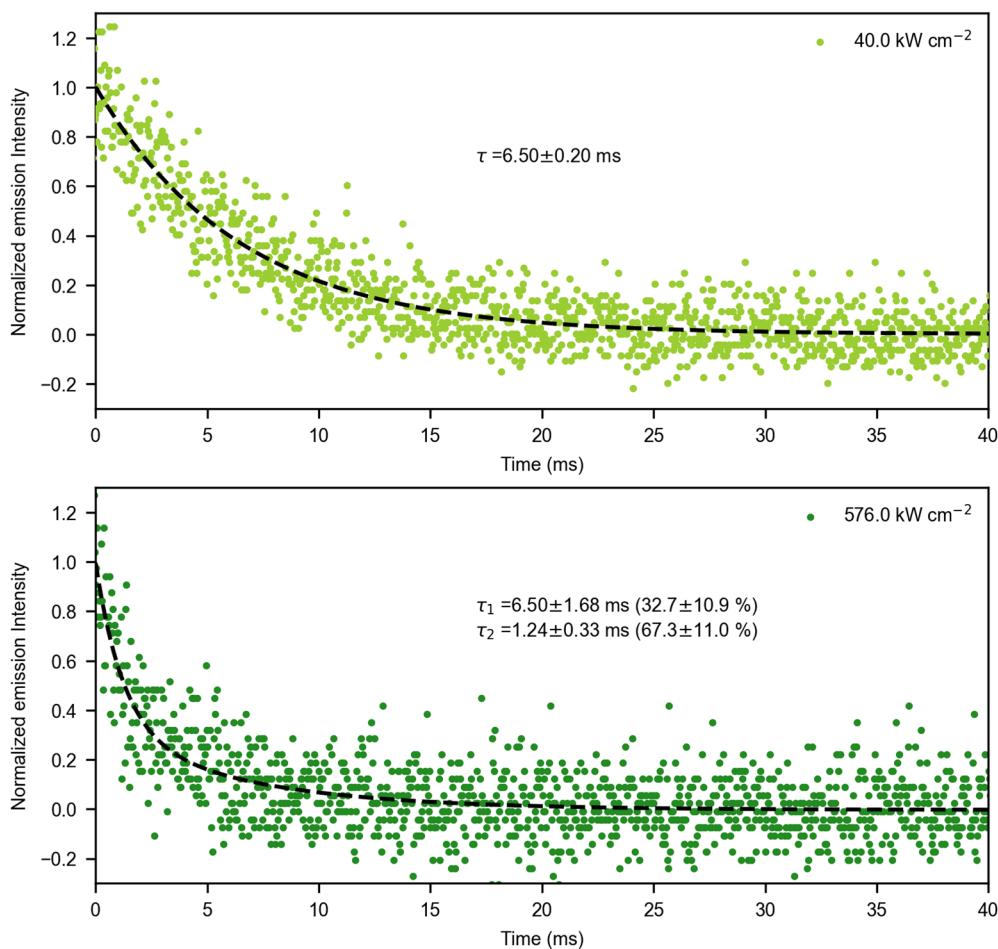


Fig. S24. Time-resolved photoluminescence of IR emission from the $\text{Tm}^{3+} {}^3\text{F}_4 \rightarrow {}^3\text{H}_6$ transition in an ensemble film of 8% Tm^{3+} 17.3/5.6 nm core/shell ANPs under 1064 nm excitation before (*top*) and after (*bottom*) photodarkening. The black dashed lines are fits of exponential functions to the data. Emission wavelengths >1750 nm were selected with a long-pass filter and collected using a superconducting nanowire single-photon detector (SNSPD; Single Quantum EOS 6). A single exponential decay is observed in the non-photodarkened ANPs (*top* panel). The lifetime curve from the photodarkened region (*bottom* panel) includes contributions from non-photodarkened ANPs within the film, resulting in a biexponential decay. The biexponential decay analysis suggests approximately two thirds of the ANPs are photodarkened in the measured region, and the photodarkened ANPs have a ${}^3\text{F}_4$ lifetime that is 5.2 times shorter, consistent with the rate equation analysis and fits in Fig. 3a.

We have added to the discussion: “Direct evidence of such nonradiative quenching is provided by our measured decrease in the ${}^3\text{F}_4$ state lifetime upon photodarkening (Fig. S24).”

2. Why should Gd be included in the shell layer? Does the phenomenon still occur when a NaYF₄ shell is used? The authors should investigate the role of Gd at different concentrations. For example, NaYF₄:Tm@NaGdF₄:Eu core-shell nanocrystals can be prepared in order to study the intensity of Eu emission as a function of the power of a 1064 nm laser.

Our reply: Gd³⁺ is added to facilitate shell growth, and we find that shell Gd³⁺ does not affect ANP photoswitching. We have measured consistent photoswitching in ANPs both with and without shell Gd³⁺, including the super-resolution imaging with the Gd³⁺-free shells (Fig. 5, Fig. S27), and we see no difference.

The Gd³⁺ is included for more consistent and complete hexagonal shell growth [following the synthetic work of X. Liu in *Nature* 463, 1061 (2010)] reducing potential ANP-to-ANP heterogeneity [J. Phys. Chem. C 125, 23976 (2021); *Nature* 589, 230 (2021)].

Changes to the manuscript: We have added the following to the main text: “Gd³⁺ was added to the shells of some batches of ANPs to help facilitate hexagonal shell growth⁴⁷ (Figs. S1,S2; Tables S1,S2).”

And

“We observe no effect on photoswitching in ANPs with shell Gd³⁺ compared to those without.”

Also, the Fig. 5 caption now explicitly notes the use of Gd-free NaYF₄ shells for ANPs in Fig. 5.

“...a, 2D map of the frame-by-frame localizations of a single 8% Tm^{3+} core-inert NaYF₄ shell ANP ($N = 403$ frames; ANP is photoswitched off and on for each frame). ...”

3. The authors reported that the luminescence can be recovered under 500-800 nm irradiation. Is it possible for the luminescence to be recovered by heating? If the electrons are being held in place by defects, luminescence should be recovered after heating since thermal stimulation can clear the traps. Thermoluminescence measurements can be performed to probe the trap depth present in nanocrystals.

Our reply: We share the reviewer’s interest in better understanding the photoswitching mechanisms and appreciate the suggestion. We have now performed temperature-dependent brightening measurements up to our maximum stage temperature of 113° C. In this study,

emission recovery from a film of 20%-Tm ANPs is measured as a function of external heating temperature, to investigate trap depths associated with the photodarkening process. The temperature-dependent data are presented in Fig. R1 below.

As the referee expected, we observe a clear increase in luminescence from photodarkened regions as temp increases, with a noticeable acceleration in thermal recovery at temperatures above 90° C. Because our sample heating stage is limited to a maximum temp of 113° C, we are unable to reach a peak in the PB-vs-temp spectrum. This sets a lower limit on trap depth energy, implying that a trap depth is >1 eV [Reddy & Rao, 1984. High-temperature X-ray irradiation induced thermoluminescence and half-life calculations in NaYF₄ polycrystalline samples. *Crystal Research and Technology*, 19(10), pp.1399-1403], consistent with our optical PB studies. In these measurements, a heating stage warms the photodarkened ANP sample to a given temperature *without any optical irradiation* incident on the sample. Once the temperature equilibrates (5 minutes), the 800 nm luminescence from photodarkened regions is probed using modest 1064nm irradiation. Taken together with the new wavelength-dependent photobrightening measurements discussed above, these experiments reveal that heating or irradiation with wavelengths below 530 nm effectively recovers photodarkened luminescence.

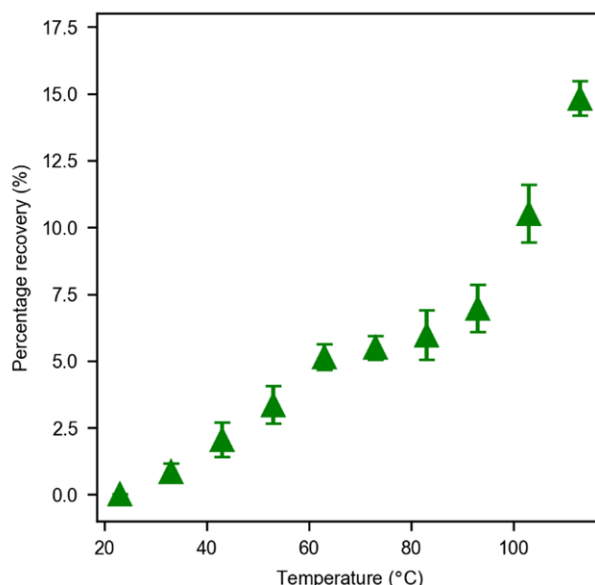


Fig. S23 | Thermal luminescence recovery of photodarkened ANP films. Recovery was accomplished with external heating only (5 min equilibration at each temperature), rather than by photobrightening. Before and after heating, 20% Tm³⁺ 10.4/2.7 nm films were photodarkened and probed with 1064 nm intensities of 320 kW cm⁻² and 64 kW cm⁻², respectively. Percentage recovery is defined as the ratio of the recovered luminescence intensity after the temperature increase to the reduced luminescence intensity after initial photodarkening. Error bars are standard deviations of emission intensities measured at the 4 different spots in the same film.

4. What exactly happened to the valance state of Tm before, during, and after the photodarkening process (using EXAFS)? A change in valance should be observed If Tm ionization can be triggered by high-power illumination from a 1064 nm laser.

Our reply: We certainly wish we could try this experiment, but unfortunately these measurements lack the sensitivity needed here based on discussions with our Advanced Light Source synchrotron colleagues. The outer shells of our ANPs pose one challenge for some methods that can only probe limited penetration depths. But the biggest issue, independent of probing depths, is the following: Single-step photodarkening and fast luminescence quenching (Fig. S3) suggest that a just few color centers (possibly even just one per NP; see Supplemental Discussion) are responsible for photodarkening, and such a small change (of $\ll 1\%$ of Tm ions) cannot be observed with these techniques.

We also note that other possible color-center-charging pathways exist (as highlighted by Ref. 3), some of which do not result in a net change in Tm valence state at all, as now noted in new Fig. S22.

Changes to the Manuscript: We have added new Fig. S22, which illustrates potential PD pathways including ones that do cause a net change in Tm valence state.

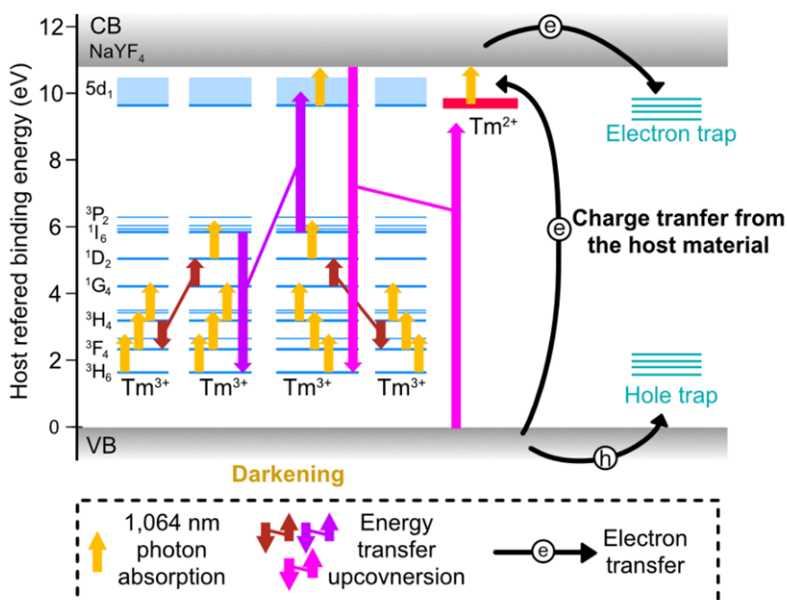


Fig. S22. Potential mechanistic pathways for photodarkening and photobrightening in ANPs via charge transfer from the host material, resulting in Tm²⁺ as an intermediate. CB: conduction band, VB: valence band, W_2 : ³F₄ relaxation rate. Hole traps in addition to electron traps can be produced through the charge transfer process, which can both potentially quench the ³F₄ excited state in Tm ions.

5. Metal oxides usually contain much more defects than fluorides and have a much smaller band gap (< 4 eV). If photoionization is the true cause of photodarkening, it should be considerably simpler to produce in metal oxides.

Our reply: We also agree that it would be interesting to try other nanocrystal hosts. However, we have not been able to synthesize Ln-doped oxide nanoparticles that exhibit photon avalanching, and as we show, PA is critical to photoswitching. This is consistent with various computational models, which show that the higher phonon energy of oxides compared to

fluorides can interfere with upconversion or avalanching. And, to the best of our knowledge, nobody has observed photodarkening in Ln-doped oxide UCNPs.

Changes to the manuscript: We have added language about recent work on alternative low-phonon-energy host materials that are potentially promising for realizing PA: “These results motivate the search for new ANP compositions demonstrating diverse photoswitching parameters⁶²,...”

6. For 2D and 3D microscale optical writing, erasing, and rewriting of stable near-infrared light-switchable ANPs, the pixel size for writing is limited by the beam focal spot size (hundreds of nanometers). What are the writing and erasing efficiencies at different depths? I do not see any advantages associated with the use of nanoparticles as a medium for information storage compared to other optical writing methods such as photonic crystal-based methods that have superior writing speed.

Our reply: These are good points. We appreciate the opportunity to address these important questions in more detail, and to revise the manuscript to highlight the unique advantages.

The primary advantages are the apparently perfect photostability of these nanoparticles and the NIR wavelengths for all processes (photoswitching both directions, excitation, emission). We are unaware of other optical writing media with these properties, and we believe photonic crystal-based 3D printing cannot be erased and repatterned with the ease we demonstrate for ANPs.

Furthermore, photoswitchable ANPs open new pathways for high-density nanoscale optical writing by combining a steep nonlinear optical response with advanced nanoparticle patterning methods [see Gu *et al.*, “Nanomaterials for optical data storage,” *Nature Reviews Materials* 1, 16070 (2016); Barad *et al.*, *ACS Nano* 2021, 15, 5861–5875], whose massively parallel and nm-precise assembly can significantly increase data storage capacity on their own. Notably, the extreme nonlinearity of ANPs means that the theoretical *far-field* writing pixel size is ca. 70 nm, *considerably smaller than the beam focal spot size*. The basis for this is described in detail in our previous ANP publication [*Nature* 589, 230 (2021)].

We achieve efficient photoswitching at 3.5 μm depth, the thickness of our ANP film, and based on literature imaging experiments with these wavelengths (1064 nm excitation, 800 nm emission, 700 nm photobrightening), we expect writing at depths of 100 μm or more should be possible (Kobat *et al.*, “Deep tissue multiphoton microscopy using longer wavelength excitation,” *Opt. Express* 17, 13354-13364, Turcotte *et al.*, “Dynamic super-resolution structured illumination imaging in the living brain.” *PNAS* 116.19 (2019): 9586-9591, Tian *et al.*, “Low irradiance multiphoton imaging with alloyed lanthanide nanocrystals.” *Nature communications* 9.1 (2018): 1-8.). We also provide a theoretical framework below about scattering loss at 100 μm depth, which should be just about 10% for these wavelengths.

Detailed estimation of write/read performance vs. depth: Since nonlinear upconversion through NIR photon absorption by Tm ions is the main excitation pathway for photodarkening, we are also able to demonstrate 3D patterning in ANP ensemble films by scanning a focal volume. We used a 3.5-micron thick film; we did not observe any measurable decrease in writing efficiency for films of this thickness. The two main photon loss pathways for optical penetration into the nanoparticle ensemble films are (i) Rayleigh scattering if the gaps in the film are not filled with

index-matched material, and (ii) photon absorption by Tm ions. If we assume the core/shell ANP size is 25 nm with a core diameter of 20 nm, the Tm dopant concentration of the core is 8%, and the excitation power density is 10 kW cm^{-2} , the number of 1064-nm pump photons in a 25-nm diameter circular area per second, N_{pump} , the number of scattered photons per second by a single nanoparticle N_{sc} , and the number of absorbed photons per second by the single ANP, N_{ab} , are estimated as:

1. $N_{\text{pump}} = (\text{Excitation intensity})/(\text{energy of a 1064 nm photon}) \times (\text{the area of a 25 nm diameter circle}) = (10000 \text{ W cm}^{-2})/(1.86 \times 10^{-19} \text{ J/photon}) \times (3.14/4 \times (25 \times 10^{-7} \text{ cm})^2) = 2.6 \times 10^{11} \text{ photons/sec}$
2. $N_{\text{sc}} = (\text{Rayleigh scattering cross section of a 25-nm sphere}) \times (\text{Excitation intensity})/(\text{energy of a 1064 nm photon}) = (8.2 \times 10^{-17} \text{ cm}^2)(10000 \text{ W cm}^{-2})/(1.86 \times 10^{-19} \text{ J/photon}) = 4.4 \times 10^6 \text{ photon/s}$
3. $N_{\text{ab}} = (\text{absorption cross section of a Tm ion at 1064 nm}) \times (\text{Excitation intensity})/(\text{energy of a 1064 nm photon}) \times (\text{The number of Tm ions in a 20 nm core}) = (4 \times 10^{-21} \text{ cm}^2)(10000 \text{ W cm}^{-2})/(1.86 \times 10^{-19} \text{ J/photon}) \times (4500 \text{ ions in a core}) = 9.6 \times 10^5 \text{ photon/s}$

Considering these calculated values, in a hexagonal closest-packed film that ignores the volume of ANP surface ligands, we expect ~10% of laser loss for a film thickness of 100 μm . We note that scattering can be complicated and can be further exacerbated by coherent effects such as Anderson localization. Also, the shorter wavelength photobrightening irradiation, from 400 nm to 800 nm, will propagate shorter distances in the film due to wavelength-dependent higher scattering cross-sections. But importantly, scattering loss can be mitigated significantly by filling the gap between particles with an index-matched material (in principle, this is straightforward to achieve since NaYF_4 has an index very similar to glass).

Changes to the manuscript:

We have revised the text/discussion to more-clearly highlight the unique advantages offered by our system for some optical storage/read-write applications:

“Recent work has stressed the benefits of nanomaterials and photoswitchable emitters for optical data storage^{8,52,53}.... These results highlight unique advantages offered by ANPs, including the combination of exceptional photostability, extreme nonlinearity, sub-100 nm pixel sizes²³ and the use of NIR wavelengths for all read-write-reset processes.”

7. The authors demonstrate the application of a single nanoparticle photoswitching for PALM super-resolution imaging and demonstrate that ANP-based imaging has higher localization accuracy due to the high number of collected photons (averaging nearly 330,000 per frame). This comparison is not fair; the author should specify the parameter of the frame rate. It might take several hours to construct a single image since the switching processes take seconds, which is several orders of magnitude longer than those employed in conventional PALM. Also, a very small field of view can be achieved as a high power must be applied.

Our reply: The fundamental difference between INPALM and stochastic superresolution techniques lies in the lack of photobleaching of ANPs: an unlimited number of darkening-brightening cycles can be acquired without measurable degradation, so as many photons can be collected for each ANP as desired, leading to exceptionally precise localization accuracies. In addition, the INPALM brightening step is predominantly deterministic, rather than

stochastic, and the darkening does not rely on irreversible photobleaching of the probe. These are the main advances that lead to improved localization accuracies.

As noted by the referee, increased localization accuracy versus measurement time is a tradeoff here. For many applications, having short acquisition times is of secondary importance, such as with fixed cells or other non-living systems. And because ANPs/UCNPs do not photobleach even under extended high fluences, high 1064-nm laser powers also become a secondary consideration. In addition, much previous work, such as with laser trapping, has shown live cells can tolerate 1064 nm fluences higher than the ones we use (e.g., Ashkin, *Nature* 330, 769 (1987)). Because they are so common for laser trapping, powerful 1064 nm CW lasers are readily available.

That said, our proof-of-principle INPALM experiments in Fig. 5, where frame rates were indeed seconds, were optimized for photons and localization accuracy, rather than for imaging speed. To address the referee's concern, we have now investigated whether photoswitching exposure times can be reduced. We find that 91.4 ± 0.5 % of ANPs are turned off with an exposure time of 188 ms ($I_{\text{ex}, 1064 \text{ nm}} = 252 \text{ kW cm}^{-2}$, 1064 nm) and 33.8 ± 1.3 % of ANPs are turned on with an exposure time of 14.6 ms ($I_{\text{ex}, 700 \text{ nm}} = 240 \text{ kW cm}^{-2}$, 700 nm). We note that these times can be considered upper limits, as minimizing the number of active emitters is needed for PALM imaging to prevent the overlapping of emitter point spread functions (PSFs). Thus, the measured photoactivation probability of 33.8 % is ~10-500-fold too high depending on ANP density, and either the exposure time or irradiation intensity needs to be further reduced.

For signal acquisition times, under the conditions of Fig. 5, the average number of detected photons for a single ANP is ~50,000 counts for 500 ms (i.e., similar to signal levels for fluorescent proteins in reported PALM experiments), which enables localization accuracy of a few nm (see Figs. 5a,f). Higher accuracy can then be achieved by collecting from the same particle for multiple frames if desired. Thus, total frame rates of <1 s are readily achievable, which is marginally higher than the typical values reported in the PALM literature, spanning roughly 10 ms to 600 ms [Nature Biotechnology 26, 1035 (2008); Nature Methods 6, 131 (2009); Nature Biotechnology 29, 942 (2011)].

Regarding the field of view (FOV), we agree that ultimately this will be limited by the laser power available on a given setup. In our experience, commonly available laser powers allow for FOVs of tens to hundreds of microns in diameter. When fast switching is desired, then higher laser powers will be needed. For example, photodarkening of a 100 μm -diameter field of view in <200 ms will require ~8 W 1064nm laser power. Such lasers are relatively common and widely used in laser trapping biophysical studies. But for more relaxed time requirements, lower power can be used. For example, in our system, we find we are often limited by the photobrightening beam. To reach a photoswitching probability of ~4% with 700nm excitation requires an irradiation intensity of ~500 W cm^{-2} . Thus, to get a 100 μm -diameter field of view, a laser power of 39 mW is needed at the sample plane, which is well within the power budget of most commercially available lasers at this wavelength.

Changes to the manuscript:

We have added a new figure to the SI showing shortened photobrightening times:

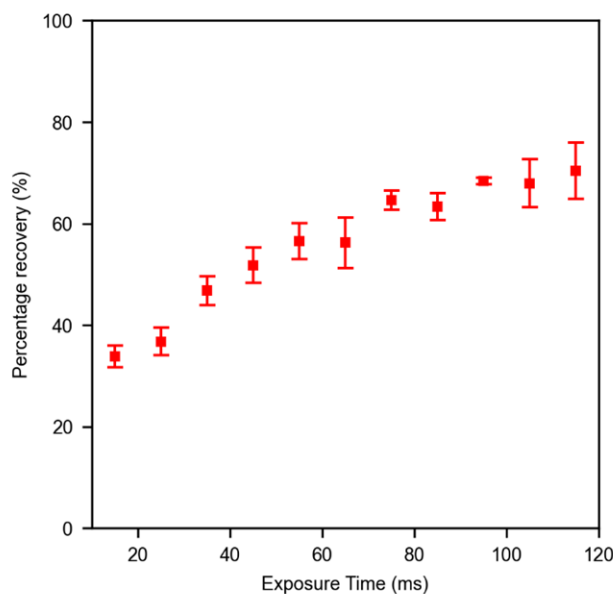


Fig. S32 | Photobrightening recovery as a function of exposure time. Photodarkened regions of a film of 20% Tm^{3+} 10.4/2.7 nm core diameter/shell thickness ANPs were exposed to a 700 nm photobrightening beam for short exposure times, and the luminescence recovery was measured as a function of exposure time. The 1064 nm pumping and photodarkening intensities are 51 kW cm^{-2} and 252 kW cm^{-2} , respectively. 700 nm photobrightening excitation intensity is 240 kW cm^{-2} . The photodarkening exposure time is 188 ms. Error bars are standard deviations of data points measured at the 4 different spots in the same ensemble sample.

We have added the following discussion to the main text: “As with most superresolution imaging techniques, there is a tradeoff between imaging speed and localization accuracy, which depends on the number of photons collected (Fig. 3c). While the INPALM experiments in Fig. 5 were optimized for high localization accuracy, faster frame rates are also possible, down to 15 ms exposure time for photobrightening of 34% of ANPs (Fig. S32). Specifically, we find that $91.4 \pm 0.5 \%$ of ANPs are turned off with an exposure time of 188 ms ($I_{\text{ex},1064 \text{ nm}} = 252 \text{ kW cm}^{-2}$, 1064 nm) and $33.8 \pm 1.3 \%$ of ANPs are turned on with an exposure time of 14.6 ms ($I_{\text{ex},700 \text{ nm}} = 240 \text{ kW cm}^{-2}$, 700 nm). For signal acquisition times, under the conditions of Fig. 5, the average number of detected photons for a single ANP is $\sim 50,000$ counts for 500 ms (similar to signal levels for fluorescent proteins in reported PALM experiments³¹), which enables localization accuracy of a few nm (see Figs. 5a,f). Better accuracy can then be achieved by collecting from the same particle for multiple frames if desired.”

8. What is the error of the positioning accuracy of 0.098 nm and 0.058 nm, and what is the accuracy of the algorithm that removes the influence of sample movement on positioning? Because the authors did not give a full discussion of localization accuracy, the data do not provide sufficient evidence to support their claims.

Our reply: We appreciate the point and realize we omitted these details in the first submission. The uncertainty associated with drift correction is $0.20 \text{ \AA}$, which we have now added to the Fig. 5 caption. This uncertainty is added in quadrature to the uncertainty of the INPALM-imaged

particles originating from the collected photon number, the pixel size, and background noise. We have also added a more complete discussion of the localization accuracy measurements and calculations to the main text and Methods.

In brief: the localization accuracy is first calculated for each ANP image spot within a frame by fitting to a 2D-Gaussian function, yielding a mean position and the standard error of that mean (the localization accuracy). After each frame acquisition, the active ANPs within the region of interest are photodarkened, and then a photobrightening beam is briefly applied to activate another random subset of ANPs within the same region. Since unlimited frames can be acquired without photodegradation, we can get a dense cloud of hundreds of localizations for each ANP as shown in Figs. 5a and 5f, which is challenging for other photobleaching photoswitches.

To compensate the sample movement, we also image reference ANPs outside the region of interest, which remain active in each frame (Methods; Figs. S28-S30). Using these reference PSFs, we can correct translational and rotational sample drift, enabling clustering of the individual frame localizations (Fig. S28-S30).

Changes to the manuscript:

We have now added details regarding the accuracy of our method for compensating sample drift in the **caption of Fig. 5**, and discussion of our localization accuracy measurements to the **main text and Methods section**:

Addition to Fig. 5 caption: “Localization accuracy values include the contribution of 0.20 Å from drift correction.”

Added discussion to main text: “For INPALM, we acquire diffraction-limited images of photoactivated ANPs in each frame. The ANP image spots within a frame are fit to a 2D-Gaussian function, yielding a mean position and the standard error of that mean. This error, i.e. localization accuracy, scales inversely with the square root of the number of collected photons (Figs. 5c,i). After each frame acquisition, the active ANPs within the region of interest are photodarkened, and then a photobrightening beam is briefly applied to activate another random subset of ANPs within the same region. After repeated photoswitching cycles, a composite superresolution image is generated by first overlaying the centroid positions from each frame (Figs. 5a,f; the diameters of the red circles correspond to the localization accuracy of each centroid), then clustering the localizations using a Gaussian mixture method (Fig. 5g; Methods), and finally fitting the centroid position maps (weighted by acquired photon number) to a 2D-Gaussian function (Figs. 5b,h; Methods)⁵⁸. This final fit provides the overall INPALM centroid positions for each ANP and their respective localization accuracies. Because an unlimited number of photodarkening-PB cycles can be acquired without photodegradation, as many photons can be collected for each ANP as desired, leading to exceptionally precise localization accuracies. Sample drift and rotation are corrected for by imaging reference ANPs outside the region of interest, which remain active in each frame (Methods; Figs. S28-S30).”

Added details in Methods: “First, each ANP image spot within a frame is fit to a 2D-gaussian function, yielding a mean position and the standard error of that mean (the localization accuracy). These fittings are then performed for each frame. Next, a composite image is generated by overlaying the centroid positions from each frame. Sample drift and rotation result in a dispersion of the collection of centroids for each ANP within this composite (Fig. S28c and S29). For Fig. 5, correction for lateral and rotational drift was achieved by imaging, in addition to

the photoswitched ANPs, 6 reference ANPs within each frame that are not photoswitched during INPALM frame collection (see Fig. S28). Corrections based on the PSF centroid fits of these 6 ANPs yields ANP centroid positions from all the frames that are tightly clustered within ~20 nm regions in the corrected composite image (Fig. S30). Each of these centroid clusters (see, for example, Figs. 5a,f are then fit to a 2D-gaussian function (Figs. 5b,h), with each centroid weighted by the number of photons collected for its evaluation, which provides the overall INPALM positions and localization accuracies for each ANP.

For ANPs in nanoscale proximity to one another (e.g., touching), data-processing using a Gaussian mixture method offers a straight-forward way to separate a mixture of localizations into several groups (Fig. 5f, g)⁶⁸...”

9. The time trace of photoblinking of just one nanoparticle is shown in Figure 2f, with a large separation between emission peaks. Under the same excitation conditions, do the random emission combinations of all nanoparticles conform to the statistical distribution required for PAML imaging, or are they all concentrated within the same shorter time period? This determines the effectiveness and imaging speed of PALM imaging. Although the authors show that the temporal distribution of photoblinking is intensity-dependent, it is practically difficult to control the excitation intensity of each nanoparticle during imaging.

Our reply: We rarely observe spontaneous blinking during INPALM experiments, nor is blinking necessary. INPALM differs from the stochastic superresolution techniques such as STORM and SOFI in this regard. Also, because photobleaching is not an issue in these experiments, blinking is not particularly disruptive when it does occur, since as many frames as needed can be acquired for adequate signal.

Regarding its effects on imaging speed: under the conditions for fast photodarkening described in response to comment #7 above, there is a high probability (>90%) of photodarkening within 188 ms, while blinking (i.e., subsequent photobrightening with 1064nm pumping) remains rare.

Under 1064 nm illumination (i.e., when no additional photobrightening beam is used), blinking only becomes common at high powers since releasing a trapped carrier from a colour centre using 1064 nm light is an inefficient multiphoton process. The blinking shown in Fig. 2f is occurring under 1064 nm pump intensity that is more than 25 times greater than the avalanching threshold, and is relatively infrequent and short-lived. Higher pump intensities are needed for more regular blinking (e.g. Fig. S4) for intensities ~100x above the avalanching threshold. We note that in our experience blinking is only observed under intense 1064 nm illumination, and *not during illumination with wavelengths used for photobrightening*.

Changes to the manuscript: We have clarified the laser intensities required for blinking to occurs: “Photoblinking statistics from single ANPs under 1064 nm irradiance show that the blinking is intensity dependent, predominantly occurs at intensities 2 orders of magnitude higher than needed for ANP imaging, and...”

10. The influence of the shape and luminous efficiency of nanoparticles on the positioning accuracy needs to be discussed. According to the information presented in Fig. S3 and S4, an

increase in switching speed results in a decrease in switching efficiency.

Our reply: Nanoparticle shape will not have significant effects on calculated localization accuracy, which is the calculated spatial average of detected photon position in the x-y plane for a given ANP. If the emitters within the ANP are evenly distributed, this spatial average will be the physical center of the nanoparticle (flattened into 2D) as long as the ANP is overall centrosymmetric (for example: spherical, elliptical/prolate, or rod-shaped). Localization position for noncentrosymmetric shapes (e.g., a triangular prism) would be affected in the same way as the center of mass. Uneven distribution of Tm ions might have a significant effect if, say, they all localized to one end of a high-aspect ratio nanorod, but the aspect ratios of our ANPs are reasonably close to 1, as seen in Fig 2. Indeed, the results from Fig 5 show the centroid fittings are very close to the centers of ANPs determined by EM, within experimental limits.

For switching speed, the referee is correct that an increase in switching speed results in a decrease in switching efficiency *for a constant excitation intensity*. This reduction in efficiency can be compensated for by increasing excitation intensity, as long as the sample is amenable to these conditions. See also our discussion of this tradeoff in response to comment 7 above.

Changes to the manuscript: We have added additional discussion in the manuscript about the localization accuracy measurements in Fig. 5, which includes a statement on the influence of the shape of particles.

“The high-precision localization is apparent in a 2D map of the localizations from more than 400 INPALM frames on a single ANP (Fig 5a). We collected 8.90×10^7 total photons from the ANP without measurable degradation, averaging nearly 200,000 per frame. This enables calculation of localization accuracies of 0.76 Å and 0.50 Å in long and short axes, respectively (Fig. 5c and Methods). These values are considerably smaller than the nanocrystal diameters, or even those of individual unit cells²¹, which raises questions of how calculated localization accuracies should be interpreted in various experimental settings. For the Fig. 5f-i experiments in resolving components of a nanoparticle cluster, the calculated values are the spatial averages of detected photons⁵⁹, reflecting a luminescence centre of mass of emitting Tm³⁺ ions, flattened into 2 dimensions, for each ANP. Because our ANPs are reasonably symmetric (Fig. 2e), we observe that the localization centroid fittings are very close to the centers of ANPs determined by EM, within experimental limits (Fig. 5e). For possible bioimaging experiments in which targeted optical probes are serving as markers of biomolecule position, the localization accuracies are limited by factors such as the size and stiffness of the nanoparticle surface passivation and biomolecule-NP linker⁶⁰. For kinetic studies of biomolecule motion, tracking of positional changes is limited largely by the stiffness of the surface passivation and linker, which determine how faithfully probe motion tracks biomolecule motion⁶¹.”

Referee #2 (Remarks to the Author):

The manuscript written by Lee et al. describes the photoswitching effect of Tm-doped avalanching nanoparticles. First of all, I would like to congratulate all the authors for such magnificent work and the detailed study of the photoluminescent properties of these fashionable nanoparticles. The authors have demonstrated an unknown behavior of ANPs such as the reversible (up to 1158 irradiation cycles) and controllable photodarkening/photo brightening

process. Nevertheless, there are some uncertainties in the current version that I encourage the authors to address before publication.

Our reply: We thank the reviewer for “magnificent work” and other kind words about our study. We appreciate the careful reading and have worked to fully address the reviewer comments here and in the revised manuscript.

1- The representative scheme in Figure 1a shows the photoswitchable ANPs. The ANPs are composed of a core based on Tm-doped NaYF₄ NPs and a NaGdF₄ inert shell whose dopant amount and layer thickness was tuned. In this scheme, it is highlighted and activated quencher, however, the authors don't use these words along the manuscript in the main text. There are indeed some references about photodarkening in Tm-doped bulk materials but the understanding of the manuscript would be aided by the inclusion of a short explanation about why 1064 nm light quenches the emission and 510-800 nm induce a brightening.

Our reply: We thank the Referee for the suggestion and have added experiments and language to clarify the photodarkening and photobrightening mechanisms. The new experiments, which comprise 12 new figures, include: Tm excited-state lifetimes establishing the quenching of Tm upon photodarkening (Fig. S24); photobrightening (PB) dependencies on core size, shell thickness, and Tm concentration that reveal colour centre location and Tm's role in IR PB (Figs. S18-S20); UV emission spectroscopy that establishes the correlation between highly-excited Tm ions with photodarkening (Fig. S21); as well as expanded and improved wavelength-dependent PB behaviors that elucidate trap-depth energies and uncover multiple wavelength-dependent pathways for PB (new Fig. 3 and Figs. S16,S17).

Changes to the manuscript: Based on the new insights from these experiments, we have included additional discussions of the potential photodarkening and photobrightening mechanisms:

“Plots of luminescence recovery *versus* photobrightening (PB) wavelength (Figs. 3b, S16), termed here the PB activity spectra, show between ~5 – 100% recovery in ANP films, depending on illumination wavelength. The PB activity spectra (Fig. 3b, S16) include broad features at wavelengths <530 nm attributable to colour centres, as well as peaks at both 800 nm and 700 nm, which correspond directly to Tm³⁺ ³H₄ and ³F_{2,3} transitions. There is a sharp dip in the activity spectrum at 450 nm, corresponding to higher-energy ¹G₄ and ¹D₂ Tm³⁺ transitions, which may enhance upconversion to UV manifolds to induce further photodarkening rather than luminescence recovery (Fig. S17). Increasing the irradiation intensity or the exposure time also enhances PB (Fig. 3c), so that nearly full luminescence recovery can be achieved in a few seconds with 700 nm light (Fig. 3c). Further, we observe that PB efficiency increases as ANP core diameters decrease. In addition, for 700 or 800 nm irradiation, PB becomes more efficient as Tm³⁺ content increases from 8% to 30% (Figs. S18, S19). The dependence on core size suggests that the number of active colour centres is determined by the core size. The strong Tm-content dependence of PB for 700 or 800 nm excitation, which correspond to Tm³⁺ transitions, suggests these wavelengths may induce PB by undergoing efficient upconversion to visible wavelengths (higher Tm content leads to shorter interionic distances, which increases the probability of Tm-based upconversion), which then releases carriers trapped in the colour centres”

and

“Taken together, our models and observations are consistent with a photodarkening scheme in which extended ANP photoexcitation populates high-lying Tm^{3+} excited states (e.g., $^1\text{I}_6$ and $5\text{d}^14\text{f}^{11}$ states⁴⁸; as in Fig. S21) and ultimately results in the transfer of an electron (hole) from the Tm^{3+} ion or the neighboring atoms to the NaYF_4 conduction (valence) band (Fig. 3d, Fig. S22), which is then trapped in a defect, activating a colour centre that can quench the $\text{Tm}^{3+} {}^3\text{F}_4$ -to-ground-state transition. Direct evidence of such nonradiative quenching is provided by the decrease in the ${}^3\text{F}_4$ state lifetime upon photodarkening (Fig. S24). Measurements of thermal luminescence recovery in photodarkened ANPs (Fig. S23) are consistent with trap depth energies suggested by the PB activity spectra (Figs. 3b, S16). Because the process is fully reversible (Fig. 4 and *vide infra*) and does not involve surface quenching, any active colour centers are in the ANP interior, likely at the core/shell interface⁴². These interfacial point defects can arise during synthesis but are too minor to be imaged within the nanoparticle interior, even by high-resolution TEM (Fig. 2e). As reported previously, such colour centres can have excited states that are capable of quenching the ~ 0.7 eV ${}^3\text{F}_4$ transition of Tm^{3+} via energy transfer^{18,19,42,49}, which increase W_2 (Fig. S24) and, critically, shift I_{th} as observed²³ (Figs. 1b, 3a, S14, Supplementary Methods). Studies of other NP systems have shown that a single trap site is sufficient to quench luminescence for nanoparticles of similar dimensions as ANPs^{46,50}.”

Finally, for this topic it is worth emphasizing that the characterization of atomic colour centres and their direct correlation with specific optical functionalities can be exceptionally challenging, and so there will necessarily be gaps in our understanding of the photoswitching mechanisms. We note in the manuscript that detailed identification of all transitions involved in the current system will require further in-depth study.

Added Figures:

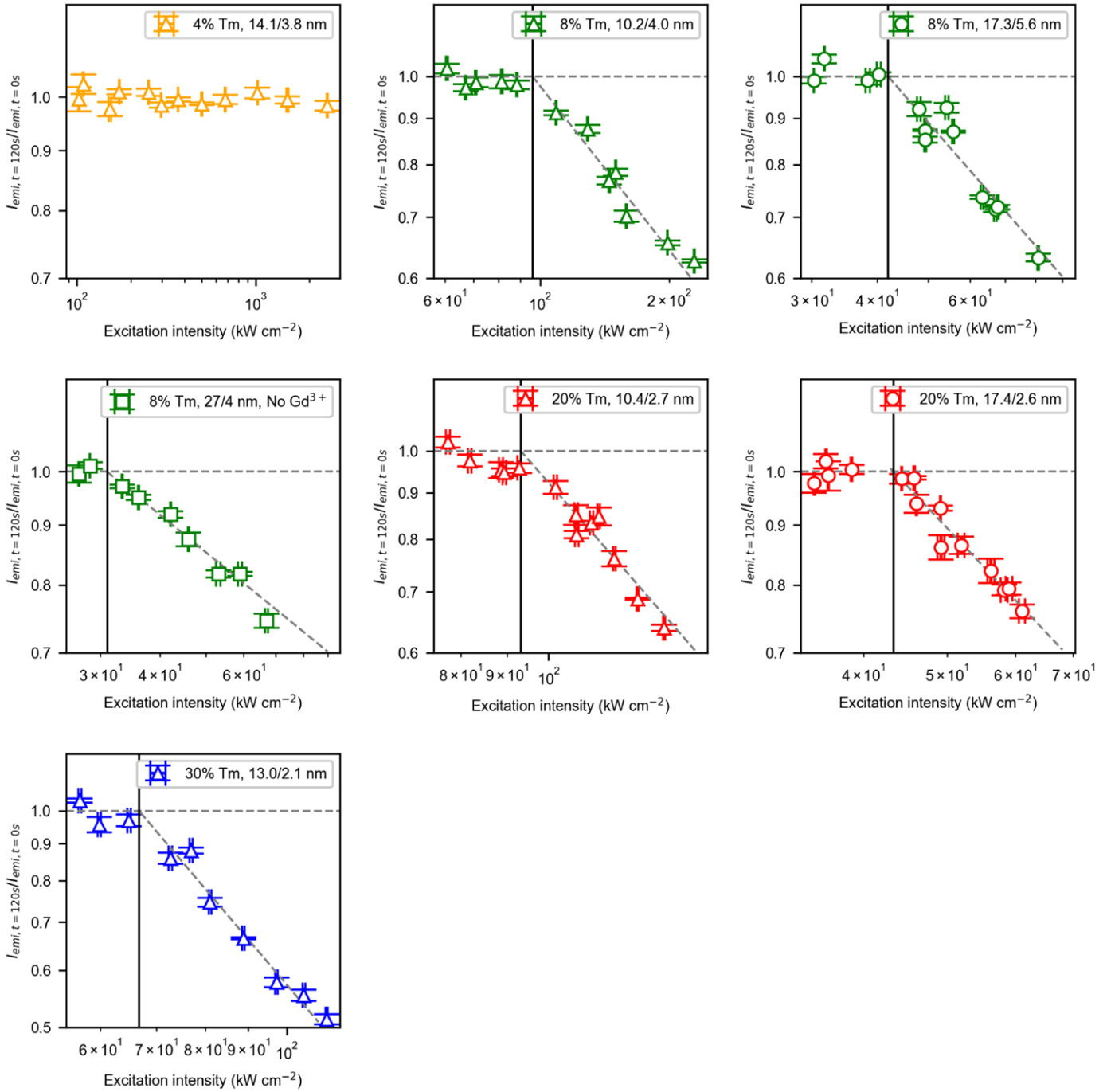


Fig. S12. Determination of photodarkening intensity I_{pd} for different ANP compositions. Each plot shows the ratio between 800 nm emission intensities (I_{emi}) at $t = 0$ and after 120 s of continuous 1064 nm exposure versus excitation intensity. These data allow us to define a photodarkening threshold intensity (I_{pd}) as the 1064 nm pump intensity where emission at $t = 120$ s decreases below the initial value. Error bars are standard deviations of 4 data points measured at the same spot within 0.4 s.

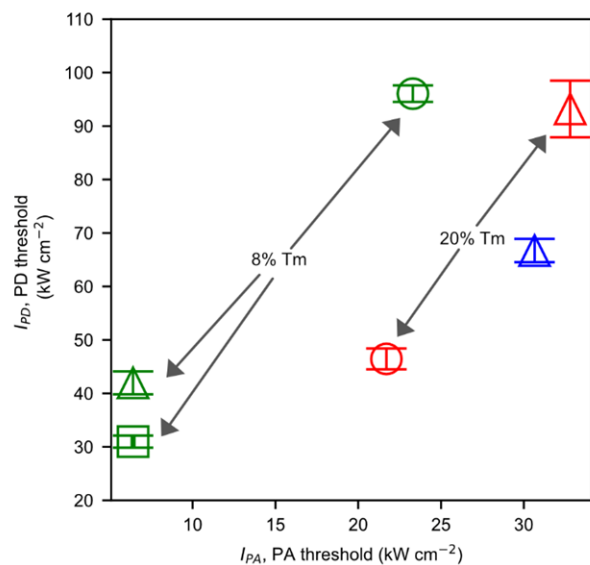


Fig. S13. Photodarkening threshold versus pre-darkened photon avalanche threshold for various ANP compositions. Symbol definitions are the same as in Fig. S12 above. Error bars are standard deviations derived from the curve fitting of the power-dependent photodarkening analysis shown in Fig. S12.

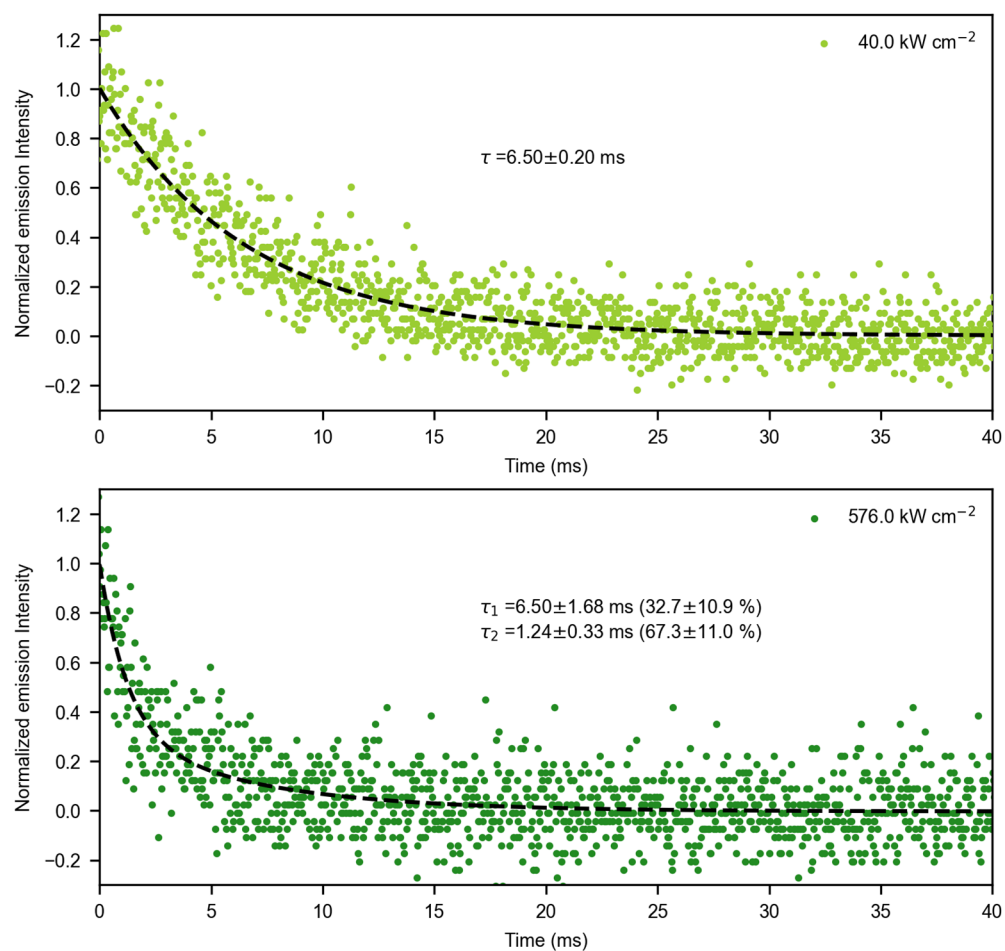


Fig. S24. Time-resolved photoluminescence of IR emission from the $\text{Tm}^{3+} {}^3\text{F}_4 \rightarrow {}^3\text{H}_6$ transition in an ensemble film of 8% Tm^{3+} 17.3/5.6 nm core/shell ANPs under 1064 nm excitation before (*top*) and after (*bottom*) photodarkening. The black dashed lines are fits of exponential functions to the data. Emission wavelengths >1750 nm were selected with a long-pass filter and collected using a superconducting nanowire single-photon detector (SNSPD; Single Quantum EOS 6). A single exponential decay is observed in the non-photodarkened ANPs (*top* panel). The lifetime curve from the photodarkened region (*bottom* panel) includes contributions from non-photodarkened ANPs within the film, resulting in a biexponential decay. The biexponential decay analysis suggests approximately two thirds of the ANPs are photodarkened in the measured region, and the photodarkened ANPs have a ${}^3\text{F}_4$ lifetime that is 5.2 times shorter, consistent with the rate equation analysis and fits in Fig. 3a.

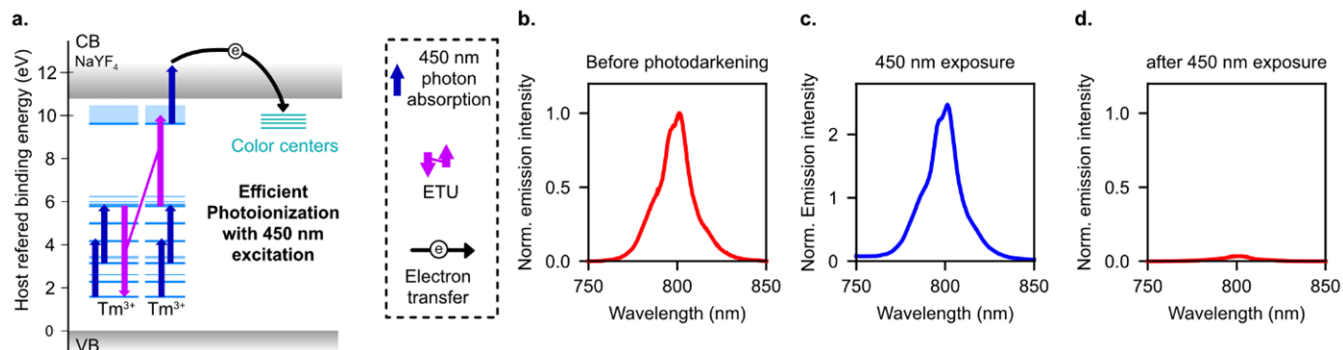


Fig. S17 | ANP Photodarkening with 450 nm excitation. **a**, Potential mechanistic pathways for photodarkening in ANPs under 450 nm pumping. Emission spectra from an ensemble film of 20% 10.4/2.7 nm core diameter/shell thickness ANPs before photodarkening (**b**), during 450 nm exposure (**c**), and after photodarkening with 450 nm exposure (**d**). 1064 nm illumination is used for pumping the luminescence in panels (**b**) and (**d**). The 1064 nm intensity is 75 kW cm^{-2} , and the 450 nm excitation intensity is 222 kW cm^{-2} . The emission intensities (panels **b,c,d**) are normalized to the maximum intensity in plot **b**.

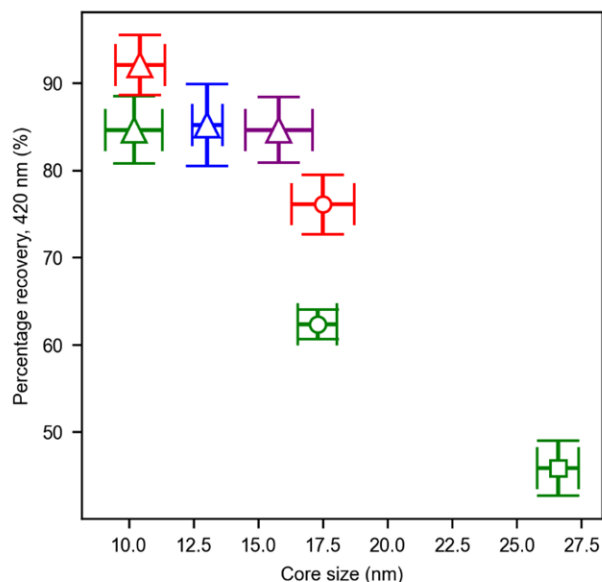


Fig. S18| Photobrightening recovery as a function of ANP core size. Excitation (at 1064 nm) and photobrightening (at 420 nm) of ensemble film samples with intensities of 54 kW cm^{-2} and $277.87 \text{ kW cm}^{-2}$, respectively, for materials marked by the triangle symbols (see caption in Fig. S16). Excitation and photobrightening intensities are 33 kW cm^{-2} and 167 kW cm^{-2} , respectively, for the samples marked by the circle and square symbols. Exposure

times for photodarkening and photobrightening are 1.2 and 1.3 sec, respectively. The 420-nm laser power at the sample is 0.6 mW for photobrightening.

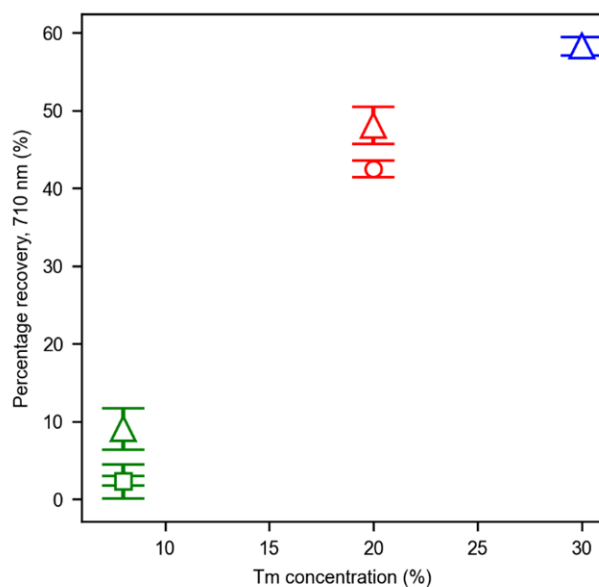


Fig. S19 | Photobrightening recovery percentage as a function of ANP Tm^{3+} concentration. Excitation (at 1064 nm) of ensemble film samples with intensities of 54 kW cm^{-2} for materials marked by the triangle symbols (see caption in Fig. S16). For photobrightening, the 710-nm laser power at the sample is 0.6 mW. Excitation intensities for the samples marked by the circle and square symbols are 33 kW cm^{-2} . Exposure times for photodarkening and photobrightening are 1.2 and 1.3 sec, respectively.

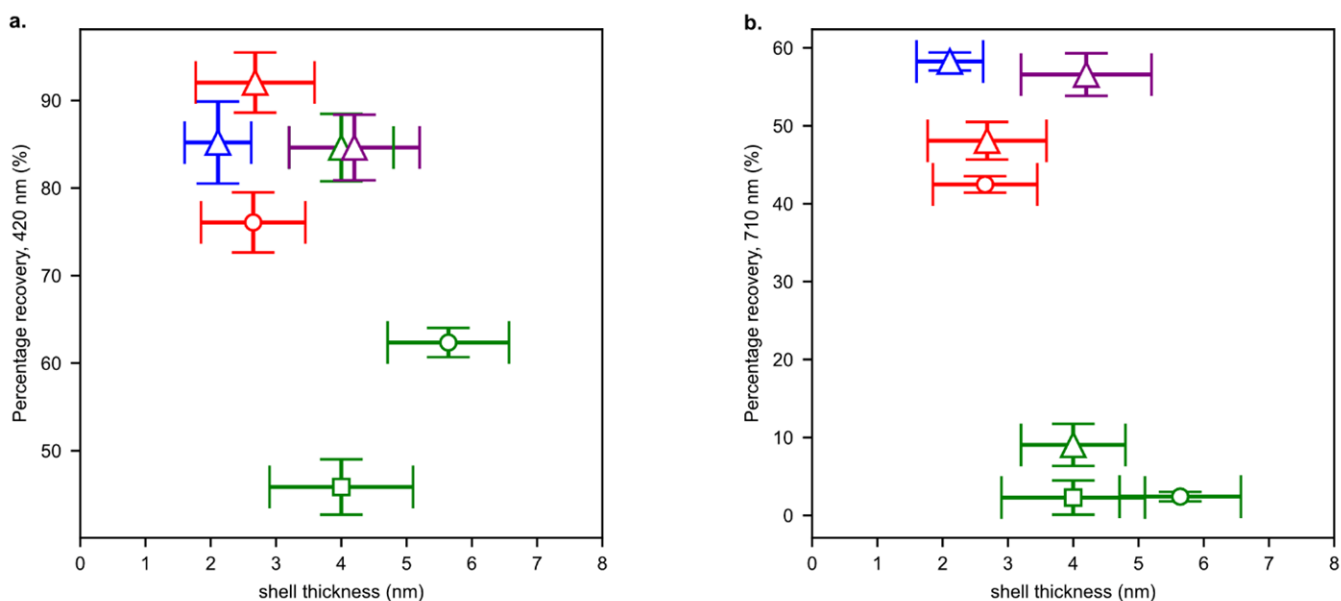


Fig. S20 | Photobrightening recovery percentage of various ANP ensemble film samples versus shell size. Pumping (at 1064 nm) and photobrightening (at 420 nm in (a), 710 nm in (b)) intensities are 54 kW cm^{-2} and 277.87

kW cm^{-2} , respectively, for the triangle symbols. Pumping and photobrightening intensities are 33 kW cm^{-2} and 167 kW cm^{-2} , respectively, for the circle and square symbols. Exposure times for photodarkening and photobrightening are 1.2 and 1.3 sec, respectively. The laser power at the sample for 710 nm and for 420 nm is set to 0.6 mW. (Marker-Tm content, core/shell sizes); green triangle-8%, 10.2/4.0 nm, green circle-8%, 17.3/5.6 nm, green square-8%, 26.6/4.0 nm, red triangle-20%, 10.4/2.7 nm, red circle-20%, 17.4/2.6 nm, blue triangle -30%, 13.0/2.1 nm. Y-axis error bars are standard deviations of data points measured at the 4 different spots in the same ensemble sample. X-axis error bars: EM-determined variability in shell thickness for each batch, from Table S1.

In addition, **new Figs. 3b,c and S16 have been added**, which are **shown below** in our response to comment #2.

2- The authors claimed at the beginning of the MS that photobrightening occurs when using wavelengths between 510 and 800 nm, nevertheless, they use 700 nm for the experiments. What is the reason? From figure 3b one can say that the best recovery is produced at ca. 600 nm. Why did you not use that one?

Our reply: We chose 700 nm for photobrightening because NIR excitation is better at subsurface penetration than visible wavelengths and is considered particularly benign for living systems. This will be important for a variety of applications that benefit from lower-energy, low-scattering NIR illumination.

Also, based on the reviewer's question, we have carefully remeasured the photobrightening action spectra for our materials. Notably, we have improved our experimental set-up and now include an improved plot in new Figure panel 3b, as well as an updated Methods section, quoted below. As shown in the new plot, we find that photobrightening at 700 nm or 800 nm are nearly as efficient as for wavelengths below 500 nm. We also find that photobrightening efficiency is dependent both on core size and Tm content (new Figs. S18, S19).

Changes to the manuscript:

New Methods: "For measuring the photobrightening activity spectra, we used a reflective objective lens (Thorlabs, LMM40X-P01), which does not show significant chromatic aberration, and selected filters (Thorlabs, FESH700 and FESH750) to block residual light from the 800 nm pump source."

New Fig 3b: We have updated Fig. 3b with the corrected data, showing that for 20% Tm ANPs photobrightening at 700 nm is nearly as efficient as for wavelengths below 500 nm.

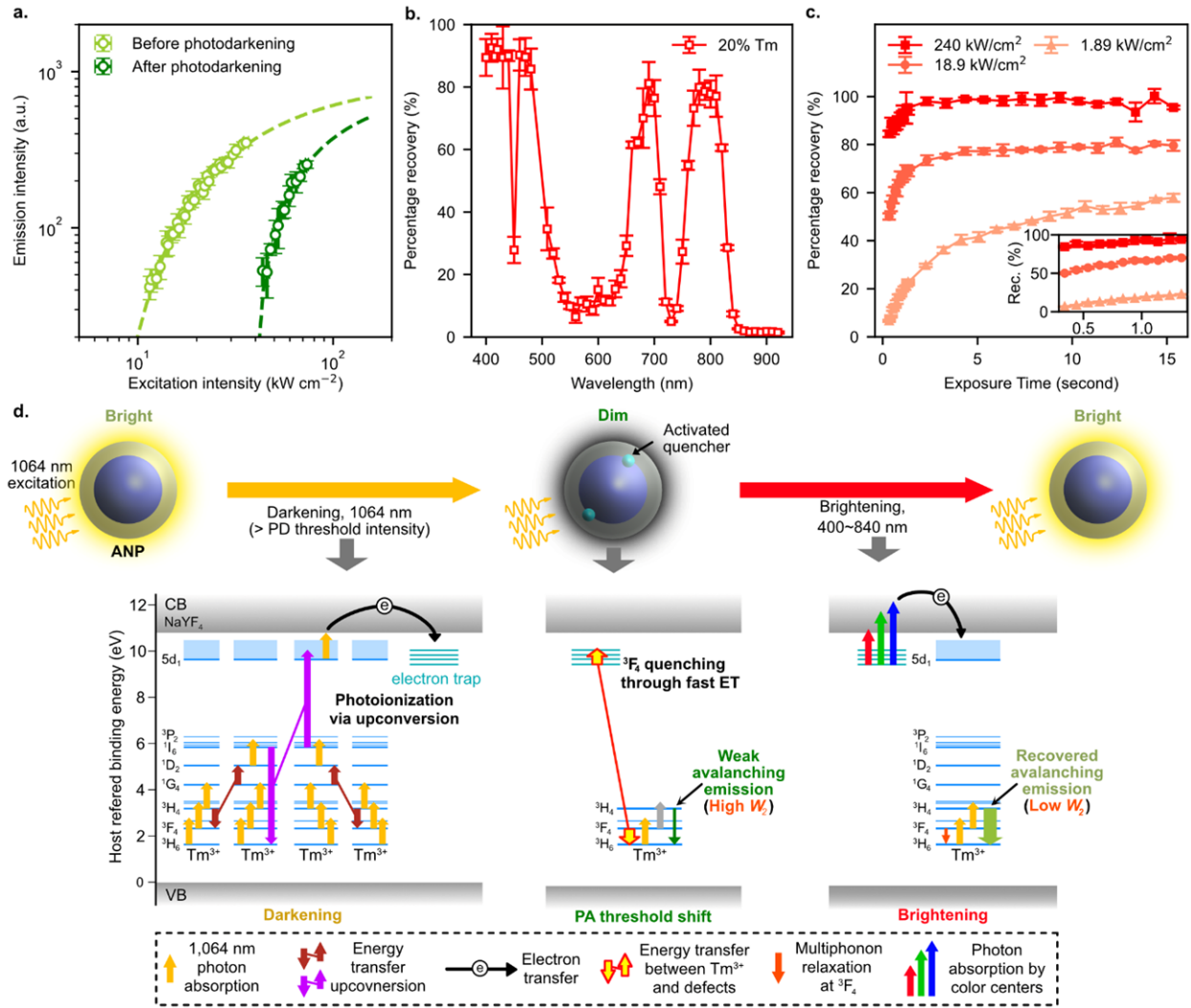


Fig. 3 | ANP Photobrightening and photodarkening. **a**, Emission intensity (800 nm) versus excitation intensity (1064 nm) of a single 8% Tm^{3+} 17.3/5.6 nm (core diameter/shell thickness) ANP before and after photodarkening. Switching between bright and dim states in ANPs is induced by shifting of the PA threshold, using 1064 nm light to darken and wavelengths ≤ 840 nm to brighten. **b,c**, Photobrightening recovery of a photodarkened 20% Tm^{3+} 10.4/2.7 nm core/shell ANP ensemble film sample versus **(b)** irradiation wavelength or **(c)** exposure time. The photobrightening laser power at the sample is set to 600 μW for all wavelengths in **b**, which corresponds to 90 kW cm^{-2} at 700 nm. Photobrightening exposure times are 1.3 sec in **b**. Photodarkening exposure times are 1.2 sec in **b** and **c**. 1064-nm laser intensities for luminescence pumping and photodarkening are 69 and 345 kW cm^{-2} , respectively, in **b** and **c**. 700-nm irradiation intensities for photobrightening are shown in the legend in **c**. The inset in **c** is a magnified plot in the exposure-time range from 340 ms to 1340 ms. Error bars are standard deviations of data points measured at the 4 different spots in the same ensemble sample. **d**, Potential mechanistic pathways for photodarkening and photobrightening in ANPs. CB: conduction band, VB: valence band, W_2 : $^3\text{F}_4$ relaxation rate.

New SI fig: We have also added new SI Fig. S16, showing photobrightening activity spectra for the seven different ANP designs studied here.

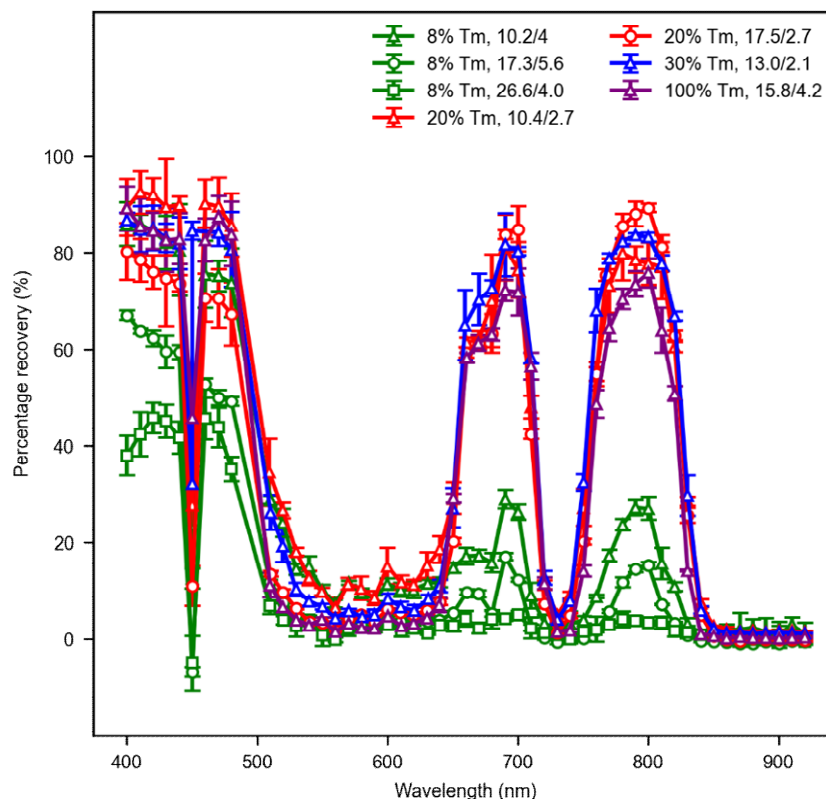


Fig. S16. Photobrightening recovery of darkened ANP films as a function of irradiation wavelength. The Tm contents for green, red, blue, and purple markers are 8%, 20%, 30%, and 100%. Excitation (1064 nm) and photobrightening intensities are 54 kW cm^{-2} and $277.87 \text{ kW cm}^{-2}$, respectively, for green triangles (10.2/4 nm), red triangles (10.4/2.7), blue triangles (13.0/2.1 nm), and purple triangles (15.8/4.2 nm). Excitation (1064 nm) and photobrightening intensities are 33 kW cm^{-2} and 167 kW cm^{-2} , respectively, for green circles (17.3/5.6 nm), green squares (26.6/4.0 nm), and red circles (17.5/2.7 nm). Exposure times for photodarkening and photobrightening are 1.2 and 1.3 s, respectively. Error bars are standard deviations of data points measured at the 4 different spots in the same ensemble sample.

New language in the text: “The PB activity spectra (Fig. 3b, S16) include broad features at wavelengths $<530 \text{ nm}$ attributable to colour centres, as well as peaks at both 800 nm and 700 nm , which correspond directly to $\text{Tm}^{3+} {}^3\text{H}_4$ and ${}^3\text{F}_{2,3}$ transitions. There is a sharp dip in the activity spectrum at 450 nm , corresponding to higher-energy ${}^1\text{G}_4$ and ${}^1\text{D}_2$ Tm^{3+} transitions, which may enhance upconversion to UV manifolds to induce further photodarkening rather than luminescence recovery (Fig. S17). Increasing the irradiation intensity or the exposure time also enhances PB (Fig. 3c), so that nearly full luminescence recovery can be achieved in a few seconds with 700 nm light (Fig. 3c).”

3- Figure 2 caption says: “blue markers are 50% Na/50% Y, Y, F, and Na ions”, I guess the authors would say 50% Na/50% Y, Gd, F.

Our reply: We agree that the description of the labeling was confusing. This stems in part from the complexity of NaYF₄ structure, in which one site is either Na or Y, so there are actually 2 possibilities even within the same crystal. We have updated the legend for clarity. These ion locations are from *Nature* 463, 1061–1065 (2010).

Changes to the manuscript: We have updated this sentence of the Figure 2 caption to read: “NaYF₄ crystallographic positions are 50% Na/50% Y (red circle); Y (orange); F (green); Na (blue)”

4- Some abbreviations are not explained the first time used in the main text, for example, PA (line 55), AFM (Fig 2 caption), DRE (Fig 1 caption)...Please check carefully this issue throughout the MS.

Our reply: Thank you for catching these, and we have added definitions for the acronyms.

Changes to the manuscript: These have been corrected in the text:

“ANPs are UCNPs with the steepest nonlinear emission response of any nanoscale material^{23,44,45}, enabled by the photon avalanching (PA) upconversion mechanism.”

“(a) Atomic force microscopy (AFM) and (b) confocal scanning images of a single and a cluster of 4 ANPs”

“The plots are derived from the differential rate equation (DRE) fits of experimental data in Fig. 3a.”

“Plots of luminescence recovery *versus* photobrightening (PB) wavelength...”

5- Figure S1 and S2, table S2 and S3 are not mentioned/discussed in the main text.

Our reply: We are grateful to the Referee for noting these oversights.

Changes to the manuscript: We have checked to make sure all supplemental figures and tables are now referenced in the text. Figs. S1, S2 and Tables S2, S3 are now called out as follows:

“Gd³⁺ was added to the shells of some batches of ANPs to help facilitate hexagonal shell growth⁴⁷ (Figs. S1,S2; Tables S1,S2).”

and

“To better explain this, we fit the experimental power-dependent emission curves for darkened and undarkened ANPs to a rate equation model that we previously developed (Supplementary Methods; Table S3) ...”

6- Concerning the reproducibility of the experiments, in some plots, the error bars are missed e.g., Fig 3a, Fig S7, Fig S12, or Fig S13. Could the authors include that information? In some

figure legends, it is written that the error bars are calculated as the standard deviation of 4 different measurements. These 4 measurements are made from 4 different films or are the same ANP film sample repeated four times? And if it is the first option, are all of them from the same batch of NPs? Otherwise, I recommend the authors revise all the figure legends to include how the error bars were calculated since in some of them the information is missed.

Our reply: We are grateful for the careful reading. We have added the error bars (one standard deviation from the mean) in Fig. 3a, S7, S12, and S13.

To derive the error bars, we performed 4 separate measurements on the same single ANP (for single-ANP measurements) or on different regions of an ensemble film. For photodarkening and photobrightening experiments with ensemble films, we move 3 μm away from the previously measured position to select a spot which is not photodarkened. We have clarified how we calculated the error bars in the Methods section and relevant figure captions.

Changes to the manuscript: We have added error bars in Figure 3a (*see response to comment 2 above*), S7, S14, and S15. Also, error bar information has been added to captions.

We have updated Methods to explain how multiple measurements were made and error calculated: “Where appropriate, the error bars in optical data plots were derived by performing 4 separate measurements on the same single ANP (for single-ANP measurements) or on different regions of an ensemble film. For photodarkening and photobrightening experiments with ensemble films, we move 3 μm away from the previously measured position to select a spot which is not photodarkened.”

Updated Fig. 3a, S14, and S15 captions now include the following: “Error bars are standard deviations derived from four separate measurements on the same single ANP.”

Error bars added to the following SI figures:

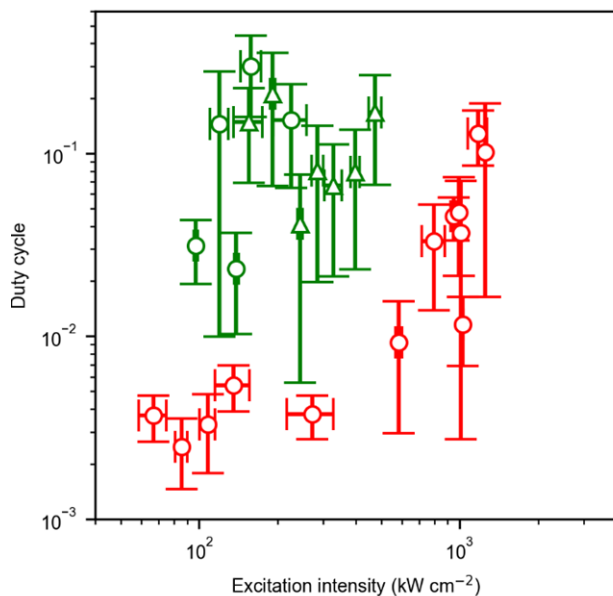


Fig. S7. Study of photoblinking by single ANPs under 1064 illumination. Duty cycle (average on time divided by sum of average on and average off times) versus 1064-nm excitation intensity of single 8% and 20% Tm³⁺ ANPs. Green triangle-8%, 10.2/4.0 nm, green circle-8%, 17.3/5.6 nm, red circle-20%, 17.4/2.6 nm. Error bars are derived from the standard deviations of the average on and average off times in the measured time traces.

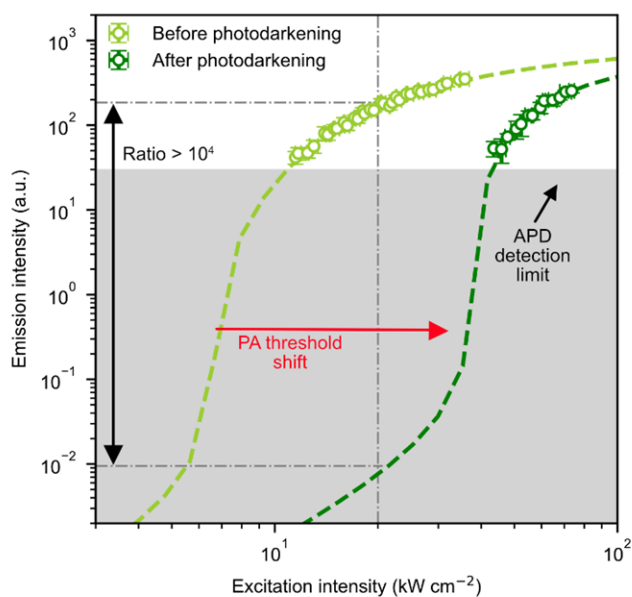


Fig. S14. Measurement (light and dark green circles) and differential rate equation model fitting (green dashed lines) of 800-nm emission intensity versus 1064-nm excitation intensity of a single 17.3/5.6 nm 8% Tm³⁺ (17 nm core with 5.6 nm shells) ANP before and after photodarkening. Error bars are standard deviations derived from four separate measurements on the same single ANP.

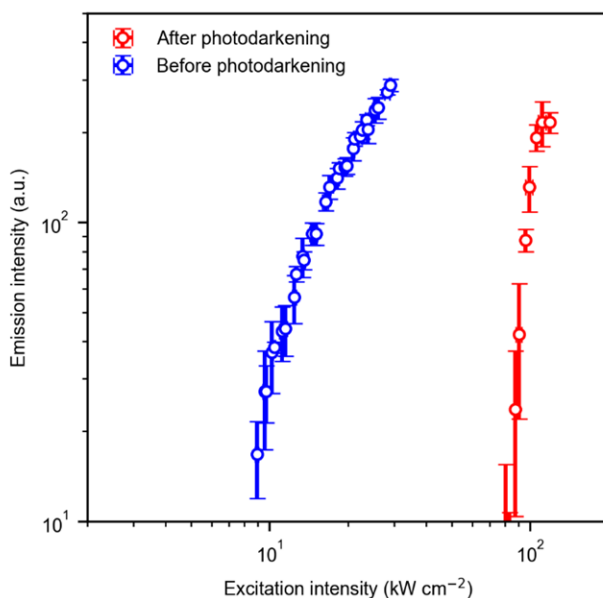


Fig. S15. Emission intensity (800 nm) versus excitation intensity (1064 nm) from a single 8% Tm³⁺ 17.3/5.6 nm core/shell ANP (different than the one in Fig. S14), before and after photodarkening. Error bars are standard deviations derived from four separate measurements on the same single ANP.

7- Supporting information: I might suggest carefully reviewing the caption of all tables and figures and be sure that there is no missed information: sample composition, excitation/emission wavelengths, or error bars. E.g., the excitation wavelength is missed Figure S3.

Our reply: We have reviewed all captions and done our best to add in all the information related to each measurement.

Changes to the manuscript. We have updated these figure captions to include missing details (changes underlined here, but not in the manuscript):

“Fig. S3. Time-Tagged Time-Resolved data of photodarkening in individual ANPs. Time steps in **a. b. and c.** are 2 ms, 2 ms, and 3 ms, respectively. Before 150 ms, the ANPs are in the on state, and photodarkened after 150 ms. Excitation wavelength and intensity are 1064 nm and 55 kW cm⁻², respectively. The single ANPs are from sample batch 3 in Table S1.”

8- Line 77: surface quenching does not play a major role. Could the authors include a Figure showing this statement or a reference?

Our reply: The absence of surface quenching is due to the presence of inert NaYF₄ shells, as has been observed in ANPs previously [Nature 589, 230 (2021); JPCC 125, 23976 (2021)]. Following the reviewer’s suggestion, we now include a plot of photobrightening recovery versus

inert shell thickness. No correlation is evident, revealing that energy or charge transfer between the surface and the Tm-doped core is not a key factor. For example, ANPs with ~2nm shells can have the same recovery percentage as those with ~4nm shells under identical 700-nm excitation conditions (same intensity and exposure time).

Changes to the manuscript: We have added **new SI Fig. S20**, showing that no correlation exists between photobrightening and shell thickness:

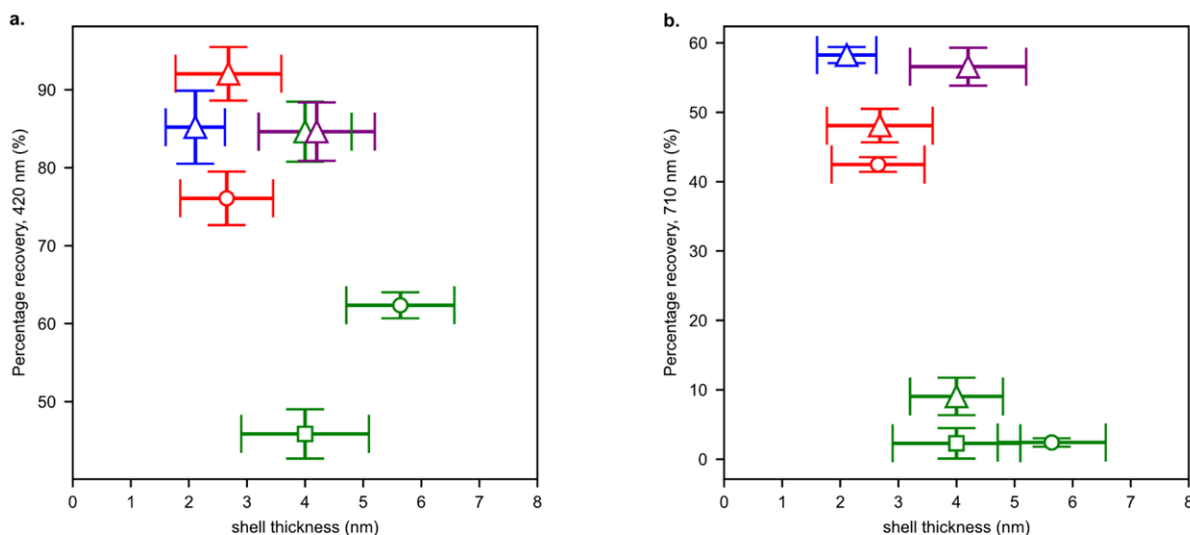


Fig. S20 | Photobrightening recovery percentage of various ANP ensemble film samples versus shell size.

Pumping (at 1064 nm) and photobrightening (at 420 nm in (a), 710 nm in (b)) intensities are 54 kW cm^{-2} and $277.87 \text{ kW cm}^{-2}$, respectively, for the triangle symbols. Pumping and photobrightening intensities are 33 kW cm^{-2} and 167 kW cm^{-2} , respectively, for the circle and square symbols. Exposure times for photodarkening and photobrightening are 1.2 and 1.3 sec, respectively. The laser power at the sample for 710 nm and for 420 nm is set to 0.6 mW. (Marker-Tm content, core/shell sizes); green triangle-8%, 10.2/4.0 nm, green circle-8%, 17.3/5.6 nm, green square-8%, 26.6/4.0 nm, red triangle-20%, 10.4/2.7 nm, red circle-20%, 17.4/2.6 nm, blue triangle -30%, 13.0/2.1 nm. Y-axis error bars are standard deviations of data points measured at the 4 different spots in the same ensemble sample. X-axis error bars: EM-determined variability in shell thickness for each batch, from Table S1.

Added discussion to the main text: "...the ability to photodarken is unrelated to factors affecting surface quenching (*i.e.*, surface ligands, water/O₂, or shell thickness). Similarly, PB performance shows no correlation to shell thickness (Fig. S20)"

9- The authors repeatedly say that the process is reversible (e.g., line 95) but the experiments about reversibility and stability over photodarkening/photo switching cycles are shown in the final part of the MS, and it is quite confusing because one is expecting to find this experiment. Probably, it is better to erase such a claim and leave it just when the experiment appears.

Our reply: We have moved most discussion of reversibility to the latter part of the main text to accompany Fig. 4. However, given its importance in the manuscript, it is still mentioned in the title and abstract, which we think is unavoidable.

Changes to the manuscript: We now introduce reversibility with Fig 4:

“We further investigated consistent and reversible photoswitching behavior by measuring the emission of single 8% Tm³⁺ core/shell ANPs while sequentially exposing them to repeated cycles of 1064 nm photodarkening followed by 700 nm photobrightening in ambient (Fig. 4a,c) and aqueous environments (Fig. S26).”

Referee #3 (Remarks to the Author):

Referee report ‘Indefinite and Bidirectional Near Infrared Nanocrystal Photoswitching’ by Lee et al.

The manuscript by Lee et al. describes experiments demonstrating reversible photoswitching in Tm³⁺-doped photo-avalanching NaYF₄ nanoparticles. Convincing experiments are reported and explained showing that at high 1064 nm excitation densities darkening occurs (ascribed to filling of defects) which induces non-radiative decay channels that shifts the onset for photo-avalanching to an order of magnitude higher excitation densities. In addition to reporting a highly robust photoswitchable nanoparticle, potential applications in high resolution imaging and patterning are demonstrated. The results are very interesting and the interpretation is generally sound and supported by experiments. The novelty is high and warrants publication in Nature. Still, there are points that are not clear (to me) and at some places a more thorough evaluation and discussion of the fidelity of the switching with these nanoparticles would be helpful.

Our reply: We thank for the Referee for the strong support for our work, noting it is “very interesting”, “sound”, and “the novelty is high and warrants publication in Nature”. We appreciate the careful reading, and we have addressed the reviewer’s comments here and in the revised manuscript.

In the comparison of photoswitchable probes the disadvantages of existing (bio-organic) probes are stressed vs. advantages of the present nanoparticles. A more balanced discussion would be appreciated. How harmful is ‘the phototoxic dose’ of UV or visible light compared to the NIR doses used for switching the NPs? What is the difference in time resolution, complexity of the imaging system and reproducibility/fidelity of the switching of the different probes? The organic probes, albeit not very stable, are successfully applied to realize high quality superresolution imaging. It may be good to also mention that reversible photoswitching in inorganic spin-crossover systems (including nanoparticles) has been demonstrated. Often in publications a one-sided view is presented on the disadvantages of existing methods to stress the importance of results without indicating potential drawbacks of the reported new system/method. This is not necessary, especially for highly innovative findings as reported in this manuscript.

Our reply: This is a good point, and we agree with the Referee. We have now updated, within the space limits of an Article, the comparison to be more balanced and less focused on the disadvantages of existing probes. Additionally, we have updated the text and SI to better describe potential trade-offs between different probes (see also the response to Ref. 1, comment 7), which includes new measurements of photoswitching temporal responses in ANPs (new Fig. S32). We also thank the reviewer for bringing up the spin-crossover systems; we now mention these along with citations.

In terms of phototoxic doses of UV or visible light, this depends on the living system being studied, exact wavelengths, and the nature of the laser (e.g., pulsed vs CW) and imaging conditions. This quickly gets more involved than we have space for, and so we can refer readers to existing comparisons of NIR to UV/visible.

Changes to the manuscript:

We have revised the Abstract to now read: "...These photoswitchable probes, including organic fluorophores and proteins, can be prone to photodegradation, and often operate in the ultraviolet (UV) or visible spectral regions. Colloidal inorganic nanoparticles^{8,10,11} can offer improved stability, but the ability to switch emission bidirectionally, particularly with near-infrared (NIR) light, has not been reported in such systems...."

We have added the following discussion to the main text: "As with most superresolution imaging techniques, there is a tradeoff between imaging speed and localization accuracy, which depends on the number of photons collected (Fig. 3c). While the INPALM experiments in Fig. 5 were optimized for high localization accuracy, faster frame rates are also possible, down to 15 ms exposure time for photobrightening of 34% of ANPs (Fig. S32). Specifically, we find that 91.4 ± 0.5 % of ANPs are turned off with an exposure time of 188 ms ($I_{\text{ex}, 1064 \text{ nm}} = 252 \text{ kW cm}^{-2}$, 1064 nm) and 33.8 ± 1.3 % of ANPs are turned on with an exposure time of 14.6 ms ($I_{\text{ex}, 700 \text{ nm}} = 240 \text{ kW cm}^{-2}$, 700 nm). For signal acquisition times, under the conditions of Fig. 5, the average number of detected photons for a single ANP is ~50,000 counts for 500 ms (similar to signal levels for fluorescent proteins in reported PALM experiments³¹), which enables localization accuracy of a few nm (see Figs. 5a,f). Better accuracy can then be achieved by collecting from the same particle for multiple frames if desired."

References to inorganic spin-crossover systems have been added to the Abstract (new refs 10 and 11).

The final line of the abstract section reports sub-Angstrom localization. I had a hard time understanding this claim – also later in the manuscripts where the results are presented that would show sub-Angstrom localization. It is not my field of expertise and it will be good if an expert with experience in this area comments on this claim and the experiments. A better description and explanation for non-experts of what sub-Angstrom localization means and how this follows from the experiments will be helpful. A first response is that at room temperature optical experiments are not capable of sub-Angstrom localization as this corresponds to a fraction of the size of a single atom/is less than half bond length between atoms.

Our reply: We appreciate this point, as the real-world meaning of the localization accuracy is not intuitive. It is important to keep in mind that localization accuracy is a calculated average, and this is technically different from resolution. These terms are often conflated (hence, the battles over the term "superresolution", which is typically localization accuracy), and we realize from the referee's question that we should do a better job of explaining our measurements and clarifying their meaning.

The INPALM localization (and, more generally, the centroid fitting-based method used in many superresolution techniques) is essentially reporting on the luminescence center of mass for a given particle, which is the 2D average position of detected photons emitted by a nanoparticle.

The Referee's intuition is correct that localization accuracies within this context can be prone to overinterpretation, so we want to make sure that mistake is not made here. As a value derived from an average (over both time and position), the localization accuracy is not conveying information about the position of a specific lanthanide ion or a specific chemical bond or the ability to resolve neighboring objects; it is just reporting on the precision with which one knows the statistical center of the emission spot, i.e., the luminescence center of mass.

For our ANPs, this centroid position will be related to, among other things, the spatial distribution of emitters (Tm ions) within the nanoparticle core. Because there is a finite acquisition time, the quantity also averages over any nanoparticle/emitter movements during that time, which can be both systematic (some types of instrument drift) and stochastic (e.g., Brownian motion). Similarly, for single fluorescent molecules or proteins, this quantity averages over all locations and orientations surveyed by the molecule during the signal acquisition time. These motions, such as arising from a floppy biomolecular linker between probe and target, are often significant and can complicate interpretation of localization accuracies. We have added a discussion, quoted below, of considerations in interpreting calculated localization accuracies in various types of experiments.

Importantly, the accuracy with which this center of mass can be located depends fundamentally upon the Poisson process of photon detection, so the most important variable is the total number of photons detected above background (see, e.g. Moerner, "New directions in single-molecule imaging and analysis," PNAS 104, 12596 (2007)).

Specifically:

$$localization\ accuracy \propto 1/\sqrt{N}$$

where N = the total number of detected photons. Therefore, collecting more photons leads to better localization accuracy. Because we know the imaging point spread function of our microscope, which is well-approximated by a Gaussian, the localization accuracy represents the standard error the mean centroid position determined from the 2D gaussian fit of the luminescence spot [Acc. Chem. Res. 38, 574 (2005)].

One can collect the photons either all at once over one extended exposure, or over multiple frames/exposures (taking on a small added cost of more detector read noise). The latter is useful when there are nanoparticles clustered close together (within a diffraction-limited spot), as is usually the case in single-molecule localization microscopies (SMLMs).

To demonstrate the potential power of our photostable probes for localization microscopy, we chose to collect $>10^8$ photons for an individual ANP in Fig. 5a-c. In contrast, the total photon budget for most photoswitchable molecules is $\sim 10^6$ or less, limited by permanent photobleaching, which thus limits their achievable localization accuracy [Angewandte Chemie International Edition 54, 8067 (2015)]. Said another way, existing probes simply cannot supply the same total number of photons as the photostable ANPs, and therefore cannot theoretically achieve the same localization accuracies. We note that the trade-off here is time: collecting more photons will generally mean you have to acquire signal for longer times. This will be acceptable for some applications, but not all. In our case, the longer collection times meant sample drift was a main challenge. To overcome this, we simultaneously measure

luminescence from 6 other particles on the sample as reference PSFs for registration, allowing us to correct the translational and rotational drifts.

Changes to the manuscript:

We have added the following discussion to the text:

“The high-precision localization is apparent in a 2D map of the localizations from more than 400 INPALM frames on a single ANP (Fig 5a). 8.90×10^7 total photons were collected from the ANP without signs of degradation, averaging nearly 200,000 per frame. This enables calculation of localization accuracies of 0.76 Å and 0.50 Å in the long and short axis, respectively (Fig. 5c and Methods). These values are considerably smaller than the nanocrystal diameters, or even those of individual unit cells²¹, which raises questions of how calculated localization accuracies should be interpreted in various experimental settings. For the Fig. 5f-I experiments in resolving components of a nanoparticle cluster, the calculated values are the spatial averages of detected photons⁵⁹, reflecting a luminescence centre of mass of emitting Tm^{3+} ions, flattened into 2 dimensions, for each ANP. Because our ANPs are reasonably symmetric (Fig. 2e), we observe that the localization centroid fittings are very close to the centers of ANPs determined by EM, within experimental limits (Fig. 5e). For possible bioimaging experiments in which targeted optical probes are serving as markers of biomolecule position, the localization accuracies are limited by factors such as the size and stiffness of the nanoparticle surface passivation and linker⁶⁰. For kinetic studies of biomolecule motion, tracking of positional changes is limited largely by the stiffness of the surface passivation and linker, which determine how faithfully probe motion tracks biomolecule motion⁶¹.”

An important aspect of photoswitching is that it is similar and reproducible for all (nano)probes. When looking at Figures 2c and 2d it seems that the threshold for bleaching varies between particles. In 2d we see a first partial drop in intensity already for the lowest excitation power (~80 kW/cm² – bleaching of 1 of the 4 nanocrystals?) and complete bleaching of all four at 110 kW/cm². In Figure 2c for a single nanoparticle the intensity continues to increase up to the highest power (164 kW/cm²) and then quenches completely. For the ensemble of four the particles the intensity switches on and off at the highest power (similar to what is also observed in Figure 2f). This aspect (explanation and implications) is not discussed clearly. Why do the particles behave different and how does this affect the fidelity in applications? For applications of reversible photoswitching the uniformity of switching thresholds and bleaching behavior is important and also turning off and on in the bleaching regime seems undesired.

Our reply: The referee raises a good question about inhomogeneous photoswitching behavior of single ANPs from the same batch and its influence on applications. This behavior is unsurprising in single particle experiments and is due primarily to: (1) the stochastic nature of blinking, most commonly under high 1064 nm excitation powers; (2) known heterogeneities in ANP PA threshold intensities for particles with thin or heterogeneous shell thicknesses, as well as other related minor atomic differences between NPs. For (1), under 1064 nm illumination (i.e., when no additional photobrightening beam is used), blinking only becomes common at high powers since releasing a trapped carrier from a colour centre using 1064 nm light is an inefficient multiphoton process. The blinking shown in Fig. 2f is occurring under 1064 nm pump intensity that is more than 25 times greater than the avalanching threshold, and is relatively

infrequent and short-lived. Higher pump intensities are needed for more regular blinking (e.g. Fig. S4) for intensities $\sim 100\times$ above the avalanching threshold. We note that in our experience, blinking is only observed under intense 1064 nm illumination, and *not during illumination with wavelengths used for photobrightening*.

For (2), inhomogeneities in particle structure result in variations in photon avalanche behavior of the single ANPs. Our group reported that single ANPs from the same batch show slightly different threshold intensities mainly due to the differences in the shell thicknesses [Kwock, et al., J. Phys. Chem. C. 125, 23976 (2021)]. These PA threshold intensity variations can translate into changes in excitation intensity needed to induce photodarkening (see new Figs. S12 and S13).

Finally, we note that we rarely observe spontaneous blinking during INPALM experiments. Also, because photobleaching is not an issue in these experiments, blinking is not particularly disruptive when it does occur, since as many frames as needed can be acquired for adequate signal.

Changes to the manuscript: We have clarified the laser intensities required for blinking to occurs:

“Photoblinking statistics from single ANPs under 1064 nm irradiance show that the blinking is intensity dependent, predominantly occurs at intensities 2 orders of magnitude higher than needed for ANP imaging, and...”

We have also added **new SI Figs. S12 and S13**, that show relationships between the PA threshold intensity and the excitation intensity needed to induce photodarkening for various ANP compositions:

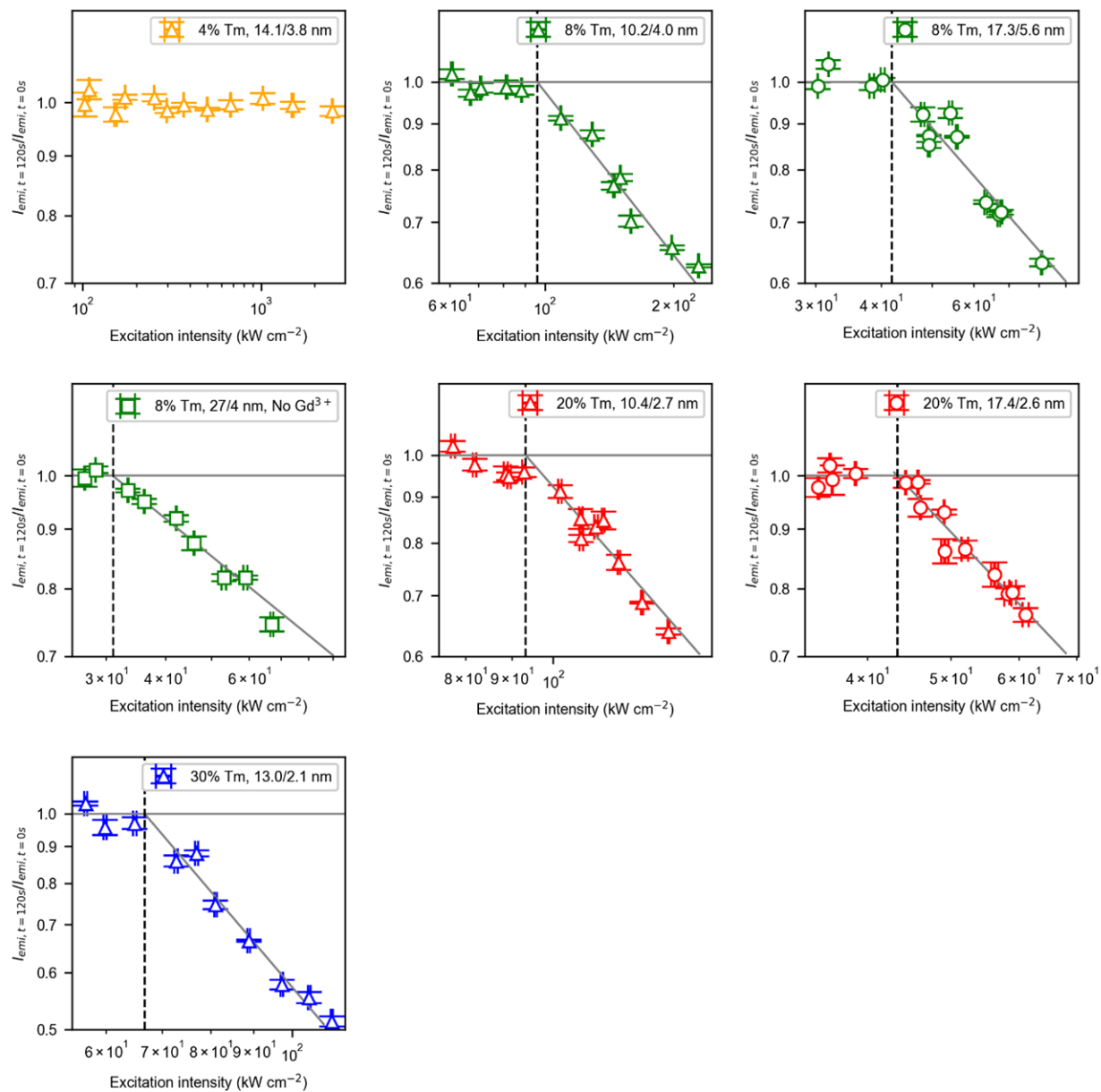


Fig. S12. Determination of photodarkening intensity I_{pd} for different ANP compositions. Each plot shows the ratio between 800 nm emission intensities (I_{emi}) at $t = 0$ and after 120 s of continuous 1064 nm exposure versus excitation intensity. These data allow us to define a photodarkening threshold intensity (I_{pd}) as the 1064 nm pump intensity where emission at $t = 120$ s decreases below the initial value. Error bars are standard deviations of 4 data points measured at the same spot within 0.4 s.

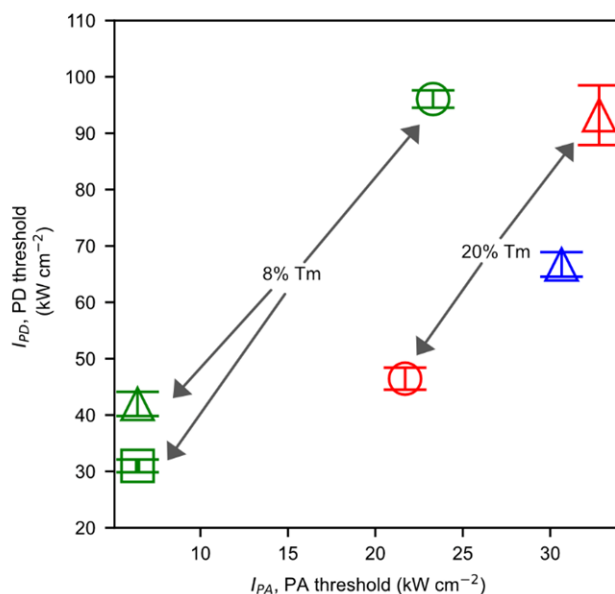


Fig. S13 | Photodarkening threshold versus pre-darkened photon avalanche threshold for various ANP compositions. Symbol definitions are the same as in Fig. S12 above. Error bars are standard deviations derived from the curve fitting of the power-dependent photodarkening analysis shown in Fig. S12.

The concern raised above is related to a point that is not addressed but is crucial for future applications: the switching relies on filling of defects. Defects are poorly understood in luminescent materials and it will be very difficult to reproducibly produce nanoparticles that all have the desired defect(s). The variation in behavior is probably caused by variations in the number, maybe type, and localization of defects, even for these well-controlled synthesized batches of ANPs. To develop a reliable product/technique for high resolution imaging based on the action and presence of defects seems difficult. For a future direction, it may be an option to intentionally introduce trapping centers for charges that have the same effect as a defect/color center (quench NIR Tm^{3+} emission). Very similar to afterglow materials where photo-ionization leads to controlled trapping of charge carriers (e.g. by $\text{Eu}^{2+}/\text{Dy}^{3+}$ or $\text{Eu}^{2+}/\text{Nd}^{3+}$ pairs) that can be thermally (or photo!) stimulated, a reversible and better controlled switching of ANPs can possibly be realized if trapping centers can be intentionally incorporated in known (low) concentrations.

Our response: We agree with the Referee about the importance of reproducible behavior. We note that we do not see poor reproducibility, even in our experience synthesizing these NPs by different methods and in different labs (either in Berkeley or Korea). So far, 100% of all ANPs we have studied are photoswitchable and operate reproducibly under a well-understood set of parameters.

To better quantify this, we have now investigated a collection of 112 individual ANPs and small clusters, showing that 112 of 112 photoswitch (both darken and brighten) under essentially identical illumination intensities – see **new Fig. S25** below. As touched on in the above

response, deterministic behavior is achieved under one range of illumination conditions, while stochastic behavior can be selected under a different set of excitation conditions. As discussed more below, we attribute this reproducibility to (i) the existence of an ample number of activatable point defects within every nanoparticle (e.g. traps such as F-centers, Frenkel defects, and Schottky defects [J. Phys. Chem. C 123, 11151 (2019)]; and (ii) mechanisms in which a very small subset of these (possibly just 1) need be activated to effectively photodarken the NP (see Supplementary Discussion).

“Point defect” is necessarily an ambiguous term, because it is currently impossible to clearly identify single-atom heterogeneities within a nanocrystal, but we wish to suggest a possibility: most faces of the core ANP surface are coated with oleate anions, which interact with Y^+ or Tm^+ cations on the surface (see Liu *et al.* "Three-dimensional controlled growth of monodisperse sub-50 nm heterogeneous nanocrystals." Nature Commun 7.1 (2016): 1-8). This suggests F are missing in some unit cells at the core surface. These F- vacancies can be filled during shell growth, as the same reagents for generating F- as during core growth are present.

We suspect the point defects arise when even a small number of unit cells at the core/shell interface remain without a full complement of F atoms, as $NaYF_3^+$ or $NaTmF_3^+$. Based on our experience with either TFA- or oleate-based shell growth, and many reports in the literature, these appear to be inherent to $NaYF_4$ core/shell syntheses, and we believe these interfacial Y^+ or Tm^+ ions could serve as point defects as described in the manuscript. Nevertheless, we cannot currently identify these (or other possible) point defects by TEM or other experiments.

As also discussed in the response to the previous comment, The NP-to-NP variability in Fig 2 photodarkening is well within the standard range for analyzing single particles as seen for single ANP analysis of avalanching thresholds [Kwock, et al., J. Phys. Chem. C. 125, 23976 (2021)], and single nanoparticle characterization generally.

Finally, we thank the referee for the excellent suggestion about intentionally engineering other traps/defects with desirable and controllable properties, which could further expand performance. We feel this could be a very exciting future direction.

Changes to the manuscript:

New figure S25 has been added, demonstrating that 100% of measured ANPs are photoswitchable (see below).

The following has been **added to the main text** regarding the need for new characterization tools to better understand and address atomic-scale heterogeneity in these materials:

“...detailed identification of all transitions involved in the current system will require further in-depth study and development of more sensitive characterization tools, as it remains challenging for current methods to identify single-atom heterogeneities that can have major effects on ANP optical properties.”

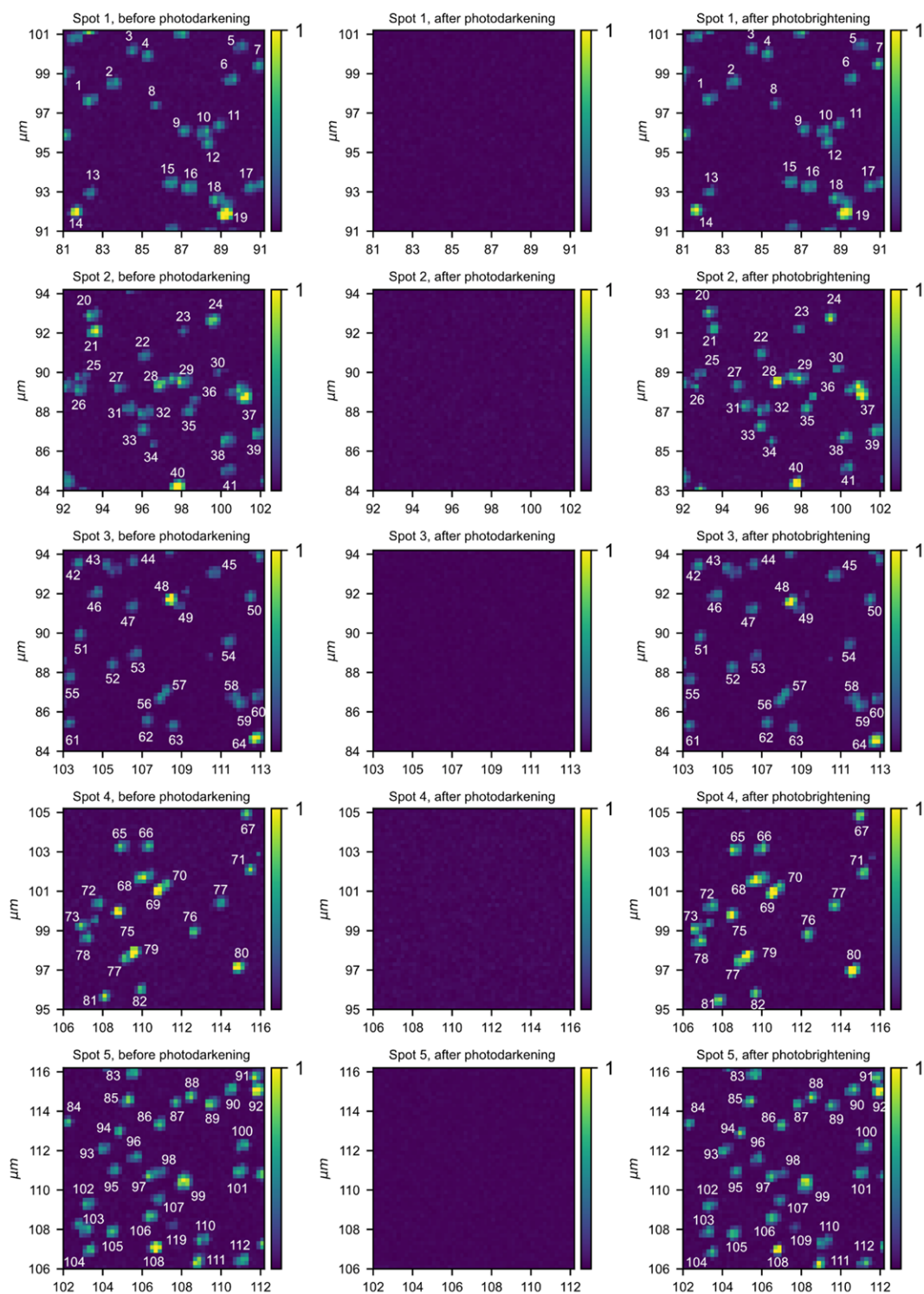


Fig. S25 | Consistent photoswitching of single ANPs. Single-particle imaging of ANPs before photodarkening (*left*), after photodarkening (*middle*), and after photobrightening (*right*), of 112 nanoparticles (mostly singles, with a few dimers or small clusters). Rows are different regions of the sample. The imaging and photodarkening intensities (1064 nm) of 8% Tm 17.4/2.6 nm ANPs are 28 kW cm^{-2} and 140 kW cm^{-2} , respectively. The photobrightening condition is 400 nm irradiation at 25 kW cm^{-2} . Photoswitching is observed in 112/112 ANPs.

In Figure 3 convincing evidence is presented for the on and off of switching of the photo-avalanching. Still, the figure raises some (minor) questions.

- The error bars in Figure 3b are very small and this would suggest that the structure observed (e.g. dip at 685 nm and 710 nm in the grey curve) is real. I assume this is not the case – a structureless broad band is expected for the colour centre to conduction band absorption. The error bars should be reconsidered.

Our reply: We thank the referee for catching this. Motivated by this question, we performed our photobrightening (PB) studies again under improved experimental conditions (details below) and have clearer data. Notable peaks in the PB excitation spectrum are now clearly observed at both 800 nm and 700 nm, corresponding directly to $\text{Tm}^{3+} \ ^3\text{H}_4$ and $\ ^3\text{F}_{2,3}$ transitions, as well as for wavelengths <530 nm. There is also a sharp dip at 450 nm, corresponding to higher-energy Tm^{3+} transitions, which can enhance upconversion to UV and induce further photodarkening rather than luminescence recovery (see also **new SI Fig. S17**). Fig. 3c now shows that for 700 nm pumping, luminescence recovery from PB can be quantitative at higher intensities.

We have updated our Methods section, included the new PB activity spectrum as Fig 3b, and updated our discussion in the main text.

Changes to the Manuscript: Fig. 3 has now been updated (see below) to include these corrected measurements. Fig. S14 has been removed and replaced by **new Fig. S16** showing PB activity spectra for different ANP compositions.

New Discussion: “The PB activity spectra (Fig. 3b, S16) include broad features at wavelengths <530 nm attributable to colour centres, as well as peaks at both 800 nm and 700 nm, which correspond directly to $\text{Tm}^{3+} \ ^3\text{H}_4$ and $\ ^3\text{F}_{2,3}$ transitions. There is a sharp dip in the activity spectrum at 450 nm, corresponding to higher-energy $\ ^1\text{G}_4$ and $\ ^1\text{D}_2$ Tm^{3+} transitions, which may enhance upconversion to UV manifolds to induce further photodarkening rather than luminescence recovery (see also Fig. S17). Increasing the irradiation intensity also enhances PB (Fig. 3c), so that nearly full luminescence recovery can be achieved in a few seconds with 700 nm light (Fig. 3c).”

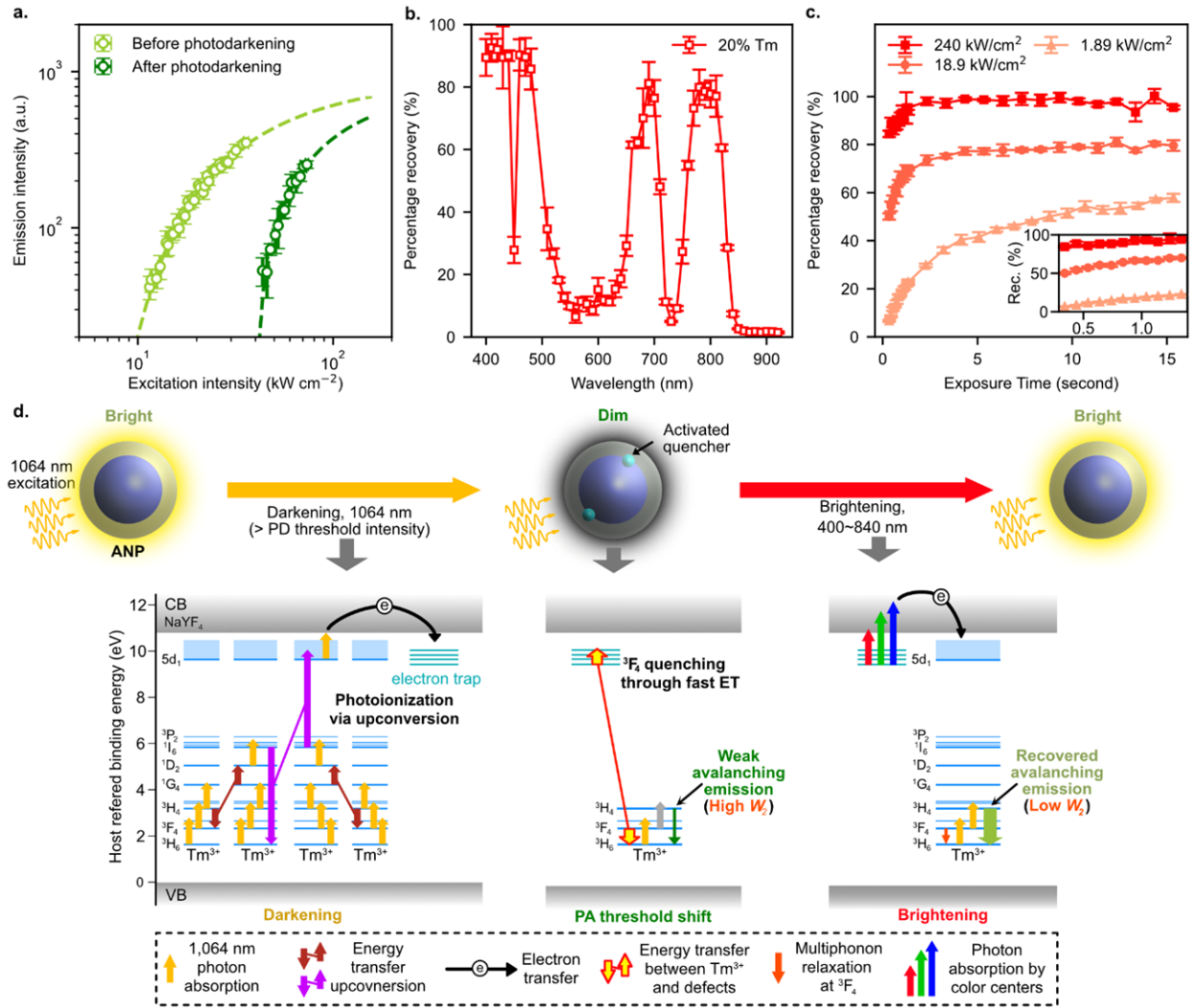


Fig. 3 | ANP Photobrightening and photodarkening. **a**, Emission intensity (800 nm) versus excitation intensity (1064 nm) of a single 8% Tm^{3+} 17.3/5.6 nm (core diameter/shell thickness) ANP before and after photodarkening. Switching between bright and dim states in ANPs is induced by shifting of the PA threshold, using 1064 nm light to darken and wavelengths ≤ 840 nm to brighten. **b,c**, Photobrightening recovery of a photodarkened 20% Tm^{3+} 10.4/2.7 nm core/shell ANP ensemble film sample versus **(b)** irradiation wavelength or **(c)** exposure time. The photobrightening laser power at the sample is set to 600 μW for all wavelengths in **b**, which corresponds to 90 kW cm^{-2} at 700 nm. Photobrightening exposure times are 1.3 sec in **b**. Photodarkening exposure times are 1.2 sec in **b** and **c**. 1064-nm laser intensities for luminescence pumping and photodarkening are 69 and 345 kW cm^{-2} , respectively, in **b** and **c**. 700-nm irradiation intensities for photobrightening are shown in the legend in **c**. The inset in **c** is a magnified plot in the exposure-time range from 340 ms to 1340 ms. Error bars are standard deviations of data points measured at the 4 different spots in the same ensemble sample. **d**, Potential mechanistic pathways for photodarkening and photobrightening in ANPs. CB: conduction band, VB: valence band, W_2 : $^3\text{F}_4$ relaxation rate.

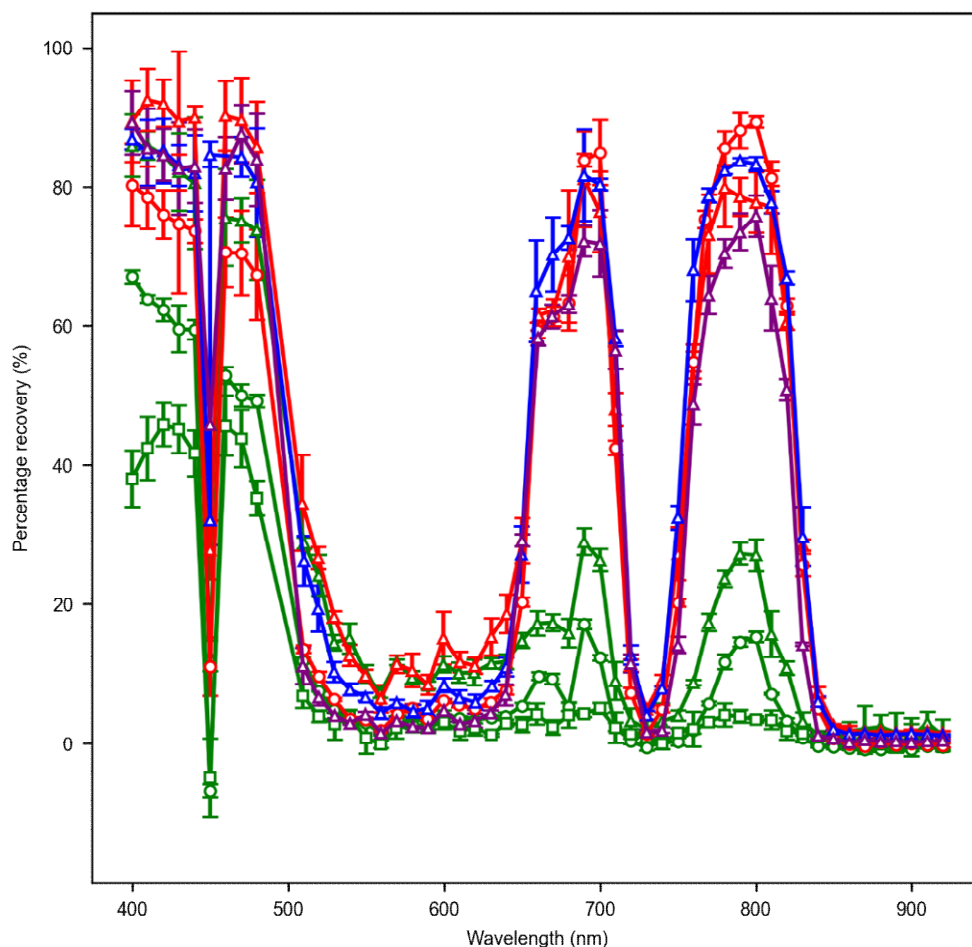


Fig. S16. Photobrightening recovery of darkened ANP films as a function of irradiation wavelength. The Tm contents for green, red, blue, and purple markers are 8%, 20%, 30%, and 100%. Excitation (1064 nm) and photobrightening intensities are 54 kW cm^{-2} and $277.87 \text{ kW cm}^{-2}$, respectively, for green triangles (10.2/4 nm), red triangles (10.4/2.7), blue triangles (13.0/2.1 nm), and purple triangles (15.8/4.2 nm). Excitation (1064 nm) and photobrightening intensities are 33 kW cm^{-2} and 167 kW cm^{-2} , respectively, for green circles (17.3/5.6 nm), green squares (26.6/4.0 nm), and red circles (17.5/2.7 nm). Exposure times for photodarkening and photobrightening are 1.2 and 1.3 s, respectively. Error bars are standard deviations of data points measured at the 4 different spots in the same ensemble sample.

New Methods: “For measuring the photobrightening activity spectra, we used a reflective objective lens (Thorlabs, LMM40X-P01), which does not show significant chromatic aberration, and selected filters (Thorlabs, FESH700 and FESH750) to block residual light from the 800 nm pump source.”

- Comparing Figure 3b with Figure S14 shows a very different wavelength onset for the photobrightening efficiency (onset at 800 nm vs. 600 nm). In the caption of S14 the lower photobrightening efficiency is mentioned/explained but the difference in spectral shape/onset is not discussed.

Our reply: The difference was primarily due to the use of different power densities in those figures. However, as detailed above, we have performed the wavelength-dependent

measurement again under improved experimental conditions and presented them in a more comparable format.

Changes to the manuscript: See updated Fig. 3 and new Fig. S16 (both above).

- In Figure 3c the recovery seems almost immediate – already after 1s 50% (for 107 kW/cm²) or 90% (for 213 kW/cm²). It would be interesting to follow the recovery dynamics on a shorter sub-s time scale. Has this been attempted?

Our reply: Following this suggestion, we have now performed time-dependent photoswitching experiments with exposure times below 1 s (new Fig. 3c). The results show that $91.4 \pm 0.5\%$ of particles are turned off with the exposure time of 188 ms ($I_{\text{ex},1064 \text{ nm}} = 252 \text{ kW cm}^{-2}$, 1064 nm) and $33.8 \pm 1.3\%$ of particles are turned on with the exposure time of 14.6 ms ($I_{\text{ex},700 \text{ nm}} = 240 \text{ kW cm}^{-2}$, 700 nm). Considering that less than one emitter per diffraction limited spot is ideal for PALM-type imaging to prevent overlapping of PSFs, the measured photoactivation probability of 33.8% is high, meaning that exposure time or irradiation intensity can be further reduced for optimized INPALM performance.

Changes to the manuscript: Fig. 3c has now been updated and Fig. S32 has been added to include shorter recovery time down to 14.6 ms.

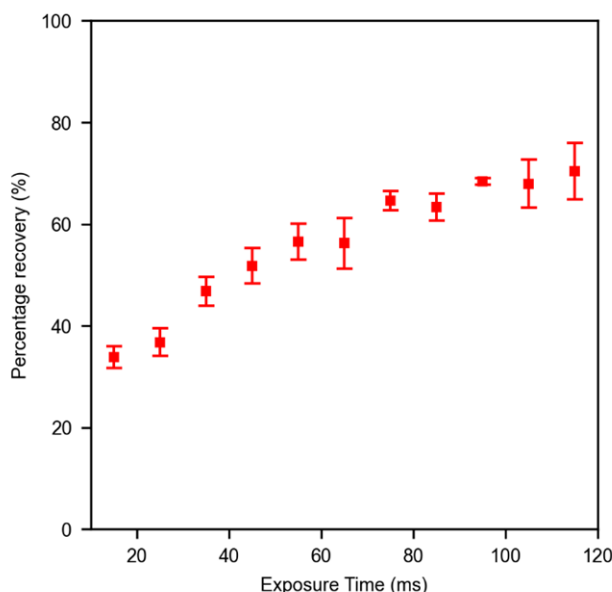


Fig. S32. Photobrightening recovery as a function of exposure time. Photodarkened regions of a film of 20% Tm³⁺ 10.4/2.7 nm core diameter/shell thickness ANPs were exposed to a 700 nm photobrightening beam for short exposure times, and the luminescence recovery was measured as a function of exposure time. The 1064 nm pumping and photodarkening intensities are 51 kW cm⁻² and 252 kW cm⁻², respectively. 700 nm photobrightening excitation intensity is 240 kW cm⁻². The photodarkening exposure time is 188 ms. Error bars are standard deviations of data points measured at the 4 different spots in the same ensemble sample.

We have also **added brief discussion of faster photoswitching**: “While the INPALM experiments in Fig. 5 were optimized for high localization accuracy, faster frame rates are also

possible, down to 15 ms exposure time for photobrightening of 34% of ANPs (Fig. S32). Specifically, we find that 91.4 ± 0.5 % of ANPs are turned off with an exposure time of 188 ms ($I_{\text{ex}, 1064 \text{ nm}} = 252 \text{ kW cm}^{-2}$, 1064 nm) and 33.8 ± 1.3 % of ANPs are turned on with an exposure time of 14.6 ms ($I_{\text{ex}, 700 \text{ nm}} = 240 \text{ kW cm}^{-2}$, 700 nm)."

The 4% Tm-doped NCs do not show the same photodarkening (Figure S10). It is interesting to see that the intensity does drop for higher excitation densities – for 70 kW/cm² a two times higher intensity is observed than for 2.3 MW/cm². It would be good to discuss this gradual decrease of emission intensity upon increasing the laser power. In relation with this, the manuscript often makes a parallel of the present mechanism for photodarkening with what is observed in Tm-doped glasses/fibers. At the same time it is inferred that photo-avalanching is crucial for photodarkening 'When doping is increased to 8% Tm³⁺, the NPs exhibit pronounced PA and noticeable photodarkening...' while it not observed for the 4% Tm³⁺ NPs (without PA) (lines 69-75). Darkening/quenching is observed in glasses/fibers but I do not think that in these glasses/fibers PA occurs or does it? It would help if the authors clearly discuss if and why PA is required for photodarkening or if it can also occur without PA.

Our reply: We are grateful for the reviewer's careful analysis of our figures, and we see our original data presentation was confusing, which we have now fixed. There is no decrease in emission for higher pump powers; in fact, it increases as might be expected. Previously, for Fig. S10, we had normalized the PL signals for each power to the $t=0$ value, and then offset the PL timetraces so they did not overlap. When offsetting the timetraces, we had put the lowest power excitation on top and the highest on the bottom. We have now modified Fig S10 to plot the each normalized PL signal on its own set of axes (see **new Fig. S10** below).

Darkening has indeed been observed in Tm³⁺ glasses/fibers, and avalanching processes – both lasing and PA – are known to occur in these bulk materials, although a number of these reports do not include explicit discussion of PA. Our data and models suggest that optical avalanching processes, which include both lasing and photon avalanching as distinct types of avalanching behaviors, effectively amplify the photodarkening effect. Notably, nearly all previous reports of PD in Tm-doped material were in fiber lasers and laser amplifiers. And in our nanoparticles, photon avalanching is of course present when pumped beyond threshold. Avalanching processes lead to a large build-up of energy within the system. In both the fiber lasers/amplifiers and our ANPs, this enhances the probability of upconversion to blue and UV wavelengths, which can activate colour centres. It is worth noting that in Tm-doped fibers, photon avalanching has commonly been used as the mechanism for efficiently achieving the population inversion necessary for lasing (thereby further linking PA and lasing in these systems).

The quenching colour centers activated upon photodarkening increase the threshold intensity for achieving photon avalanching or lasing, and the generation rate of such quenchers is shown to have a nonlinear dependence on excitation power [e.g., Optics Letters 20, pg. 2484-2486, 1995]. We do believe that some select photodarkening cases in lanthanide-doped fibers may have occurred without an optical avalanching process but when pumped with exceptionally high power densities. However, we so far have not been able to induce photodarkening in non-PA-supporting Ln-doped nanoparticles. A summary of these trends and their supporting evidence can be found in a recent review article on photodarkening in rare-earth-doped fibers (Sun, et al,

“Progress and Summary of Photodarkening in Rare Earth Doped Fiber,” *Appl. Sci.* 11, 10386 (2021)), which we have now included in our reference list.

Changes to the manuscript:

The above discussion was **added to the SI as the Supplementary Discussion** titled “Relationship between avalanching processes and photodarkening in Ln-doped materials”.

We have **updated Fig. S10** for clarity, plotting each excitation power in its own set of axes.

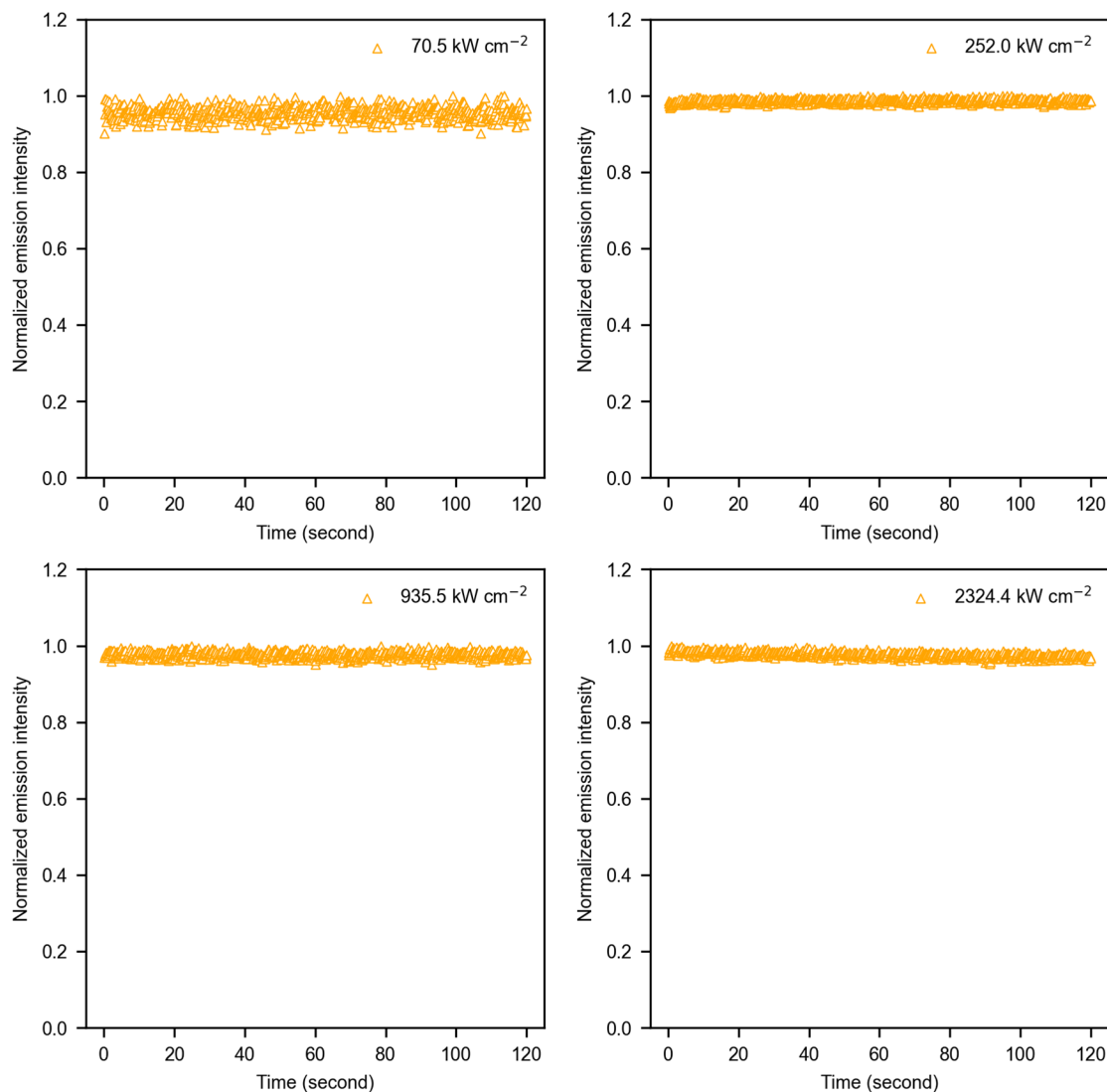


Fig. S10. Time dependence of 800 nm emission intensity at various 1064-nm excitation intensities, for 4% 14.3/3.7 nm (core diameter/shell thickness) Tm³⁺ nanoparticle ensemble films. UCNPs with Tm³⁺ doping $\leq 4\%$ do not show avalanching behaviour (ref. 23 in main text), and no photodarkening here.

The mechanism for photodarkening, although not completely proven, is plausible. It would be interesting to record the luminescence decay curves of the 3F_4 emission directly after darkening to confirm the higher W2NR (faster and non-exponential decay expected, converting to longer and more single exponential upon photobrightening). This would also provide independent quantitative information on W2NR. In addition, it would be a relatively simple experiment to see if the colour centres disappear upon heating. Optical stimulation (emptying) of traps around 800 nm is observed and based on this onset wavelength it can be expected thermal emptying at temperatures below 100°C is possible (note that the thermal trap depth is always much smaller than the optical trap depth). Recovering the PA at a low power threshold after a heat treatment would provide further support for trapped charge/colour centre model presented in Figure 3d.

Our reply: We share the reviewer's interest in unraveling the photoswitching mechanism and appreciate these suggestions for additional experiments. While some of these are out of our reach given our experimental set-ups, we have now performed: (a) time-resolved measurements of luminescence from the 3F_4 state of Tm^{3+} in both bright and photodarkened ANPs (**new SI Fig. S24**); and (b) thermal-only brightening measurements, in which emission recovery from a film of 20%-Tm ANPs is measured as a function of external heating temperature, to investigate trap depths associated with the photodarkening process (Fig. R1, below).

In our direct measurements of ANP Tm^{3+} 3F_4 lifetimes before and after photodarkening (new Fig. S24), we observe a reduction in lifetime by more than a factor of 2 (although this limited by residual emission from non-photodarkened ANPs within the film), providing direct evidence of nonradiative quenching of the 3F_4 state upon photodarkening. The lifetime curve from the photodarkened region of the film includes contributions from non-photodarkened ANPs within the film; thus photodarkened ANPs have a 3F_4 lifetime shorter than the measured 3.35 ms, consistent with DRE analysis.

For thermal brightening studies, as the referee suggests, we observe a clear increase in luminescence from photodarkened regions as temperature increases, with a noticeable acceleration in thermal recovery at temperatures above 90° C. Our sample heating stage is limited to a maximum temp of 113° C, and so we are unable to reach a peak in the PB-vs-temperature plot. This sets a lower limit on trap depth energy, implying that a trap depth is >1 eV [Reddy & Rao, 1984. High-temperature X-ray irradiation induced thermoluminescence and half-life calculations in NaYF₄ polycrystalline samples. *Crystal Research and Technology*, 19(10), pp.1399-1403]. In these measurements, a heating stage warms the photodarkened ANP sample to a given temperature *without any optical irradiation* incident on the sample. Once the temperature equilibrates (5 minutes), the 800 nm luminescence from photodarkened regions is probed using modest 1064nm irradiation. Taken together with the new wavelength-dependent photobrightening measurements discussed above, these experiments reveal that heating or irradiation with wavelengths below 530 nm effectively recovers photodarkened luminescence. The results match well with the TSL spectra of X-ray-irradiated NaYF₄ nanocrystals [J. Phys. Chem. C 124, 24940 (2020); Cryst. Res. Technol. 19, 1399 (1984)] and the previously noted optical absorption bands of Tm-doped fibers photodarkened under ~1.1 um irradiation [Optics Letters 21, 348-350 (1996); Optics Letters 20, 2195-2197 (1995); Optics letters 20, 2484-2486 (1995); Optics Letters 18, 799-801 (1993)].

Changes to the manuscript: Our 3F_4 state lifetime measurements have been added as new Fig. S24:

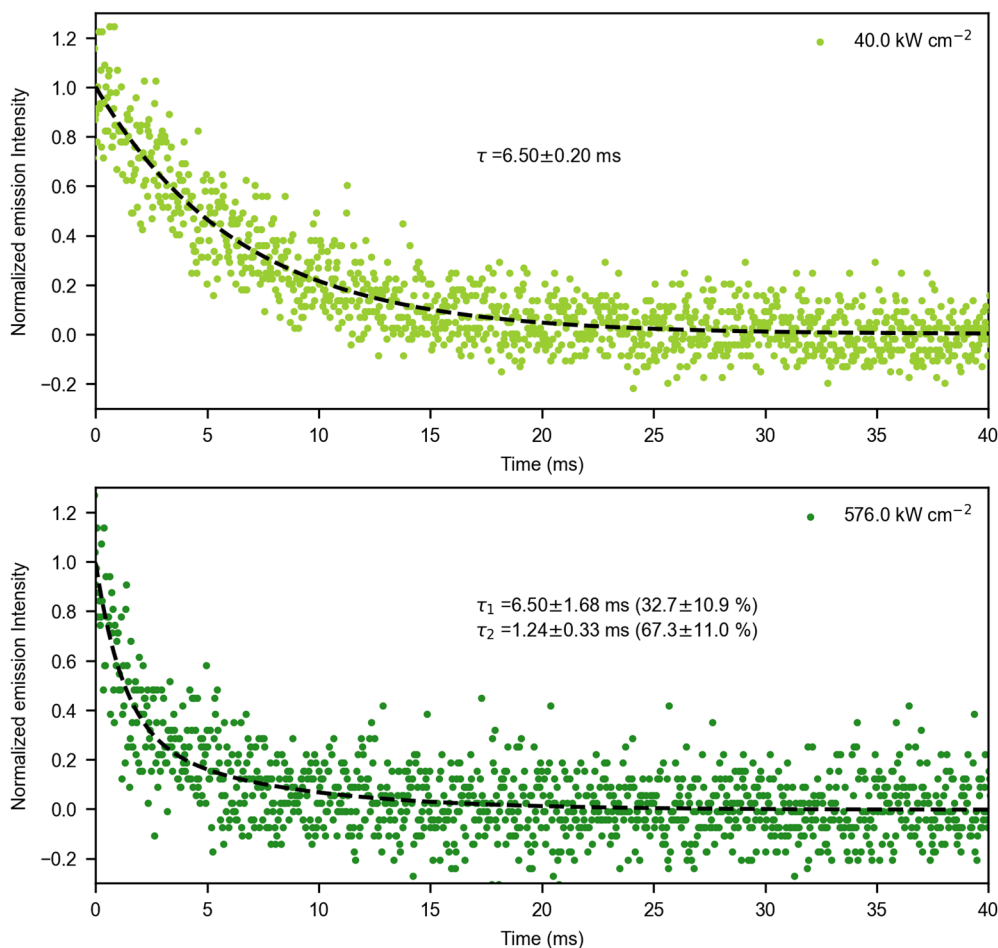


Fig. S24. Time-resolved photoluminescence of IR emission from the $Tm^{3+} \ ^3F_4 \rightarrow \ ^3H_6$ transition in an ensemble film of 8% Tm^{3+} 17.3/5.6 nm core/shell ANPs under 1064 nm excitation before (*top*) and after (*bottom*) photodarkening. The black dashed lines are fits of exponential functions to the data. Emission wavelengths >1750 nm were selected with a long-pass filter and collected using a superconducting nanowire single-photon detector (SNSPD; Single Quantum EOS 6). A single exponential decay is observed in the non-photodarkened ANPs (*top* panel). The lifetime curve from the photodarkened region (*bottom* panel) includes contributions from non-photodarkened ANPs within the film, resulting in a biexponential decay. The biexponential decay analysis suggests approximately two thirds of the ANPs are photodarkened in the measured region, and the photodarkened ANPs have a 3F_4 lifetime that is 5.2 times shorter, consistent with the rate equation analysis and fits in Fig. 3a.

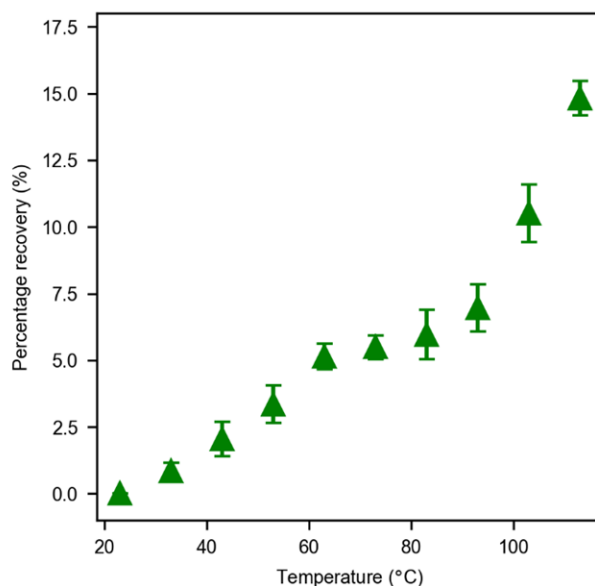


Fig. S23 | Thermal luminescence recovery of photodarkened ANP films. Recovery was accomplished with external heating only (5 min equilibration at each temperature), rather than by photobrightening. Before and after heating, 20% Tm³⁺ 10.4/2.7 nm films were photodarkened and probed with 1064 nm intensities of 320 kW cm⁻² and 64 kW cm⁻², respectively. Percentage recovery is defined as the ratio of the recovered luminescence intensity after temperature increase to the reduced luminescence intensity after initial photodarkening. Error bars are standard deviations of data points measured at the 4 different spots in the same film.

We have also added a brief discussion of thermal luminescence recovery: “Measurements of thermal luminescence recovery in photodarkened ANPs (Fig. S23) are consistent with trap depth energies suggested by the PB activity spectra (Figs. 3b, S16).”

The filling of traps via the 1I₆ level is suggested. This is a valid mechanism. Instead of showing emission lines around 490 nm and 510 nm as evidence for 1I₆ emission, it would be more convincing to show shorter wavelength UV emission. The spectra do show clear evidence for 1D₂ emission around 360 nm. Evidence for 1I₆ emission would be obtained from emission around 290 nm (to 3H₆) and 350 nm (to 3F₄). The reason for looking for UV emission is that the weak lines around 490 and 510 nm could also originate from 1G₄ emission to higher crystal field components of the 3H₆ ground state. The power dependence of the intensities (Figure S17) seems to be the same for the 490 nm and 510 nm emission as for the 450-470 nm 1G₄ emission lines while a stronger power dependence would be expected for 1I₆ emission lines (higher order upconversion process). In the inset of Figure S16 one can observe a weak 350 nm emission that I would assign to 1I₆-3F₄ emission.

Our reply: These are great points and suggestions. We have now performed additional UV luminescence measurements, showing a clear emission peak around 345 nm as well as the set of peaks near 365 nm (new Fig. S21), providing further evidence for 1I₆ emission. Notably, power dependence of the 345 nm emission is more nonlinear than that of 365 nm emission, suggesting the 345 nm emission originates from a higher energy state.

Changes to the manuscript: We have added a new UV luminescence plot in new Fig. S21.

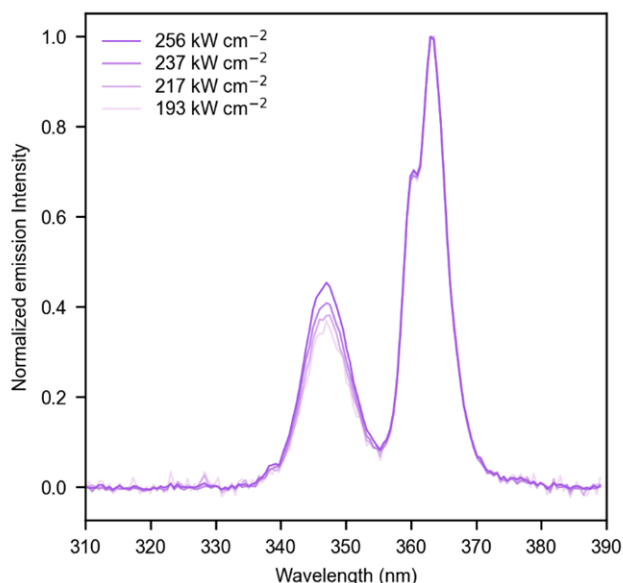


Fig. S21. Ultraviolet emission spectra of 8% Tm^{3+} 17.3/5.6 nm ANP ensembles at 1064-nm excitation intensities above photodarkening threshold intensity (I_{PD}). Spectra are normalized to the 365 nm peak. The 345 nm and 365 nm emission peaks are attributed to the excited-state transitions from the $^1\text{I}_6$ state to the $^3\text{F}_4$ state and the $^1\text{D}_2$ state to the $^3\text{H}_6$ state, respectively.

In figure 3d the position of the 5d1 level is too high in energy. The lowest 5d states for Tm^{3+} in fluorides are around 160 nm corresponding to 7.75 eV above the $^3\text{H}_6$ ground state. In the diagrams in Figure 3d this would place the 5d1 state around 9.5 eV above the valence band edge (not 11.3 eV). The lower energy 5d1 state would also allow for 1D_2 - 1I_6 energy transfer to populate the 5d1 state. One also has to be careful to assume that photoionization results in expelling an electron from Tm^{3+} to give Tm^{4+} + a conduction band electron as suggested here. Although harder to depict in a band diagram as Figure 3d, conversion to Tm^{2+} + hole in the valence band that can be trapped to form a colour centre is also possible. The two processes are completely analogous. It is good to realize that Tm^{4+} is notoriously unstable while Tm^{2+} is a well-known valence state for Tm. Overall, for the manuscript this is not an important point as the nature of the trap/defect/colour centre is not known.

Our reply: We agree that the energy state of the 5d1 level should be lower than what was previously sketched and a more accurate representation is needed. The previous value was off due to a conflation of values that occurred during assembly of the original figure. To determine the energetic position of the 5d1 state, we referenced emission from the 5d-4f transition of Ce^{3+} ions in hexagonal NaYF_4 crystals [Journal of Alloys and Compounds 658, 952 (2016)]. The 5d-4f transition has an absorption peak at 259 nm (4.78 eV) and an emission peak at 309 nm (4.01 eV). Considering that the 5d states of lanthanides in the same host material are located at a similar position [Journal of Luminescence 128, 578 (2008)], and the energy difference between the ground states of Ce^{3+} and Tm^{3+} in NaYF_4 is approximately 4 eV [Journal of Luminescence 136, 122 (2013)], the extrapolated value matches well with the referee's suggestion for the position of the Tm^{3+} 5d1 state. In addition, the energy gap of hexagonal NaYF_4 in our figure has

now been updated based on a Tauc plot for hexagonal NaYF₄, yielding the band gap of 10.8 eV. This energy gap is similar to bandgap structures of hexagonal NaYF₄ calculated by DFT simulation [J. Phys. Chem. C 2016, 120, 18858–18870, Optical Materials 99 (2020) 109529].

We also agree with the referee about the possible generation of Tm²⁺ upon photodarkening instead of Tm⁴⁺, and that the hole centres can also act as quenching colour centres. We have updated the manuscript text to note this additional possibility, and have added Fig. S22 to schematically represent another possible photodarkening mechanism based on a Tm²⁺ intermediate.

Changes to the manuscript: We have **updated the energy level diagram in Fig. 3** with..... We have also **revised the text** to note the possible Tm²⁺-related routes for colour centre activation:

“Taken together, our models and observations are consistent with a photodarkening scheme in which extended ANP photoexcitation populates high-lying Tm³⁺ excited states (e.g., ¹I₆ and 5d¹4f¹¹ states⁴⁸; as in Fig. S21) and ultimately results in the transfer of an electron (hole) from the Tm³⁺ ion or the neighboring atoms to the NaYF₄ conduction (valence) band (Fig. 3d, Fig. S22), which is then trapped in a defect, activating a colour centre that can quench the Tm³⁺ ³F₄-to-ground-state transition.”

and **added new Fig. S22** that includes a schematic representation of a hole-mediated trap activation route.

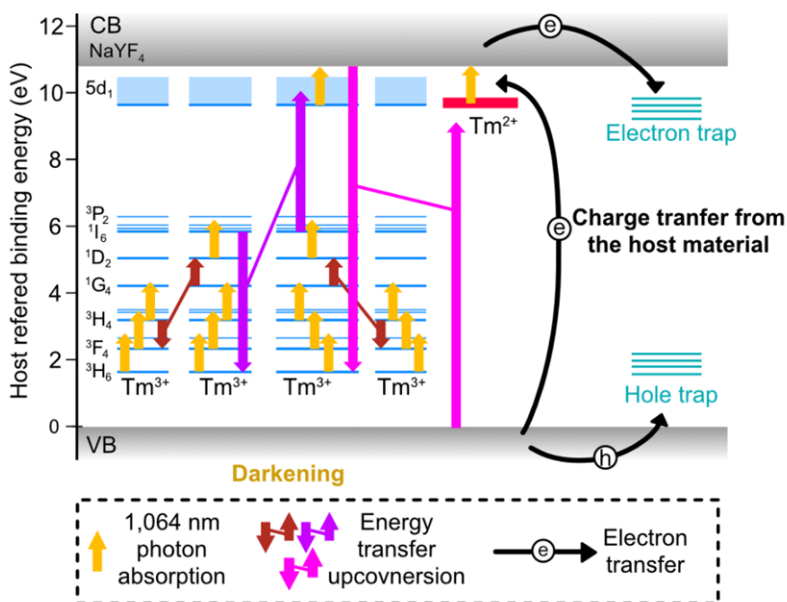


Fig. S22. Potential mechanistic pathways for photodarkening and photobrightening in ANPs via charge transfer from the host material, resulting in Tm²⁺ as an intermediate. CB: conduction band, VB: valence band, W₂: ³F₄ relaxation rate. Hole traps in addition to electron traps can be produced through the charge transfer process, which can both potentially quench the ³F₄ excited state in Tm ions.

In conclusion, this is another highly interesting manuscript from the Schuck-group and the team of collaborating groups showing the potential of photon avalanching in nanoparticles. I strongly support publication in Nature after the authors have considered my comments and questions.

Our Reply: We sincerely thank the Referee and appreciate their insight and attention to detail in helping us to improve our manuscript.

Reviewer Reports on the First Revision:

Referees' comments:

Referee #1 (Remarks to the Author):

In the revised manuscript, the authors have carried out a comprehensive set of experiments. I appreciate their efforts to improve the technical part of the study. However, despite these efforts, many of my concerns remain unaddressed. While I understand that characterizing the change in valence state can be challenging, I believe that the demonstration of these photoswitchable nanocrystals for potential STORM and PALM imaging is technically flawed in my opinion.

1) On page 4, the authors claim that "photo blinking statistics... stochastic optical reconstruction microscopy (STORM) imaging." However, the high excitation power required for photoblinking is not ideal for STORM-based bioimaging due to its potential phototoxicity. In addition, the slow photoblinking rate of lanthanide nanoprobe would significantly limit imaging speed, making this STORM technique less advantageous than STED. In fact, similar control of photobrightening and photodarkening (depletion) can be achieved in STED microscopy using lanthanide nanocrystals.

2) In Fig. 5, the authors demonstrate avalanching nanocrystals as probes for INPALM. PALM relies on random activation of fluorophores to spatially resolve molecular details. However, no evidence is provided to show how the avalanching nanocrystals can randomly activate in space, which is a fundamental requirement for PALM imaging. In fact, Fig. S25 shows consistent photoswitching of many single ANPs, which raises concerns about the use of this technique for PALM-based super-resolution imaging. The high-power excitation required for photoactivation is still a big problem for imaging living samples compared to conventional PALM systems. In addition, the imaging rate of INPALM systems is much slower than STED, and there is no promising prospect for the development of INPALM compared to lanthanide nanocrystal-based STED.

3) The central concept of this work and the presentation of the data are confusing. If the authors intended to demonstrate photoswitchable nanoprobe for super-resolution localization microscopy (e.g., PALM), then photoblinking is not necessary for PALM techniques. However, the authors have included much photoblinking data in the manuscript, and the results of the 2D pattern applications are unnecessary.

Overall assessment:

Although the experimental results are extensive and impressive, the broad significance, importance and novelty of this study are questionable, because the same authors had already demonstrated the basic principle of avalanching nanocrystals in a previous study. And the current photoswitchable setup has notable limitations that hinder its use for both STORM and PALM. The limitations of this work are critical and lead to the conclusion that its impact falls short of the standards typically expected for publication in Nature.

Referee #2 (Remarks to the Author):

The authors have improved considerably the manuscript after making a great effort to address all the reviewer's comments. New experiments and figures have been added, which were necessary to clarify key concepts for the understanding of the research carried out, mainly those related to the mechanism. The authors have addressed clearly and concisely all concerns. In my opinion, the manuscript is now ready for publication as it stands.

Referee #3 (Remarks to the Author):

Referee report 'Indefinite and Bidirectional Near Infrared Nanocrystal Photoswitching' by Changhwan Lee et al.

The authors have done an excellent job in taking into account the various comments – I am very satisfied with the manner in which all my concerns were addressed and the impressive new experiments that were conducted. Particularly, Figure S25 very convincingly demonstrates the robustness of the method, Figure S32 shows the time response in the sub-sec scale which the authors now also use to estimate the time scale required for the localization experiments. Figure S24 gives now direct evidence for the energy transfer/quenching of the 3F₄ level (not sure why it is not plotted on a log I vs. t scale, but that is not so important). It is also satisfying to see that the other experiments suggested also work (Figs. S23 and S21) and also how the mistakes were corrected. In addition, the localization accuracy is now well-explained. For the time-resolution/speed, it would be good if the time per frame is mentioned in Figure 5. What I understand from the SI and Manuscript is that one frame takes 500 ms and gives a localization accuracy of 2.5 nm and 400 frames (and thus 200 seconds) are needed to realize the sub-Angstrom resolution. It would be good if this is clearly mentioned in Figure 5 and maybe also Table S4. Overall, the revised manuscript looks almost ready to be published.

Nice new results, unfortunately, can also come with new questions. Figure S16 now shows a better photostimulation spectrum for the photobrightening with realistic error bars and an interesting structure. The peaks at 800 and 700 nm are assigned to transitions to the 3H₄ and 3F_{2,3} levels – this is correct and nice to see. The broad band below 530 nm is the colour center related absorption. But now there is also a very sharp dip at 450 nm “corresponding to higher-energy 1G₄ and 1D₂ Tm³⁺ transitions”. This is puzzling. First of all, it is a single data point while the transitions to the 3H₄ and 3F_{2,3} levels are broadened as expected for the crystal-field split levels. For the 1G₄ a broader feature (dip in this case) would be expected between 470 and 480 nm while the 1D₂ level is around 360 nm. So both the wavelength and shape do not correspond with what would be expected for transitions from the 3H₆ ground state to 1G₄ or 1D₂. Maybe the 3F₄ to 1D₂ transition could be around 450 nm but then still a broader feature would be expected. Overall the dip is puzzling and certainly cannot be explained by ground state absorption to 1G₄ or 1D₂ as is suggested now in the revised manuscript. The sharp feature is clearly reproducible – the SI figure S16 shows the same 450 nm feature for different photobrightening intensities (power densities) and sometimes even negative percentages of the photobrightening recovery are observed. I do not have a good explanation for the sharp dip but as it is now, it requires attention before publication. Overall, I recommend publication of this excellent work. I always like to prevent another round of corrections but the explanation for the sharp 450 nm dip does require correction/discussion. As long as changes are made, I would also recommend to include the time per frame in the caption of Figure 5 and the total time needed to realize the sub-Angstrom resolution in Figure 5c.

Author Rebuttals to First Revision:

Referees' comments:

Referee #1 (Remarks to the Author):

In the revised manuscript, the authors have carried out a comprehensive set of experiments. I appreciate their efforts to improve the technical part of the study.

Our reply: We thank the referee for their efforts and critical comments, and for noting the improved technical aspects of our work. We also are indebted to the referee for noting that the emphasis on blinking early in the manuscript may lead readers to expect it to be the basis of all that follows. Our observation of blinking, while scientifically interesting (to us, at least), was not intended to be a major emphasis of the study. Because it occurs with higher excitation powers than photoswitching, it is not directly relevant to the main finding of this work, and we now realize that our previous organization of the manuscript was confusing in this regard. We have made alterations in the manuscript to address this. Please see our response to Comment 3 below for more discussion of this point, and resulting manuscript changes.

However, despite these efforts, many of my concerns remain unaddressed. While I understand that characterizing the change in valence state can be challenging, I believe that the demonstration of these photoswitchable nanocrystals for potential STORM and PALM imaging is technically flawed in my opinion.

1) On page 4, the authors claim that “photo blinking statistics... stochastic optical reconstruction microscopy (STORM) imaging.” However, the high excitation power required for photoblinking is not ideal for STORM-based bioimaging due to its potential phototoxicity. In addition, the slow photoblinking rate of lanthanide nanoprobe would significantly limit imaging speed, making this STORM technique less advantageous than STED. In fact, similar control of photobrightening and photodarkening (depletion) can be achieved in STED microscopy using lanthanide nanocrystals.

Our reply:

We agree, and that was exactly the point we were trying to make about ANPs with STORM in Fig. S9. Current ANPs are *not* a great fit for STORM imaging applications due to a lack of collected photons when operating under small duty cycle pump conditions. But we see that the sentence in question is distracting, as neither STORM nor ANP blinking are directly related to INPALM, so we have removed it.

In terms of PALM, we should emphasize that INPALM is distinct from GFP-based PALM in that ANPs are impervious to photobleaching, meaning as many photons can be collected as desired, with unlimited photodimming/photobrightening cycles. This is a major advance compared to superresolution techniques that rely on organics or proteins, which are all subject to irreversible bleaching, which ultimately limits the number of photons that can be collected.

We demonstrate the potential of INPALM in Figure 5, showing the ease of generating sub-nm localization accuracies.

Changes to the manuscript:

Because of the reviewer's comments and to streamline the manuscript, we have **removed the sentence referencing STORM** in the main text.

2) In Fig. 5, the authors demonstrate avalanching nanocrystals as probes for INPALM. PALM relies on random activation of fluorophores to spatially resolve molecular details. However, no evidence is provided to show how the avalanching nanocrystals can randomly activate in space, which is a fundamental requirement for PALM imaging. In fact, Fig. S25 shows consistent photoswitching of many single ANPs, which raises concerns about the use of this technique for PALM-based super-resolution imaging. The high-power excitation required for photoactivation is still a big problem for imaging living samples compared to conventional PALM systems. In addition, the imaging rate of INPALM systems is much slower than STED, and there is no promising prospect for the development of INPALM compared to lanthanide nanocrystal-based STED.

Our reply:

The Referee is correct that INPALM (and PALM) imaging rely on the random activation of emitters, and this is demonstrated in 2 figures in the manuscript. First, Fig. 5 relies on random activation of individual ANPs within the cluster. Second, the conditions for random ANP photobrightening are shown more explicitly in the recovery percentage measurements in Fig 3c, which show that the probability of activation is controlled by intensity, exposure time, and wavelength of the photobrightening beam. Thus, activation parameters are easily adjustable to match application requirements, and can result in the sparse, random activation needed for INPALM, or in the consistent and controllable photoswitching demonstrated in Fig. S27 and Fig. 4.

We also want to highlight that this activation is distinct from the photoblinking: blinking is not observed under illumination with photobrightening wavelengths.

We agree with the reviewer that high intensities may be a concern for living samples. This is most acute with the higher energies of UV or blue light (such as the 405 nm laser for GFP photoactivation in PALM) and decreases appreciably at the NIR wavelengths. It is not a concern for other applications, such as with fixed cells or other non-living systems, as we touched on in our previous response for the last draft. In addition, the NIR PB and PD beam intensities needed for INPALM noted in the main text (penultimate paragraph) are >25-fold lower than the depletion intensities required for achieving the best reported resolution of 19 nm in lanthanide-nanocrystal-based STED imaging [Liang et al., *Nature Nanotechnology* 16, 975 (2021)].

Changes to the manuscript:

We have added a sentence to the discussion on the intensity needed for stochastic PB, and how this differs from intensities needed for the consistent single-ANP PB shown in Fig 4.

“As with photoactivated localization microscopy (PALM)⁵⁷, in INPALM, the concentration of emitting ANPs is actively controlled at a very low level by choosing a PB illumination exposure time, intensity, or wavelength corresponding to a small probability of luminescence recovery (Fig. 3b,c, Figs. S17, S29).”

3) The central concept of this work and the presentation of the data are confusing. If the authors intended to demonstrate photoswitchable nanoprobe for super-resolution localization microscopy (e.g., PALM), then photoblinking is not necessary for PALM techniques. However, the authors have included much photoblinking data in the manuscript, and the results of the 2D pattern applications are unnecessary.

Our reply:

Based on this comment, and multiple comments in the previous round, we have removed Fig 2 from the main text and replaced it with a single-ANP photoswitching figure (previously Fig S25). We think this dramatically improves the clarity of our presentation, and we appreciate the reviewer's comments pointing out that the blinking data are distracting from the central finding of the manuscript: that controllable, indefinite IR photoswitching has been realized for the first time.

We would like to emphasize that blinking is a specific phenomenon observed in the ANPs, which occurs at intensities of 1064 nm illumination higher than needed for controllable photoswitching or INPALM experiments. We had previously included it because it was the first observation of inherent blinking of any kind in UCNPs, since their invention more than 20 years ago. However, while providing some motivation for the development of controllable photoswitching, we agree that the blinking behavior is only tangentially related to our manuscript's main thrust.

Therefore, we have removed the blinking plots and related discussion from the main text and removed Fig. 2 from the main text. We do feel the blinking studies hold scholarly import for this material system, so have moved the blinking portions of that figure to the SI where it can be better contextualized.

Changes to the manuscript:

The previous Figure 2 has now been removed, with the TEM panels now becoming part of Fig. 1 (panel c), and the blinking time traces moved to the SI (Fig. S1).

To better focus the manuscript on photoswitching, as the reviewer suggests, we have a new Fig. 2 (updated from previous Fig. S25), highlighting robust, repeatable photoswitching over a collection of 112 individual ANPs:

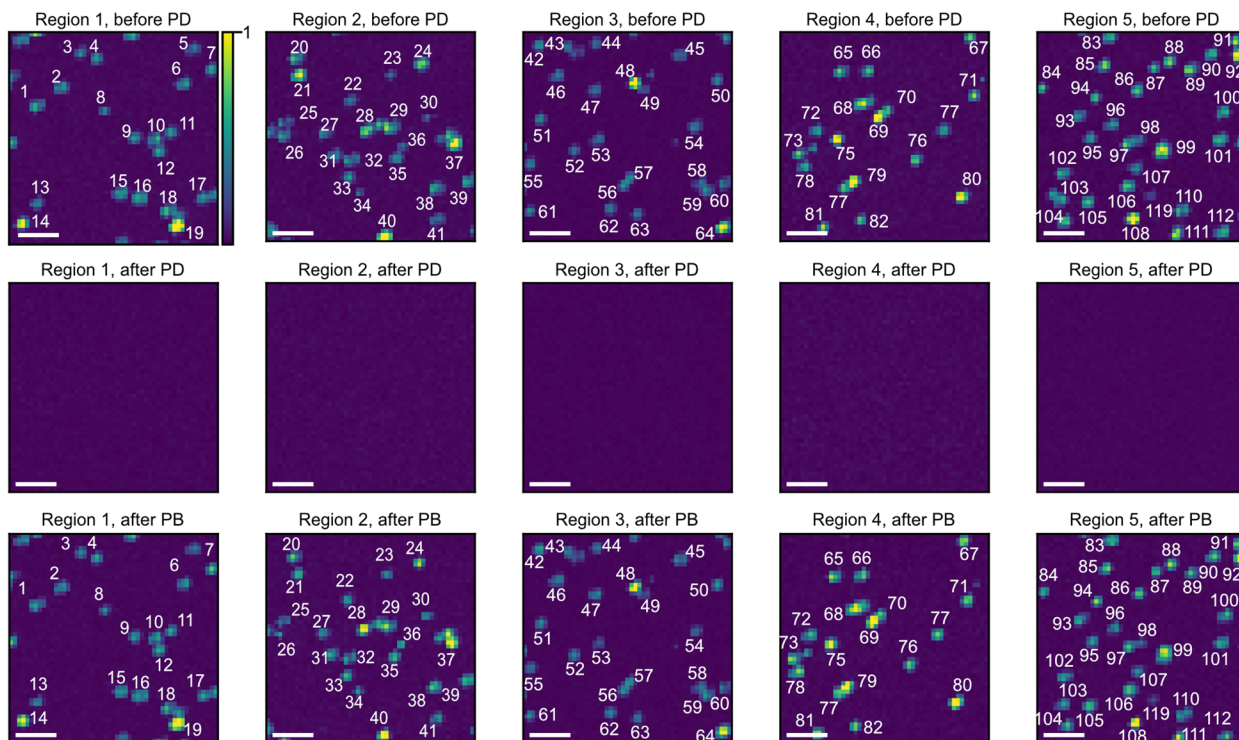


Fig. 2 | Consistent photoswitching of single ANPs. Single-particle imaging of ANPs before photodarkening (*top*), after photodarkening (*middle*), and after photobrightening (*bottom*), of 112 nanoparticles (mostly single ANPs, with a few dimers or small clusters). Columns are different regions of the sample. The imaging and photodarkening intensities (1064 nm) of 8% Tm³⁺ 17.4/2.6 nm core/shell ANPs are 28 kW cm⁻² and 140 kW cm⁻², respectively. Photobrightening is achieved with 400 nm irradiation at 25 kW cm⁻². Photoswitching is observed in all 112 ANPs.

We have also added discussion describing these experiments:

“To determine if ANP photodarkening can be reversed, we examined the emission of 112 individual ANPs under various pump conditions (Fig. 2). Illuminating these photodarkened 8% Tm³⁺ ANPs with 400 nm light results in full recovery of ANP brightness in all 112 ANPs. Further single-particle experiments (*vide infra*) have shown this recovery is consistent for all ANPs examined, including those synthesized by different protocols (Methods). “

Overall assessment:

Although the experimental results are extensive and impressive, the broad significance, importance and novelty of this study are questionable, because the same authors had already demonstrated the basic principle of avalanching nanocrystals in a previous study. And the current photoswitchable setup has notable limitations that hinder its use for both STORM and PALM. The limitations of this work are critical and lead to the conclusion that its impact falls short of the standards typically expected for publication in Nature.

Our reply:

We appreciate and value the Referee's perspective on this topic, and we have revised the manuscript to better emphasize the novelty and utility of our findings. We note that there is no discussion of photoswitching in our previous photon avalanching work.

Specifically, the work represents the realization of the long-sought goal of developing a robust, all-NIR, fully photoswitchable probe with apparently perfect photostability. We are unaware of any other nanoscale probes that 1) can be photoswitched in both directions solely by NIR light (without the need for extreme temperatures or concern about thermal relaxation) or 2) can be photowswitched indefinitely. These are both significant advances that enable both fully rewritable 3D nanoscale patterning and sub-Å localization accuracies, but also have the potential to impact a broad range of areas spanning biomedicine to nanophotonics to solid-state memory.

Referee #2 (Remarks to the Author):

The authors have improved considerably the manuscript after making a great effort to address all the reviewer's comments. New experiments and figures have been added, which were necessary to clarify key concepts for the understanding of the research carried out, mainly thore related to the mechanism. The authors have addressed clearly and concisely all concerns. In my opinion, the manuscript is now ready for publication as it stands.

Our reply: We are thankful to the reviewer for their support, and for their thoughtful comments and time in reviewing our manuscript, which we feel has notably improved its the clarity and presentation.

Referee #3 (Remarks to the Author):

Referee report 'Indefinite and Bidirectional Near Infrared Nanocrystal Photoswitching' by Changhwan Lee et al.

The authors have done an excellent job in taking into account the various comments – I am very satisfied with the manner in which all my concerns were addressed and the impressive new experiments that were conducted. Particularly, Figure S25 very convincingly demonstrates the robustness of the method, Figure S32 shows the time response in the sub-sec scale which the authors now also use to estimate the time scale required for the localization experiments. Figure S24 gives now direct evidence for the energy transfer/quenching of the 3F4 level (not sure why it is not plotted on a log I vs. t scale, but that is not so important). It is also satisfying to see that the other experiments suggested also work (Figs. S23 and S21) and also how the mistakes were corrected. In addition, the localization accuracy is now well-explained.

Our reply: We thank the reviewer for the strong support, noting the “impressive new experiments”.

For the time-resolution/speed, it would be good if the time per frame is mentioned in Figure 5. What I understand from the SI and Manuscript is that one frame takes 500 ms and gives a localization accuracy of 2.5 nm and 400 frames (and thus 200 seconds) are needed to realize the sub-Angstrom resolution. It would be good if this is clearly mentioned in Figure 5 and maybe also Table S4. Overall, the revised manuscript looks almost ready to be published.

Our reply: We agree and have added the acquisition times for imaging in the caption of Fig. 5. We note that we acquired the image frames for 1.8 s in panels a-c, and 1.5 s in panels f-i, allowing us to collect nearly 200,000 photons per frame, which provides the 2.5 nm localization accuracy per frame. The 500 ms acquisition time results in signal levels similar to those for fluorescent proteins in reported PALM experiments (see ref. 31).

Changes to the manuscript: We have added the acquisition times in the caption of Fig. 5.

“a, 2D map of the frame-by-frame localizations of a single 8% Tm³⁺ core-inert NaYF₄ shell ANP (*N* = 403 frames; ANP is photoswitched off and on for each frame). See Methods for details of constructing this map. Scale bar is 3 nm. For each frame, the signal acquisition time is 1.8 s.”

“f, Frame-by-frame localizations of the trimer in e (*N* = 172). For each frame, the signal acquisition time is 1.5 s.”

Nice new results, unfortunately, can also come with new questions. Figure S16 now shows a better photostimulation spectrum for the photobrightening with realistic error bars and an interesting structure. The peaks at 800 and 700 nm are assigned to transitions to the 3H₄ and 3F_{2,3} levels – this is correct and nice to see. The broad band below 530 nm is the colour center related absorption. But now there is also a very sharp dip at 450 nm “corresponding to higher-energy 1G₄ and 1D₂ Tm³⁺ transitions”. This is puzzling. First of all, it is a single data point while the transitions to the 3H₄ and 3F_{2,3} levels are broadened as expected for the crystal-field split levels. For the 1G₄ a broader feature (dip in this case) would be expected between 470 and 480 nm while the 1D₂ level is around 360 nm. So both the wavelength and shape do not correspond with what would be expected for transitions from the 3H₆ ground state to 1G₄ or 1D₂. Maybe the 3F₄ to 1D₂ transition could be around 450 nm but then still a broader feature would be expected. Overall the dip is puzzling and certainly cannot be explained by ground state absorption to 1G₄ or 1D₂ as is suggested now in the revised manuscript. The sharp feature is clearly reproducible – the SI figure S16 shows the same 450 nm feature for different photobrightening intensities (power densities) and sometimes even negative percentages of the photobrightening recovery are observed. I do not have a good explanation for the sharp dip but as it is now, it requires attention before publication.

Our reply: We, too, were at first surprised by the dip at 450 nm, noting that it did not directly match a ground state absorption transition but rather the excited state absorption transition noted by the Referee.

Based on these comments, we have performed two additional experiments. One is a high-resolution wavelength-dependent photobrightening measurement, and the other is a set of UV emission spectroscopy measurements that vary blue excitation every 5 nm from 440 nm to 480 nm. The high-resolution PB activity spectral study reveals that the dip has a FWHM of ~5 nm (new Fig. S19, see below). The narrowness of this dip is not totally surprising, as it is similar to the width of the 450 nm emission peak from the ¹D₂-³F₄ transition in Tm³⁺ that we measure (Fig. R1 below). The measurement of UV emission spectra (310 nm to 390 nm) under various blue excitation wavelengths shows that UV emission is the strongest under 450 nm excitation (updated Fig. S18c, see below), while no UV is observed under 480 nm excitation. This is consistent with the idea that the 450 nm excitation can lead to UV-induced photodarkening, as manifested in Fig. 3b and S17.

These findings are supported by theoretical studies we have uncovered in the literature. Brunet *et al.* reported that the 455 nm excitation can excite Tm^{3+} ions to the $^1\text{D}_2$ state ["Modeling of Tm-doped ZBLAN blue upconversion fiber lasers operating at 455 nm", *Infrared Optical Fibers and Their Applications*, SPIE, 3849, 125-135 (1999)]. Even though the energy mismatch between the $^3\text{H}_6$ - $^1\text{G}_4$ ground state absorption and 455 nm pumping photon decreases the absorption cross section, the absorption is still possible according to the calculated cross sections. This is related to the fact that the probability of phonon release in the host material can be orders-of-magnitude higher than that of phonon absorption, explaining why intense UV emission is observed under 450 nm excitation, but not under 480 nm excitation [Physical Review B, 56(22), 14344 (1997)]. It means that the key excitation transitions required for the upconversion to UV, including the combination of the $^3\text{H}_6$ - $^1\text{G}_4$ and $^3\text{F}_4$ - $^1\text{D}_2$ transitions, and the $^3\text{H}_4$ - $^1\text{I}_6$ transition, are more efficient with 450 nm excitation than with 480 nm excitation, leading to the stronger UV emission under 450 nm excitation.

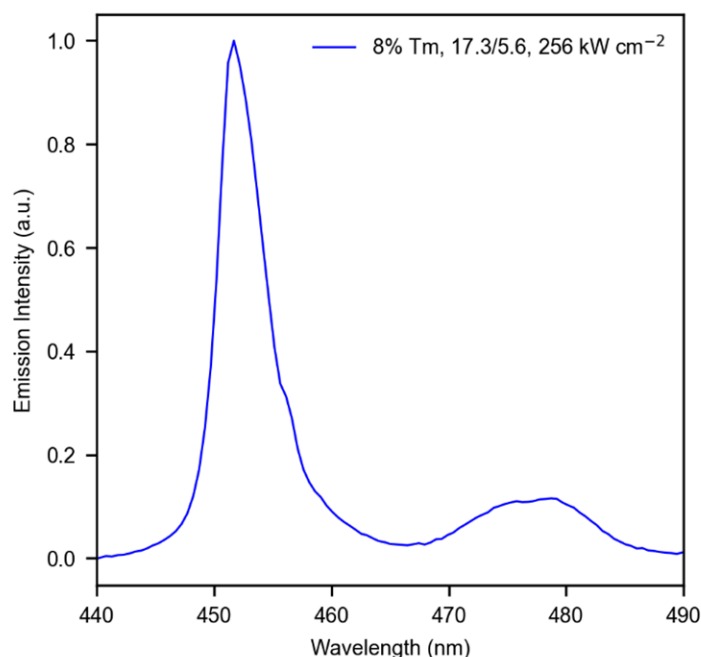


Fig. R1. Emission spectra of an 8% Tm avalanche nanoparticle film under 1064 nm excitation. The ANP composition and the excitation intensity are noted in the legend.

Changes to the manuscript:

We have **added new Fig. S18c and S19**, which shows a clear 450 nm dip at the activity spectrum and supports the photodarkening under 450 nm exposure. We also have added more possible energy transfer pathways in Fig. S18a to support the efficient UV emission under 450 nm excitation.

We have added new discussion to the main text regarding the observed photodarkening under 450nm excitation:

“There is a sharp dip in the activity spectrum at 450 nm (Figs. 3b, S17), where absorbed photons result in enhanced upconversion to photodarkening UV manifolds (Fig. S18c). Higher resolution activity spectra reveal that the dip has a full width at half maximum (FWHM) of ~5 nm (Fig. S19), and luminescence studies show that UV emission is strongest under 450 nm excitation (which is resonant with excited-state Tm^{3+} absorptions), while no UV is observed under 480 nm excitation (corresponding to the $^3\text{H}_6$ -to- $^1\text{G}_4$ ground state absorption) (Fig. S18c).”

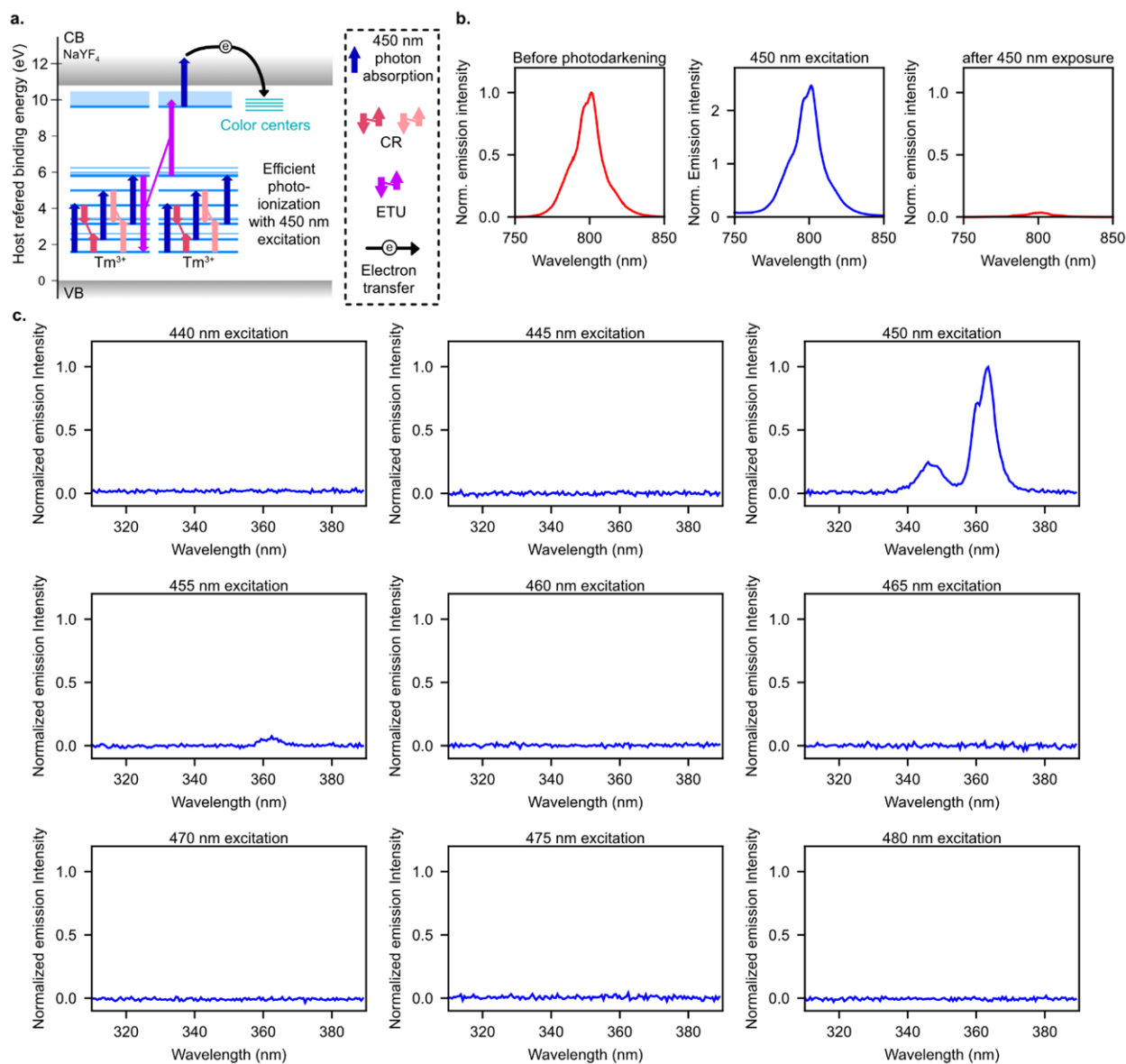


Fig. S18 | ANP photodarkening with blue-light excitation. **a**, Potential mechanistic pathways for photodarkening in ANPs under 450 nm pumping. **b**, Emission spectra from an ensemble film of 20% Tm^{3+} 10.4/2.7 nm core diameter/shell thickness ANPs before photodarkening (left), during 450 nm exposure (middle), and after photodarkening with 450 nm exposure (left). 1064 nm illumination is used for pumping the luminescence in the left and right panels. The 1064 nm intensity is 75 kW cm^{-2} , and the 450 nm excitation intensity is 222 kW cm^{-2} . The emission intensities in **b** are normalized to the maximum intensity in the left panel. **c**, UV emission spectra

of 20% Tm^{3+} 10.4/2.7 nm core diameter/shell thickness ANPs under focused blue excitation from 440 nm to 480 nm. Excitation powers are all 1 mW. The emission intensities in **c** are normalized to the maximum intensity in the panel obtained under 450 nm excitation. The 345 nm and 365 nm emission peaks are attributed to the excited-state transitions from the $^1\text{I}_6$ state to the $^3\text{F}_4$ state and the $^1\text{D}_2$ state to the $^3\text{H}_6$ state, respectively.

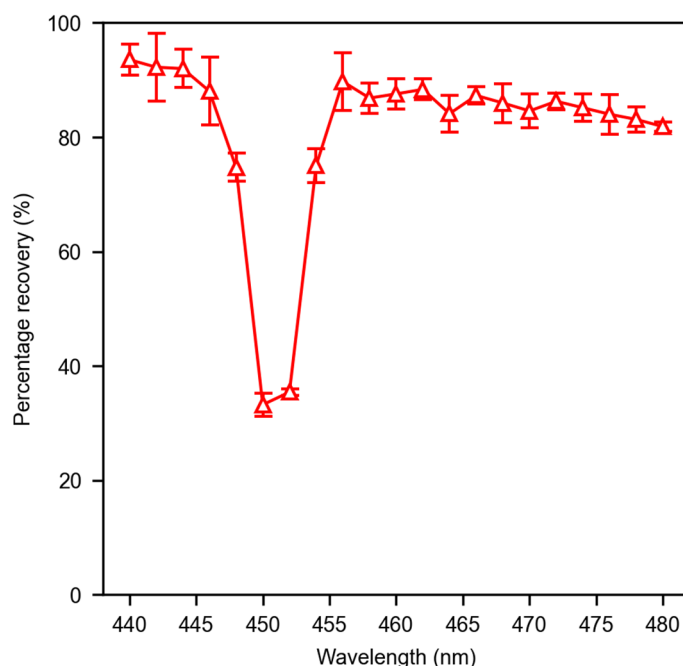


Fig. S19. Photobrightening recovery of darkened 20% Tm^{3+} 10.4/2.7 nm core diameter/shell thickness ANP films as a function of irradiation wavelength from 440 nm to 480 nm. The photobrightening laser power at the sample is set to 1 mW for all wavelengths.

Overall, I recommend publication of this excellent work. I always like to prevent another round of corrections but the explanation for the sharp 450 nm dip does require correction/discussion. As long as changes are made, I would also recommend to include the time per frame in the caption of Figure 5 and the total time needed to realize the sub-Angstrom resolution in Figure 5c.

Our reply: We are grateful to the Referee for these comments and suggestions, and we hope the additional data and discussion answer their questions. We think these data and discussion have resulted in a better mechanistic understanding and marked improvements to the manuscript.

Reviewer Reports on the Second Revision:

Referees' comments:

Referee #3 (Remarks to the Author):

The authors have convincingly addressed the one remaining concern I had and the manuscript can be published in the present form.