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Water-insensitive NIR-I-to-NIR-I down-shifting nanoparticles enable stable biomarker detection at low power thresholds in opaque aqueous environments

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Abstract

Luminescence quenching in aqueous environments poses a challenge for practical applications. Lanthanide-doped up-conversion nanoparticles (UCNPs), representative of near-infrared (NIR)-emitting phosphors, typically utilize Yb^{3+} ions as sensitizers, requiring 980 nm light. This wavelength coincides with the transitions of water molecules, interfering with population dynamics, and continuous irradiation causes unintended heating. Although Nd^{3+} ions, which absorb at 800 nm, serve as alternative sensitizers, their practical use is limited by low quantum yield (Q.Y.). In this study, we developed water-insensitive down-shifting nanoparticles (WINPs) functioning within the NIR-I range (700–900 nm) to avoid water interference. Characterization through single-particle-level spectroscopy demonstrated water-insensitive properties, with identical powers density and lifetime profiles under both dry and water conditions. The WINPs achieved a high Q.Y. of $22.1 \pm 0.9\%$, allowing operation at a detection limit power 15-fold lower than UCNPs, effectively eliminating background noise and enhancing overall performance. To assess diagnostic potential, we validated WINP-based lateral flow immunoassay (LFA) for detecting avian influenza viruses (AIVs) in 65 opaque clinical samples, achieving 100% sensitivity and an area under the curve (AUC) of 1.000 at only 100 mW cm^{-2} . These findings highlight the potential of WINPs as water-insensitive NIR phosphors that can operate at low power, even in water-rich environments.

Introduction

Near-infrared (NIR)-based optical technologies have emerged as promising tools in biomedical research because they enable real-time and highly sensitive monitoring of biomolecular interactions and high-resolution imaging in biological environments^{1–6}. The so-called “biological window,” which encompasses the NIR-I (700–900 nm) and NIR-II (1000–1700 nm) ranges, offers numerous advantages, such as low auto-fluorescence and

reduced scattering, facilitating deeper tissue penetration and yielding a high signal-to-background ratio (S/B) in biological contexts^{7–9}. Lanthanide-doped up-conversion nanoparticles (UCNPs) are anti-Stokes emitters capable of NIR-to-NIR conversion^{10–12}. Typically, NIR-emitting UCNPs are composed of ytterbium (Yb^{3+}) ions as sensitizers, which absorb infrared radiation at 980 nm and non-radiatively transfer their excitation to doped activator ions, such as thulium (Tm^{3+}) ions, resulting in NIR emission at 800 nm (980-to-800 nm). Recent studies have reported the advantages of NIR-emitting UCNP-based optical sensors as powerful tools for developing biological applications with highly sensitive detection capability^{13–15}. However, the absorption peak of Yb^{3+} ions at 980 nm significantly overlaps that of overtones of water molecules, leading to attenuation and unwanted heating

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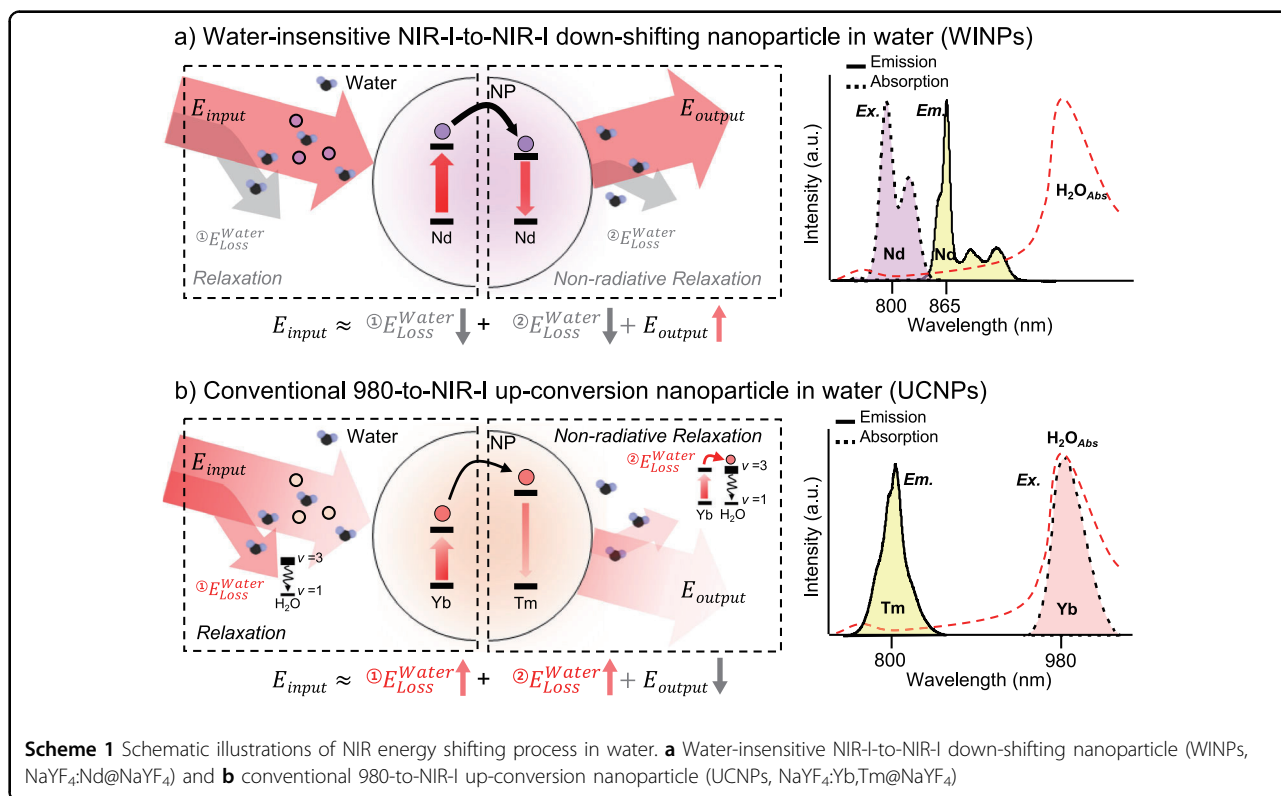
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effects in water-rich biological environments^{16–18}. To address these issues, neodymium (Nd^{3+}) ions, which absorb infrared radiation at 800 nm, have been used as alternative sensitizers. This substitution reduced the absorption coefficient of water from 0.48 cm^{-1} at 980 nm to 0.02 cm^{-1} at 800 nm, a 24-fold reduction that effectively minimizes heating effects¹⁹. Despite these improvements, Nd-sensitized up-conversion (UC) still suffers from low quantum yield (Q.Y. <1%), necessitating the use of high-power densities, which also increases the risk of photo-damage to cells and target analytes. Moreover, the need for a high power density places a strain on laser stability, potentially shortening the lifespan of the equipment and posing challenges for the commercialization of diagnostic tools owing to energy inefficiency and increased costs. Furthermore, their UC emission spectra are confined to the visible range (400–700 nm); moreover, their detection in the NIR-II region is also restricted owing to their reliance on expensive InGaAs detectors and complex cooling systems.

Here, we introduce water-insensitive nanoparticles (WINPs) designed to function entirely in the NIR-I region. Scheme 1a illustrates the NIR-I-to-NIR-I down-shifting (DS) process of WINPs in water, in contrast to the UC process (Scheme 1b). The WINPs were finely optimized at $\text{NaYF}_4:5\%\text{Nd}@\text{NaYF}_4$ to efficiently convert light absorbed at 800 nm into emission at 865 nm through the DS process. Single-particle-level characterization

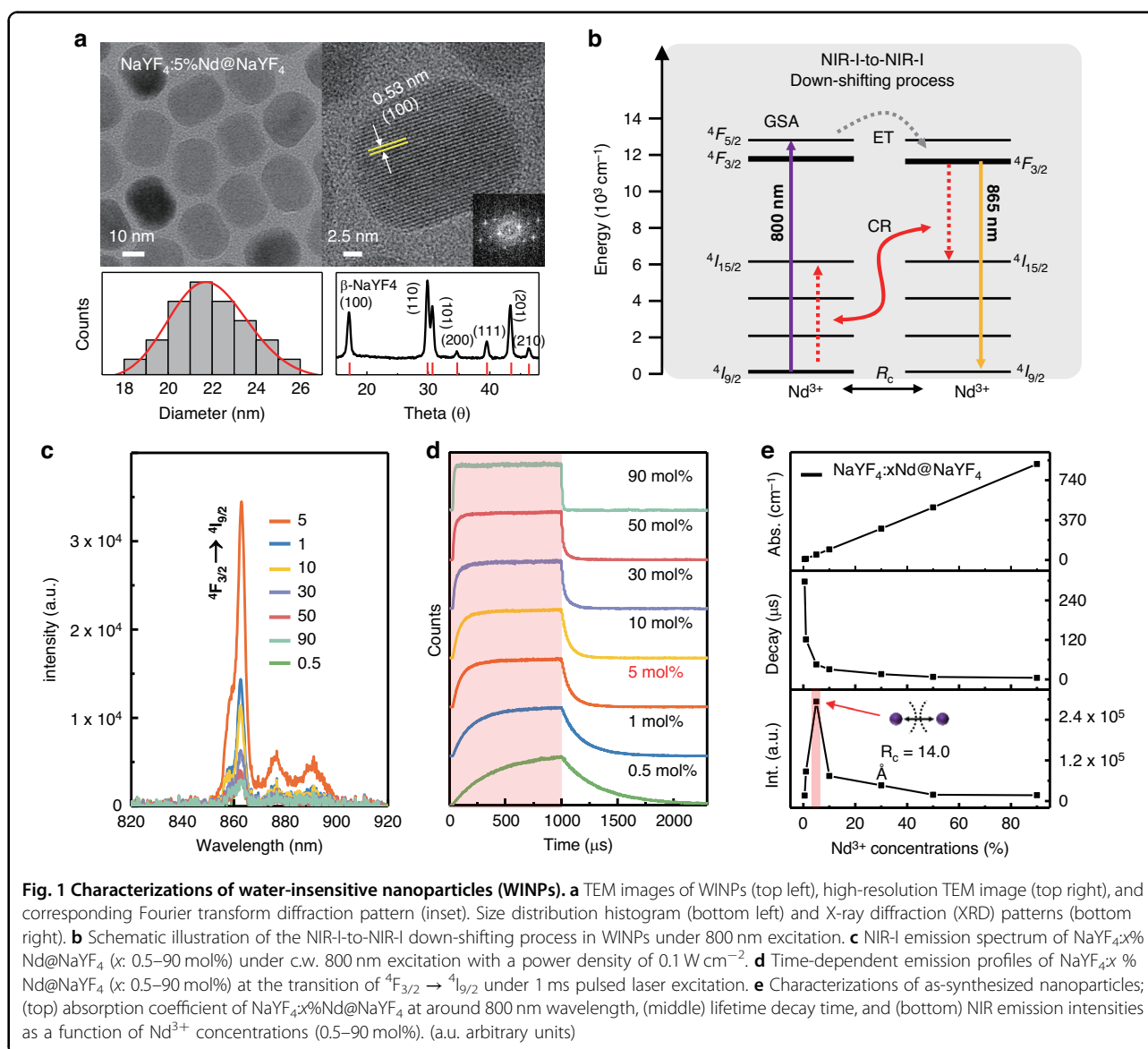
demonstrated water insensitivity and maintained a consistent detection limit power and lifetime profile in both dry and aqueous environments. Compared with UCNPs, WINPs achieved a 15-fold lower detection limit power in water, highlighting the superior efficiency of the DS. Under irradiation at 800 nm, the thermal effects were minimal, ensuring stable signal output from the WINPs, even over durations exceeding 5 min. Consequently, we applied this approach to validate water-independent, highly sensitive detection using a lateral flow immunoassay (LFA). Avian influenza viruses (AIVs) were chosen as target analytes because they are typically detected in opaque avian stools and cloacal swab samples^{20–22}, making this a suitable test for evaluating the performance of WINPs in aqueous samples under low-power irradiation. The WINP-based LFA demonstrated high diagnostic accuracy and reliability when tested against 65 opaque clinical samples, achieving a sensitivity of 100% and an area under the curve (AUC) of 1.000, even under 100 mW cm^{-2} irradiation. Therefore, the developed WINPs offer considerable potential for use as water-independent biosensors in water-rich biological contexts that operate effectively at low power densities.

Results

Characterization of NIR-I-to-NIR-I down-shifting WINPs

To optimize the WINPs, we synthesized a series of $\text{NaYF}_4:x\%\text{Nd}@\text{NaYF}_4$ (x mol%, where x ranges from 0.5 to 90)





nanoparticles via a thermal decomposition method²³. The core-shell nanoparticles were synthesized by directly growing a NaYF_4 inert shell on pre-synthesized core nanoparticles (Fig. S1). This process enhances photoluminescence (PL) through surface passivation, which minimizes surface defects and thereby improves the optical performance²⁴. As shown in Fig. S1, doping of Nd^{3+} ions into $\text{NaYF}_4:x\% \text{Nd}@\text{NaYF}_4$ does not have an observable effect on morphology and size. The as-synthesized WINPs ($\text{NaYF}_4:5\% \text{Nd}@\text{NaYF}_4$) exhibit a consistent size distribution with an average diameter of $21.9 \pm 1.8 \text{ nm}$. High-resolution transmission electron microscopy (TEM) image reveals distinct lattice fringes with a d -spacing of 0.53 nm, corresponding to the interplanar distance of (100) crystalline planes (Fig. 1a). The Fourier transform diffraction pattern and

the selected area electron diffraction (SAED) pattern indicated well-defined crystallinity. Additionally, X-ray diffraction (XRD) patterns confirmed the formation of the β -phase of NaYF_4 , which is known for its low phonon energy, reducing nonradiative losses and enabling efficient luminescence. This property makes it an effective host for facilitating specific energy transfer pathways between lanthanide ions, thereby enhancing overall photoluminescence efficiency²⁵.

Figure 1b illustrates the NIR-I-to-NIR-I DS process between Nd^{3+} ions under 800 nm irradiation. Upon absorption in their ground states (ground state absorption, GSA), the ions undergo nonradiative relaxation and quickly accumulate in the $^4\text{F}_{3/2}$ state. Typical PL emission bands of Nd, peaking at 865 nm corresponding to the $^4\text{F}_{3/2} \rightarrow ^4\text{I}_{9/2}$ transition, were observed (Fig. 1b, c)^{26–28}. In



this state, optimizing the concentration of Nd^{3+} ions is crucial for achieving an ideal spacing between the ions. This optimization is essential because decreasing the spacing between Nd^{3+} ions leads to cross-relaxation, resulting in non-radiative relaxation and a subsequent decrease in the radiative PL intensity²⁹.

To better understand the temporal characteristics of ionic interactions, we measured time-dependent PL lifetime profiles under a pulsed laser with a width of 1 ms³⁰. Figure 1d shows that the lifetime decay in the $^4\text{F}_{3/2}$ state significantly accelerates, decreasing from 297.1 to 58.2 μs as the doping concentration increases up to 5%. Generally, the PL intensity increased monotonically with increasing concentrations of Nd^{3+} ions (Fig. 1e). However, beyond a critical doping concentration, the decreased interionic distance results in stronger ion-ion interactions that enhanced non-radiative relaxation, a phenomenon known as concentration quenching, ultimately leading to a reduction in PL intensities^{31–33}. According to Blasse's equation, the critical distance (R_c) between Nd^{3+} ions at 5% concentration was calculated as 14.0 Å^{34,35} (Fig. S2). As a comparison, we synthesized $\text{NaYF}_4\text{:}48\%\text{Yb}, 2\%\text{Tm@NaYF}_4$ nanoparticles, conventional NIR-emitting UCNP known for their efficient UC (980-to-800 nm)^{36,37}. TEM image shows that these UCNP have a size of approximately 20.9 ± 1.5 nm, comparable to the WINPs used for comparison (Fig. S3). We further investigated the influence of Yb^{3+} concentration on PL efficiency by comparing the emission intensities with 10, 30, and 48% Yb^{3+} doping in both water and cyclohexane conditions. As a result, nanoparticles with lower Yb^{3+} content exhibited reduced quenching effects in water. However, for practical applications, we selected 48% Yb^{3+} doping for UCNP as it provides an optimal balance between high absolute PL and quenching, making it well-suited for further applications (Fig. S4). Quantum yields (Q.Y.) of DS and UC were calculated to be $22.1 \pm 0.9\%$ and $3.1 \pm 0.3\%$, respectively, as shown in Fig. S5. Moreover, compared to other lanthanide ions with similar downshifting mechanisms, Nd-based nanomaterials show superior absorbance and quantum yield in NIR-I region (Table S1). In addition, to minimize the influence of shell thickness, we engineered shell structures with similar thicknesses, averaging 1.55 nm for WINPs and 1.35 nm for UCNP (Fig. S6).

Single-particle-level spectroscopy for evaluating photon population mechanism in each nanoparticle

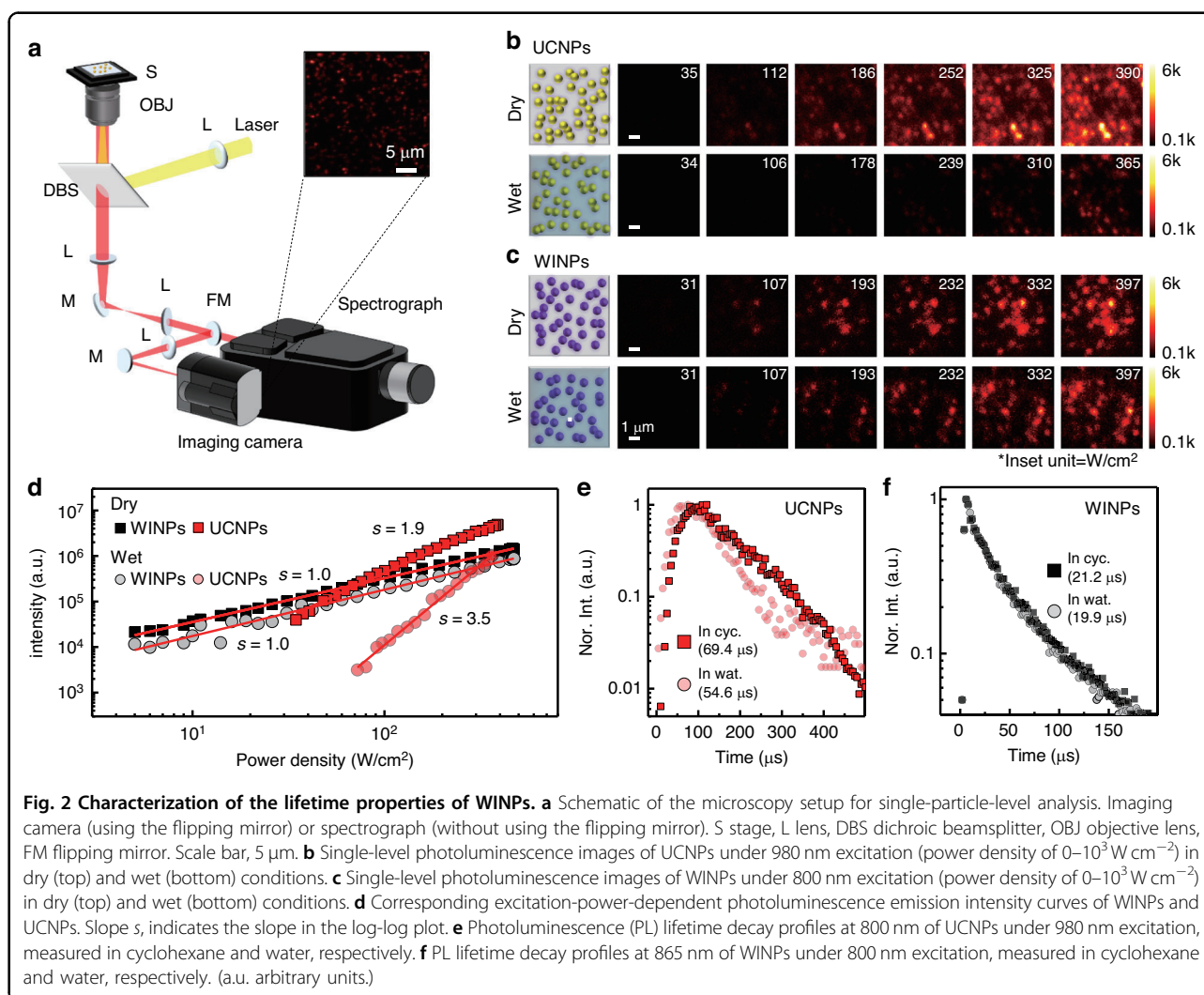
To evaluate the photon population mechanism in WINPs and UCNP, we conducted power-dependent PL measurements using a homemade wide-field spectroscopic imaging system (Fig. 2a) across a broad irradiance range of $5\text{--}10^3 \text{ W cm}^{-2}$. The as-synthesized nanoparticles, initially coated with oleic acid and soluble in

organic solvents, such as cyclohexane, underwent ligand exchange with dopamine hydrochloride via an ionization process to assess their performance in aqueous environments. Fourier transform infrared (FT-IR) spectra and zeta-potential measurements confirmed the successful replacement of the oleic acid ligand with an amine ligand (Fig. S7)^{38,39}. As shown in Fig. 2a, the dopamine-coated nanoparticles were spin-coated onto a piranha-etched cover glass to obtain single-particle-level images.

Figure 2b displays images of the PL signal from the UCNP at 800 nm under 980 nm irradiation, revealing a detection limit power density of 35 W cm^{-2} under dry conditions. In water, the detection limit power required to obtain the PL signal is approximately twice as high, i.e., 72 W cm^{-2} . The corresponding NIR spectra and power-dependence curves are shown in Figs. S8 and 2d, respectively. To explore the effects of environmental changes on the dynamics in UCNP, we analyzed the variations in their lifetime profiles, showing a decrease from 69.4 μs under dry conditions to 54.6 μs in water, a reduction of 21.3% (Fig. 2e). This change is attributed to the overtone transition of the O–H ($\nu=3$) at $\sim 10,300 \text{ cm}^{-1}$, closely matching the transition in Yb^{3+} ions ($^2\text{F}_{5/2} \rightarrow ^2\text{F}_{7/2}$) at $\sim 10,200 \text{ cm}^{-1}$ (Fig. S9). This spectral overlap resulted in a reduced lifetime and increased detection limit power owing to the enhanced quenching process, leading to a 10-fold overall reduction in the PL signals in water at the same power densities (Fig. 2d).

In contrast, the WINPs exhibited consistent PL signals at 865 nm under 800 nm irradiance with an identical detection limit power density of 5 W cm^{-2} in both dry and water environments (Fig. 2c). The negligible changes in the lifetime profiles (Fig. 2f) suggest that the energy transition in WINPs occurs independently of the water environment, significantly reducing signal quenching compared to that in UCNP, with only a 1.8-fold reduction in signal intensity. To compare these quenching mechanisms, the relative population model illustrates (Fig. S10) that, unlike WINPs, energy quenching in the $\text{Yb}^{3+}/\text{Tm}^{3+}$ system of UCNP is primarily attributed to nonradiative relaxation from Yb^{3+} to water molecules. These findings indicate that WINPs operate through a water-insensitive NIR-I-to-NIR-I DS process, requiring an approximately 15-fold lower power density because UC involves a complex multistep energy-absorption process, whereas DS is a simpler single-photon absorption process, leading to fewer energy losses during transfer. A lower-excitation source is particularly beneficial for biological environments because it effectively reduces background noise, enables highly sensitive signaling, and minimizes fluctuations by mitigating unwanted heating effects.





Characterizations of incident light and evaluating nanoparticle performance in water environments

Before applying them in practical biological applications, we examined the behavior of two types of incident light sources, 800 and 980 nm, i.e., the excitation wavelengths of WINPs and UCNPs, respectively, in aqueous environments. Using Monte Carlo simulations, we numerically analyzed the physics of light propagation in water, obtaining quantitative insights into the light distribution and penetration properties⁴⁰. As shown in Fig. 3a, the simulation result for 100 mW photon packets indicated that photons with a wavelength of 800 nm propagated through more than 95% of the 10 mm thick water medium, whereas over 45% of photons at 980 nm were absorbed by water molecules. This phenomenon is attributed to the overtone of the O–H stretching mode of H_2O ($\nu = 3$, $\epsilon_{980} = 0.48\text{ cm}^{-1}$), which absorbs significantly more than in the NIR region at 800 nm ($\epsilon_{800} = 0.02\text{ cm}^{-1}$, Fig. S9).

The practical experimental results, depicted in Fig. 3b, demonstrate that at a wavelength of 800 nm, more than 97.3% of photons penetrated to a depth of 10 mm. In contrast, at a wavelength of 980 nm, 54.6% of the photons were absorbed within the same thickness, consistent with the simulation results. Considering these findings, achieving above 90% irradiance at a depth of 10 mm underwater would theoretically require significantly higher power output, ranging from 1 to 2 W, depending on the laser target area (Fig. S11). These power levels, especially at 980 nm, tend to produce unwanted thermal effects in water. When irradiating water with two types of lasers at the same power density (2 W cm^{-2}), the 980 nm laser source resulted in a temperature increase of up to 50 degrees within 5 min, whereas the 800 nm laser source showed negligible thermal effect (Fig. 3c).

To investigate the correlation between the thermal effects and the performance of each nanoparticle, we observed time-dependent fluctuations in the intensities



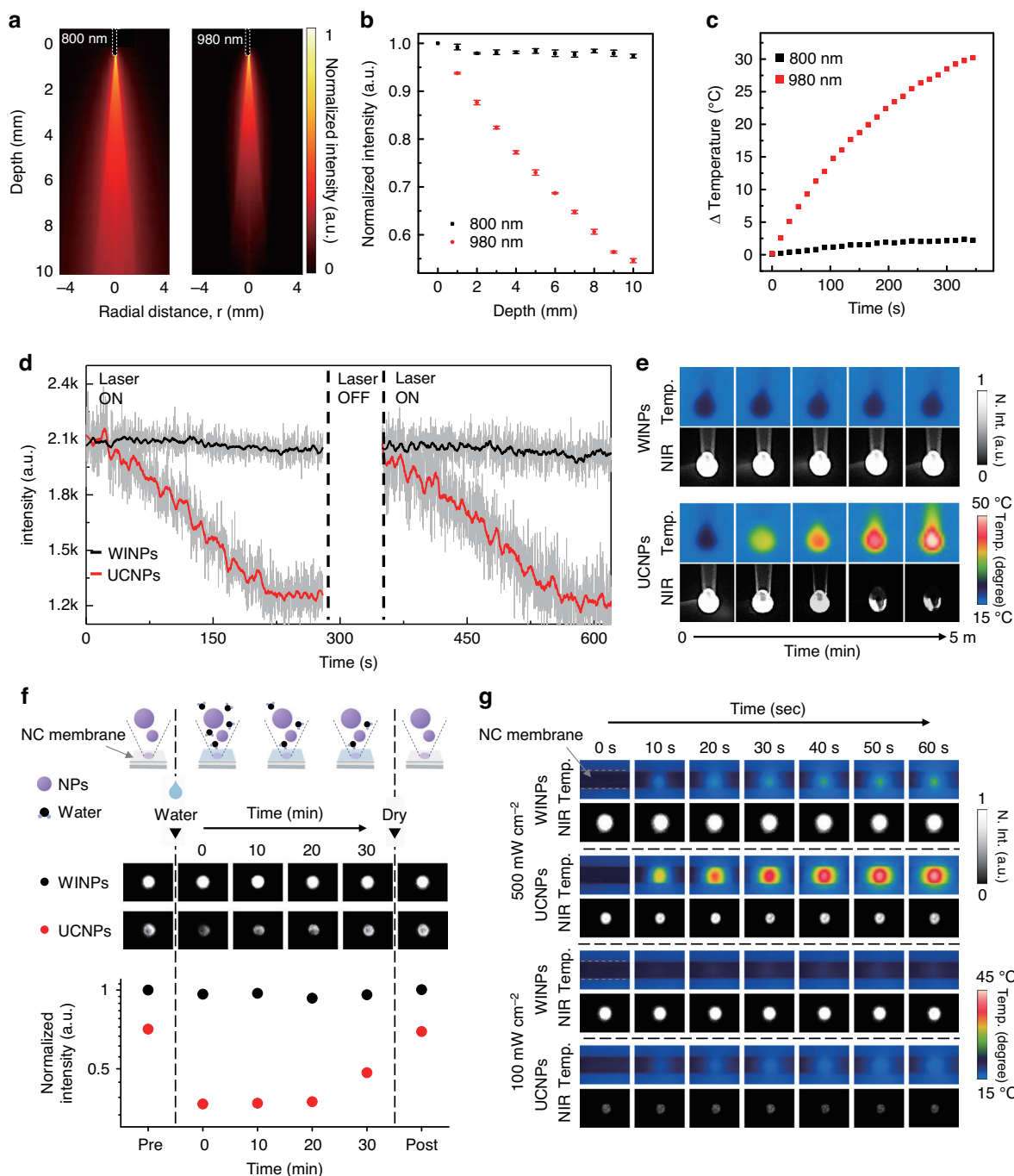


Fig. 3 Characterization of incident lights. **a** Heatmap profiles for 800 and 980 nm light originating from the fiber (100 μm , $\text{NA} = 0.22$, $g = 0.9$, output power = 100 mW) placed in water solution, obtained via Monte Carlo simulation as a function of distance. **b** Measured intensity profiles of the two lasers as function of water solution depth (power density of 100 mW cm^{-2}). **c** Temperature profiles over 5 min in a water solution, depending on the laser type, under irradiation at a power density of 2 W cm^{-2} . **d** Time-dependent intensity profiles of two types of nanoparticles over 5 min. **e** Corresponding thermal and NIR intensity images of two types of nanoparticles. **f** NIR intensity images obtained from nitrocellulose (NC) membranes drop-cast with two types of nanoparticles (200 $\mu\text{g mL}^{-1}$), then wetted with water. The membranes were stored in a desiccator, and the images were captured at 10-min intervals with a power density of 100 mW cm^{-2} . The images labeled "Post" were captured after complete removal of the water in a vacuum oven. **g** Time-dependent thermal and NIR intensity images obtained from NC membranes drop-cast with two types of nanoparticles (200 $\mu\text{g mL}^{-1}$), then wetted with water and irradiated at power densities of 500 and 100 mW cm^{-2} for 60 s. (a.u. arbitrary units)

of the NIR-I signal under continuous laser irradiation for 5 min. As shown in Fig. 3d, the intensity fluctuation from WINPs at an 865 nm wavelength remained stable within 4% under 800 nm irradiation, as the water temperature did not increase. In contrast, intensities from UCNPs at an 800 nm wavelength decreased by up to 60% as the water temperature rose to 50 degrees within 5 min. Their corresponding thermal and NIR signal images are shown in Fig. 3e, and the NIR-I emission spectra are shown in Fig. S12. The stability of the laser was confirmed through power meter measurements, showing a fluctuation of less than 1.7% over a 1 h (Fig. S13). The signal degradation observed in UCNPs was attributed to the thermally induced emission quenching, where elevated temperatures enhanced the nonradiative relaxation pathways, leading to a significant decrease in the PL efficiency^{41–44}. Although the signal attenuation from UCNPs can recover as the temperature drops, it is not suitable for applications involving small sample volumes or those requiring long-term measurements. In addition, a biomimetic environment was established using 2% intralipid phantoms to assess the NIR-I imaging capability. As shown in Fig. S14, WINPs-filled capillaries embedded in phantoms of varying thickness (~8 mm) exhibited enhanced resolution and 4-fold improvement in the S/B value under only irradiation at only 100 mW cm^{-2} . These findings indicate that 800 nm irradiation of the WINPs ensures sustained high-quality signal output, allowing effective performance at low laser power in water-rich biological environments.

Evaluation of WINPs for stable detection of target viruses under low power irradiation

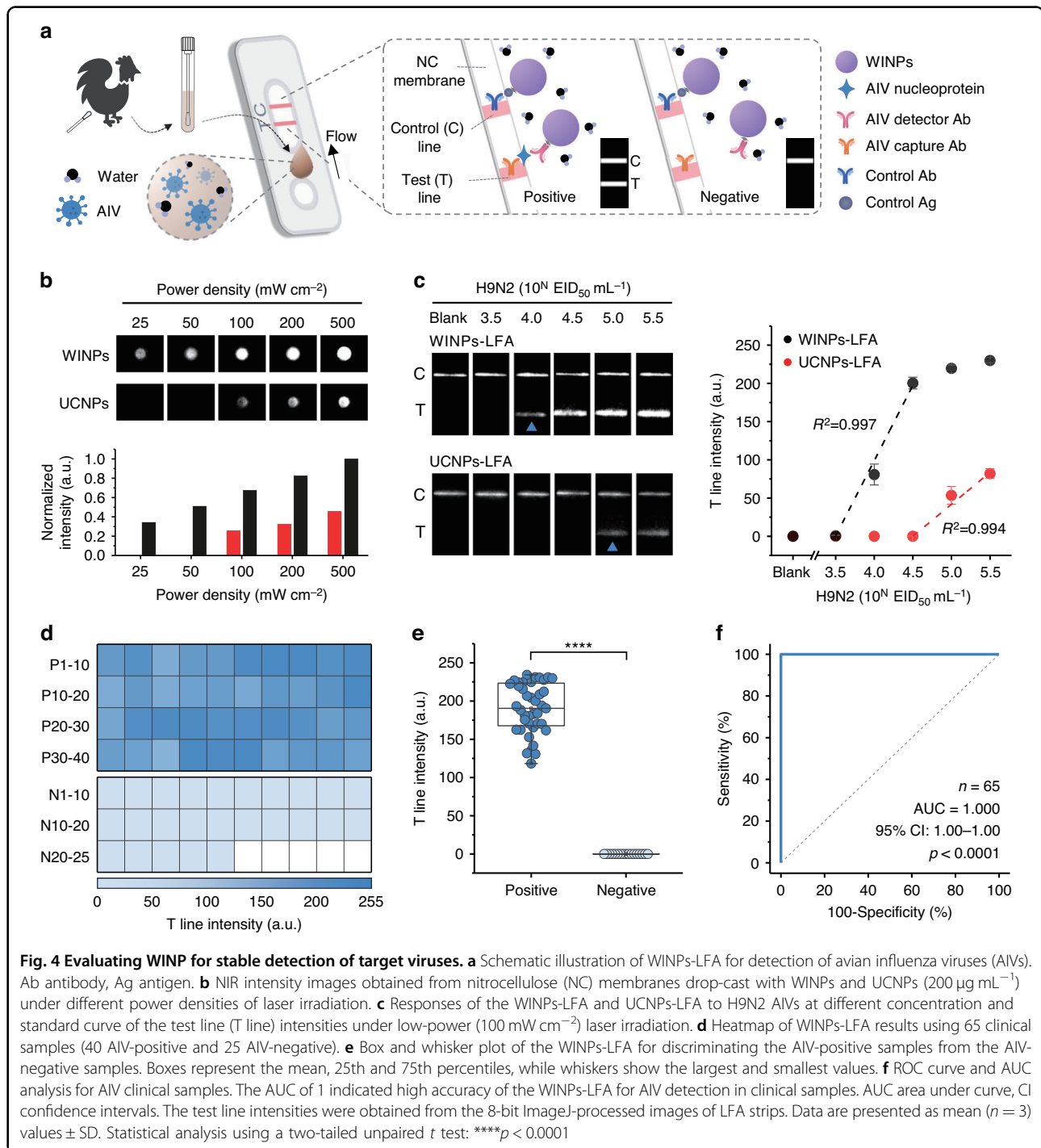
To further explore the potential for biological application, we incorporated WINPs as nanoprobe into an LFA for detecting AIVs to validate their water-independent and highly stable detection capabilities. AIVs cause highly contagious diseases in poultry, leading to significant economic losses and posing a public health risk owing to their ability to infect humans^{45–49}. Rapid and reliable detection of AIV is essential for effective prevention and control^{50–52}. AIV detection is typically performed using opaque avian stools and cloacal swab samples. However, conventional LFAs using colorimetric or fluorescent signals in the ultraviolet or visible range often suffer from strong light scattering and absorption in such opaque samples^{53,54}. These background interferences reduce signal clarity and detection reliability. In contrast, WINPs-LFA generates a background-free NIR signal, minimizing interference while maintaining high sensitivity for reliable AIV detection. To evaluate its diagnostic performance, the nucleoprotein within AIVs, a highly conserved internal viral protein less prone to mutations, was selected as the detection target^{21,55}.

To assess whether WINPs could enhance the accuracy of LFA, we compared their performance to that of UCNPs on a nitrocellulose (NC) membrane, a key component of LFA strips. After adding 100 μL of water—typical LFA conditions—the WINPs demonstrated higher signal intensity and stability, with only a 4% fluctuation, whereas UCNPs exhibited a 40% signal degradation (Fig. 3f). Even after 30 min in a desiccator, the signal intensity of the UCNPs did not fully recover, indicating their limitations for water-based LFA applications. Full recovery occurred only after the removal of water in a vacuum oven, confirming that water-induced energy loss was responsible for degradation. As shown in Fig. 3g, irradiation of the NC membrane with a 980 nm laser at 500 mW cm^{-2} increased the water temperature to 43 degrees within 1 min, resulting in a 40% decrease in the PL signal of UCNPs. In contrast, the PL signal of WINPs remained stable within 6% fluctuation, as 800 nm irradiation caused negligible heating issues. Under 100 mW cm^{-2} , thermal effects were minimal for both wavelengths; however, UCNPs exhibited significantly lower PL intensity. Consequently, WINPs, which demonstrated both higher and stable PL signal under low power conditions, are promising for biomarker detection in practical LFA applications.

The fabrication of the WINPs-LFA for detecting AIVs involved coupling the WINPs with an anti-AIV nucleoprotein detector antibody (Ab) for the test line (T line) signal and a control antigen (Ag) for the control line (C line) signal through a series of modifications: amine-, maleimide-, and Ab-modification (Fig. S15 and “Methods”). AIV detection using the WINPs-LFA begins by loading an AIV sample solution onto the sample pad of the LFA strip (Fig. 4a). The target analyte, a nucleoprotein lysed from AIV, specifically binds to the detector Ab-WINPs, forming analyte-WINP conjugates. These conjugates flowed through the NC membrane, where AIV Abs on the T line capture them, resulting in an NIR signal. In contrast, in the absence of AIVs, an NIR signal was observed only on the C line because the conjugates were not captured on the T line. For the control experiments, UCNPs were applied using an identical procedure and denoted as UCNPs-LFA. NIR signals of both WINPs-LFA and UCNPs-LFA were captured under 100 mW cm^{-2} irradiation, the minimum power density required to compare their performances (Fig. 4b).

In this study, we assessed the analytical performance of the WINPs-LFA avian influenza H9N2 at concentrations ranging from $10^{3.5}$ to $10^{5.5}$ 50% egg infectious dose (EID_{50}) mL^{-1} . H9N2 is classified as a low-pathogenic virus but is recognized as a public health risk because of its reported ability to infect humans^{56,57}. The T line intensities exhibited a linear relationship with increasing





AIV concentrations, ranging from $10^{3.5}$ to $10^{4.5} \text{ EID}_{50} \text{ mL}^{-1}$, with a high correlation coefficient (R^2) of 0.997 (Fig. 4c). Notably, the WINPs-LFA detected AIV concentrations as low as $10^{4.0} \text{ EID}_{50} \text{ mL}^{-1}$ (indicated by a triangle), whereas the UCNPs-LFA required a 10-fold higher concentration to produce a distinguishable T line intensity. The limit of detection (LOD) of the WINPs-LFA calculated using the IUPAC guidelines was $10^{3.52} \text{ EID}_{50} \text{ mL}^{-1}$, 10-fold lower

compared to that of the UCNPs-LFA ($10^{4.53} \text{ EID}_{50} \text{ mL}^{-1}$). While previous studies have reported a higher sensitivity of the $\text{NaYF}_4\text{:Yb,Tm}$ UCNPs-based LFA under high power conditions (Table S2), the WINPs-LFA demonstrated robust performance with significantly lower LODs, even under more practical, low power settings. These results can be attributed to the stable luminescence intensity of the WINPs, which maintains their

performance under low power laser irradiation without energy loss, in contrast to the UCNP that experience significant luminescence intensity reduction due to energy loss induced by water molecules.

To evaluate specificity, WINPs-LFA was tested using $10^{5.0}$ EID₅₀ mL⁻¹ of non-target avian pathogens, including infectious bronchitis virus (IBV), *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS), and Newcastle disease virus (NDV). The assay showed a significantly higher T line intensity for AIV than for the other pathogens ($p < 0.0001$), indicating the high specificity of WINPs-LFA with no detectable cross-reactivity (Fig. S16). Furthermore, the repeatability of the WINPs-LFA was evaluated by measuring coefficients of variation (CVs) for $10^{4.0}$ and $10^{5.0}$ EID₅₀ mL⁻¹ H9N2 AIVs. The intra-assay CVs were 1.14 and 4.52% and the inter-assay CVs were 2.54 and 5.28%, indicating the high repeatability of the WINPs-LFA (Fig. S17).

Clinical specimens such as avian cloacal swabs and blood are typically opaque and exhibit strong absorption in the visible range and negligible absorption in the NIR region. Consequently, the WINPs dispersed in these samples demonstrated stable NIR emission intensities without being disturbed by the background noise of the opaque sample matrix (Fig. S18a, b). We investigated the anti-interference capability of WINPs-LFA in detecting $10^{4.0}$ and $10^{5.0}$ EID₅₀ mL⁻¹ H9N2 AIVs in opaque avian cloacal swab samples and colored samples containing hemoglobin and bilirubin, respectively (Fig. S18c, d). The T line intensities of the WINPs-LFA showed no significant changes, even for AIVs spiked in these opaque solutions, demonstrating that WINPs-LFA is a reliable diagnostic tool for detecting target analytes in opaque clinical samples. Finally, we validated the clinical applicability of the WINPs-LFA in detecting AIVs under low power excitation conditions. We used 25 AIV-negative samples (cloacal samples) and 40 AIV-positive samples (oropharyngeal and vent swab samples) with different cycle threshold (Ct) values (Table S3). Figure 4d summarizes the T line intensities for each clinical sample, demonstrating significantly higher T line intensities for AIV-positive samples than for AIV-negative samples. The WINPs-LFA also yielded positive results for all AIV-positive samples without any false-negative results (p value < 0.0001) (Fig. 4e). We conducted a receiver operating characteristic (ROC) analysis, wherein the WINPs-LFA accurately distinguished AIVs in clinical samples with an area under the curve (AUC) of 1.000, indicating a sensitivity and specificity of 100% (Fig. 4f). Additionally, we compared the AIV detection results using WINPs-LFA and real-time PCR and observed a strong correlation between the two methods, as indicated by a Pearson's coefficient (r) of -0.830 (Fig. S19).

Discussion

In this study, we developed water-insensitive down-shifting nanoparticle (WINPs, NaYF₄:5%Nd@NaYF₄) that operate within the NIR-I range (700–900 nm) and demonstrated their potential as bio-suitable nanoprobe especially in water-rich environments. The optimized WINPs achieved a remarkable Q.Y. of $22.1 \pm 0.9\%$, with an operational detection limit power 15-times lower than that of conventional UCNP. These features support efficient utilization of the widely accessible 808 nm laser alongside silicon-based detectors, achieving lower background noise and enhanced performance even at low power density. Notably, single-particle-level spectroscopy confirmed that the WINPs maintained operational detection limit power under both dry and water conditions, offering consistent emission profiles regardless of the medium. Monte Carlo simulations and experimental results further demonstrated that 800 nm excitation minimizes thermal effects in water, making it more suitable for biological systems compared to the 980 nm excitation used in UCNP. In water condition, WINPs exhibited only minimal fluctuations in PL intensity ($< 4\%$), underscoring their stability against laser-induced thermal quenching and their suitability for extended use in bio-environments. Furthermore, the result of NIR-I imaging in 2% intralipid phantoms demonstrated a clear improvement in resolution and signal-to-background ratio (S/B) under irradiation of 100 mW cm^{-2} . Integrating WINPs into an LFA for detecting AIVs in opaque samples resulted in a 10-fold increase in sensitivity under a lower power irradiation of 100 mW cm^{-2} . Conventional UCNP-based LFAs in previous studies typically required lasers with 10-fold higher power (1000 mW cm^{-2})^{51,58}, which could rapidly increase temperature (> 45 degrees) on the LFA strip within 1 min. This heating issue could denature proteins and disrupt immunoreactions, compromising the assay accuracy. In contrast, low power irradiation caused negligible thermal effects on LFA strip. Therefore, WINPs-LFA, which maintains high and stable signal under low power irradiation, enables reliable and accurate biosensing. Moreover, as summarized in Table S4, other NIR-based LFAs have required high-power excitation sources (often up to several watts) or expensive InGaAs detectors with complex cooling systems, limiting their practical applicability. In contrast, WINPs-LFA exhibited high sensitivity with low-power excitation and a cost-effective silicon-based detector, making it a more practical alternative for biosensing. This system exhibited excellent specificity and repeatability, with 100% sensitivity confirmed through ROC analysis. Overall, these results highlighted the potential of WINPs to replace conventional NIR-emitting phosphors, particularly in applications requiring low-power excitation and water-rich biological environments.



Materials and methods

Reagents

Yttrium(III) acetate hydrate (99.9%), neodymium(III) acetate hydrate (99.9%), ytterbium(III) acetate hydrate (99.9%), thulium(III) acetate hydrate (99.95%), oleic acid (OA, 90%), 1-octadecene (ODE, 90%), sodiumhydroxide (NaOH, $\geq 98\%$), ammoniumfluoride (NH₄F, $\geq 99.9\%$), ethanol (absolute), methanol ($\geq 99.8\%$), cyclohexane ($\geq 99\%$), tetra-hydrofuran (THF, $\geq 99.9\%$), dopamine hydrochloride ($\geq 99.9\%$), hydro-chloric acid (HCl, 37%), dimethyl sulfoxide (DMSO, $\geq 99.9\%$), hydroxylamine hydrochloride (HH, 99%), bovine serum albumin (BSA), Tween 20, sucrose, poly-vinylpyrrolidone (PVP, 10k) and *N,N*-dimethylformamide anhydrous (DMF, $\geq 99.8\%$) were purchased from Sigma-Aldrich, St. Louis, MO, USA. HEPES buffer (1 M) was purchased from Gibco. *N*-succinimidyl *S*-acetylthioacetate (SATA), and sulfosuccinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC) were purchased from Thermo Fisher Scientific, Waltham, MA, USA. An Amicon Ultra centrifugal filter (0.5 mL, 30 K, 5 K) was purchased from Millipore, Bedford, MA, USA. The NC membrane strips, sample pads, conjugated pads, absorbent pads, anti-AIV detector antibody, malaria antigen, and the inactivated AIV antigens were provided by Median Diagnostic (Republic of Korea).

Synthesis of lanthanide-doped nanoparticles

Lanthanide-doped nanoparticles were synthesized via thermal decomposition of lanthanide acetate precursors. In a typical synthesis, Ln(CH₃CO₂)₃ (Ln = Y and Nd: 0.5–90%, total 0.4 mmol) or (Ln = Y, Yb, and Tm total 0.4 mmol) was combined with 3 mL of oleic acid and 7 mL of 1-octadecene. This mixture was heated to 150 °C for 60 min, then cooled to 50 °C. Subsequently, a methanolic solution of NaOH (1 mmol, 2 mL) and NH₄F (1.6 mmol, 4 mL) was gradually added to the oleate-lanthanide solution while stirring at 50 °C. The solution was then heated to 100 °C and degassed using vacuum pump to eliminate residual water and methanol. Next, the solution was heated to 300 °C and maintained under argon for 1 h before cooling to room temperature. The precipitated nanoparticles were collected by centrifugation, washed, and finally re-dispersed in 2 mL of cyclohexane. Core@-shell nanoparticles were synthesized following a procedure similar to that used to synthesize core nanoparticle.

Estimation of critical distance (R_c) between Nd³⁺ ions

According to the literature, R_c between Nd³⁺ ions could be derived by Blasse's equation:

$$R_c = 2 \left(\frac{3V}{4\pi x_c N} \right)^{1/3}$$

where x_c denotes the concentrations of Nd³⁺ ion, N represents the number of lattice sites within the unit cell

that can be occupied by emitter ions, V is the volume of the unit cell. In the case of hexagonal NaYF₄ (JCPDS 28-1192) with a space group of $P6_3/m$ ($N = 1.5$), the unit cell parameters are $a = 5.96$ Å and $c = 3.53$ Å, resulting in a unit cell volume of 108.6 Å³. Consequently, for a 5% doping level, R_c was estimated to be about 14.0 Å (Fig. S2).

Quantum yield measurement

Quantum yields (Q.Y.) of WINP (NaYF₄:5%Nd@NaYF₄) and UCNP (NaYF₄:48%Yb,2%Tm@NaYF₄) measured using the integrating sphere connected to a fiber optic spectrometer (Fig. S5). Initially, the setup was calibrated using a Xenon Arc light sources (THORLABS) to derive the correction factor, which was subsequently used to adjust the measured spectra of the sample. Then, micro quartz cuvette containing a cyclohexane solution of the nanoparticles was positioned inside the integrating sphere, connected to the fiber optics spectrometer (Flame, Ocean Optics), to capture the emission and excitation light signals under 980 and 800 nm excitation. A cyclohexane solution without nanoparticles was used as a reference. The Q.Y. was then calculated using the following equation:

$$Q.Y. = N_{em}/N_{abs} = \int P_{em}(\lambda) \lambda d\lambda / \int [P_{ref}(\lambda) - P_{sample}(\lambda)] \lambda d\lambda$$

where $P_{em}(\lambda)$ is the emission intensity spectrum of the sample, $P_{ref}(\lambda)$ and $P_{sample}(\lambda)$ are the intensity spectra of excitation light recorded after passing through the reference sample, respectively.

Single-particle-level wide-field imaging and spectroscopy

Two types of nanoparticles were imaged using a homemade wide-field spectroscopic imaging system⁵⁹. Before surface modification, the nanoparticles were dispersed in hexane, and those modified with dopamine were dispersed in water. Samples were prepared by spin-coating 10 μ L of each nanoparticle dispersed in hexane or water onto piranha-etched cover glass. The dry samples were imaged as spin-coated, while for wet samples, 20 μ L of water were dropped onto the spin-coated nanoparticles before imaging. The samples on the cover glass were placed on an oil immersion-type objective lens (UPlan-Sapo 60X, Olympus) in an inverted microscope (IX71, Olympus). For the excitation of each nanoparticle, a 795 nm laser from a continuous-wave laser diode (QFLD-795-100S, QPhotonics) mounted on a laser diode driver (MW-SN-0905-0029, MW Technologies) and a 980 nm laser from a CW laser diode (AC1401-0600-0980-SM, Gooch & Housego) mounted on a laser diode driver (CLD1015, Thorlabs) were used, respectively. The 795 and 980 nm laser beams were collimated using lens (LA-1027-B-ML, Thorlabs and LA1509-B-ML, Thorlabs, respectively). The nanoparticles on the samples were



excited by wide-field illumination using another lens (AC508-300-B-ML, Thorlabs). Dichroic beamsplitters (Di02-830R, Semrock for WINP and ZT1064rdc-sp, Chroma for conventional NIR nanoparticle) and optical filters (LP02-808RE-25, Semrock for WINP and FF01-890/SP-25, Semrock for conventional NIR nanoparticle) were used to block the excitation wavelength and gather the photoluminescence emitted from the nanoparticles. Emission images were collected using an electron-multiplying charge-coupled device (EMCCD; iXon3 888, DU-888E-C00-#BV, Andor). Spectroscopic measurements were performed using a CCD camera (PIXIS 400 BR, Princeton Instruments) mounted on a spectrograph (IsoPlane SCT 320, Princeton Instruments).

Monte Carlo simulation for light propagation

We conducted Monte Carlo simulation in MATLAB, employing a technique similar to that previously reported⁴⁰. Photon packet, each with an initial energy E_0 , were delivered into the water, assuming an angular distribution from an optical fiber with a numerical aperture $NA = 0.22$ and anisotropy parameter $g = 0.9$. In non-scattering media such as water, the absorption coefficients were determined to be $\epsilon_{980} = 4 \times 10^{-3} \text{ cm}^{-1} \text{ M}^{-1}$, $\epsilon_{800} = 0.1$, respectively. The index of refraction of water is calculated to be $n = 1.33$. The photon packets were uniformly distributed across a $100 \mu\text{m}$ region in the center of the simulation domain, representing a $100 \mu\text{m}$ diameter optical fiber. The simulation domain, measuring 10 mm in depth and 15 mm in width, was divided into bins.

Dopamine-modified nanoparticles

Nanoparticles (15 mg) were dissolved in THF, while dopamine hydrochloride (50 mg) was dissolved in water. These solutions were combined in a 50 mL flask and heated to 50°C with vigorous stirring. After adding hydrochloric acid (HCl), the resulting NH_2 -ligand was collected through several washing steps.

Ligand exchange with antibody-modified nanoparticles

NH_2 -modified nanoparticles (2 mg) were dispersed in $200 \mu\text{L}$ of HEPES buffer (10 mM). Sulfo-SMCC was also dispersed in HEPES buffer (10 mM), and the two solutions were mixed and incubated for 5 h. A SATA stock solution was added to the pristine AIV-detector antibody ($150 \mu\text{g}$) and incubated for 30 min. Subsequently, a hydroxylamine hydrochloride (HH) solution ($1.75 \mu\text{L}$, 0.5 M) was added and incubated for 2 h. The resulting solution was washed using a 30 kDa centrifuge filter. Thiol-modified antibodies and maleimide-modified NPs were then linked through a click reaction. Finally, the surface of the AIV detector antibody-NPs was blocked with a 1% BSA solution to minimize non-specific binding. The Ab-immobilized WINPs (Ab-WINPs) were

characterized by zeta potential measurements, FT-IR, and ultraviolet-visible (UV-vis) absorbance analysis. The zeta potential of the WINPs decreased from $+35.0 \text{ mV}$ to -36.1 mV after maleimide modification, which then increased to -15.2 mV after Ab immobilization (Fig. S14b). FT-IR analysis showed new peaks at 1542 and 1639 cm^{-1} (amide I and amide II)^{60,61} appeared after Ab immobilization (Fig. S14c). Additionally, the Ab-WINPs showed absorption peak in the 280 nm (Fig. S14d), successful antibody conjugation through a thiol-maleimide reaction between the maleimide groups on the WINPs and the thiol groups on the Abs⁵⁸.

Fabrication of the WINPs-LFA strip

A NC membrane, dispensed with a 1.0 mg mL^{-1} of anti-AIV capture antibody and 1.0 mg mL^{-1} of anti-malaria antibody in the test and control line, respectively, was provided by Median Diagnostic (Republic of Korea). A conjugate pad was pretreated with $15 \mu\text{L}$ of blocking reagents [1.5% (w/v) BSA, 2% (w/v) sucrose, 1.5% (w/v) Tween 20, and 0.25% (w/v) PVP in water]. After drying at 30°C for 30 min in a vacuum oven, anti-AIV detector antibody-conjugated WINPs and malaria antigen-conjugated WINPs were dried on the conjugate pad at 30°C for 30 min in a vacuum oven. Finally, the LFA strip was fabricated by stacking the as-prepared NC membrane, as-pretreated conjugate pad, sample pad, and absorbent pad with a 2 mm overlap on an adhesive backing card and cut to a width of 4 mm. A conjugate pad, sample pad, absorbent pad, and backing card were provided by Median Diagnostic (Republic of Korea).

AIV detection using the WINPs-LFA

We evaluated analytical sensitivity of the WINPs-LFA using different concentrations of AIV (0 , $2 \times 10^{3.5}$, $2 \times 10^{4.0}$, $2 \times 10^{4.5}$, $2 \times 10^{5.0}$, $2 \times 10^{5.5} \text{ EID}_{50} \text{ mL}^{-1}$), which were prepared by serial dilution using PBS buffer (pH 7.4). The AIV samples were mixed at a ratio of 1:1 with the loading buffer, which facilitates the lysis of the nucleoprotein (NP) within AIV. The NIR luminescence signal of WINPs was measured 15 min after adding $100 \mu\text{L}$ of AIV samples to the sample pad using an intensified sCMOS camera with an 850 nm longpass filter (Thorlabs, FELH0850), under excitation at 800 nm with an 800 nm band pass filter (Semrock, ff-01-800/12-25). An additional 75 mm focal length lens (Edmund, #54-691) was fixed at the C-mount thread of the camera. For the control experiments, UCNPs were also applied in an identical procedure, denoted as UCNPs-LFA. The NIR luminescence signal of UCNPs were measured using an intensified sCMOS camera with an 800 nm band pass filter, under excitation at 980 nm. The AIV samples and loading buffer were provided by Median Diagnostic (Republic of Korea).



We validated the clinical applicability of the WINPs-LFA using 65 clinical samples including 40 AIV positive and 25 AIV negative samples. The positive samples, collected at 1dpc (days post challenge), 3dpc, and 5dpc, were provided by Konkuk University and Kyungpook National University. The animal experiment procedures were approved by the institutional animal care and use committee of Korea University College of Medicine (Protocol Number: KUIACUC-2023-246) and performed in accordance with the regulations and guidelines of this committee. The negative samples were collected as cloacal swab of uninfected avians and provided by Median Diagnostic (Republic of Korea). The samples were confirmed by real-time PCR. 100 μ L of samples mixed at a ratio of 1:1 with the loading buffer were added to the sample pad. Then the AIV detection was performed as described above.

Statistical analysis

Statistical analyses were performed using Origin Pro 2016 and IBM SPSS statistical package (version 27). Means and $\pm$ standard deviations were calculated for each data point from at least triplicate measurements. The LOD was calculated using the IUPAC guidelines [$\text{LOD} = \text{mean} + 3.3\sigma$ of the blank; σ is standard deviation]. Statistical significance of differences between AIV positive samples and AIV negative samples was evaluated using a two-tailed unpaired *t*-test ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$).

Characterizations

Sample images were analyzed on a JEN-2100F (JEOL Ltd.) installed at the Hanyang LINC3.0 Center for Research Facilities (Seoul, Republic of Korea). The XRD patterns of the NPs were recorded by an XRD-7000 diffractometer. A Zetasizer Nano ZSP instrument (Malvern Co., UK) was used to determine the zeta potentials of the WINPs. The Fourier transform infrared (FT-IR) spectra of the WINPs were obtained by using an iS50 Fourier transform infrared spectrophotometer (Thermo Fisher Scientific Co., USA) installed at Center for Polymers and Composite Materials (Hanyang University, Seoul, Republic of Korea). The photoluminescence emission spectra were recorded using a Flame spectrometer (Ocean Optics, Inc., USA) under external excitation at 800 or 980 nm provided by an infrared diode laser (Changchun New Industries Optoelectronics Tech. CO., China). A Jasco V-700 UV-Vis spectrophotometer (Jasco., Japan) was used to record the absorbance spectra. The lifetime and images were obtained by the iStar intensified sCMOS camera (Andor) using time-gated imaging technology. Their time gate delay was 100 μ s, and the time gate width was 50 μ s. All practical images were obtained under the irradiation of

an 808 nm and 980 nm laser (CNI, MDL-H-808-5W, MDL-III-980-2W) with a power density of 0.5 W cm^{-2} . The NIR emission wavelength was selectively measured using a bandpass filter (Semrock, ff-01-800/12-25, Edmund 980 nm CWL, 12.5 mm Dia., Hard Coated OD 4.0 10 nm), longpass filter (Thorlab, FELH0850) and shortpass filter (Semrock, ff-01-950/sp-25) placed in front of an all detectors. The optical properties were measured using a tunable laser EKSPLA NT-342.

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Author contributions

D.K. carried out the synthesis of WINPs, the overall measurements, Monte Carlo simulation, and the data analysis. S.K. performed the biosensors experiments and data analysis. D.K. and S.K. wrote the original manuscript. Y.G. and M.K. carried out the measurements of single-level imaging from nanoparticles and data analysis. S.H.N. and J.L. conceived and designed the study. S.-H.L. and J.-H.K. provided the clinical samples and the data analysis. S.H.N. and J.L. are responsible for supervision of project administration, funding acquisition, and writing—review and editing.

Data availability

The authors declare that all data supporting the finding of this study are available within the paper and its Supplementary Information files. The remaining data are available within the article, Supplementary Information, or Source data file.

Conflict of interest

The authors declare no competing interests.

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