

# Improving Immunoassay Sensitivity with Upconverting Nanoparticles

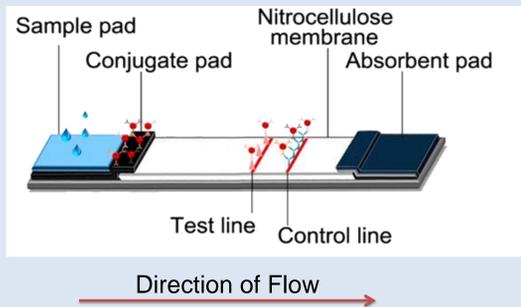
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## Abstract

Nanoparticles are now commercially available which can perform highly tunable two-photon upconversion, onto which proteins may be immobilized. When illuminated with near-IR light, these nanoparticles emit strong green light via anti-Stokes emission. These materials may have great utility in augmenting the function of lateral-flow immunoassays. For example, proteins coupled to upconverting nanoparticles have been detected at < 3 pg/mL, whereas conventional gold nanoparticles commonly used in current lateral flow immunoassays can be a thousand times less sensitive at ~10 ng/mL. The use of these nanoparticles may enable both a lower limit of detection (versus conventional optical-density based detection of gold particles) and genuine multiplexing (by using nanoparticles tuned to emit different wavelengths of light). Herein, we report on our adaptation and characterization of upconverting nanoparticles for use in lateral flow immunoassays.

## Hypothesis

The limit of detection of lateral flow immunoassays will be improved by replacing the conventional colloidal gold reporter with rare-earth lanthanide upconverting nanoparticles (UCNPs).

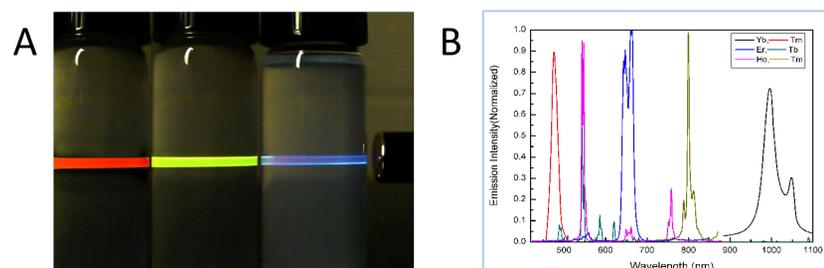


**Figure 1.** A Lateral Flow Assay. A liquid sample containing an analyte is placed on a sample pad, the fluid then migrates laterally to a site where the analyte binds to an antibody that is conjugated to colloidal gold. The analyte bound to the antibody-nanoparticle conjugate migrates further along the membrane where it binds to a second capture antibody (specific to the target) that forms the test line; unbound antibody-gold conjugate then binds to the control line (anti-species antibody), indicating a successful assay. Adapted from *Anal. Chem.* **2014**, *86*, 4995-5001.

- ✓ Lateral flow assays provide analytics with little to no sample prep
- ✓ The limit of detection of gold-based LFIs is *ca.* 10 ng/mL

## UCNPs are Tunable

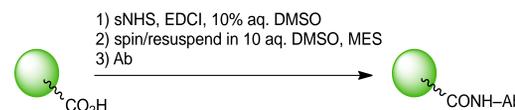
The ability to determine the absorption and emission spectra of the nanoparticles may provide an opportunity to design multiplexed LFIs.



**Figure 2.** (A) Red, Green, and Blue emission from three different UCNPs compositions. (B) Emission spectra is dependent on the elemental composition of the nanoparticles.

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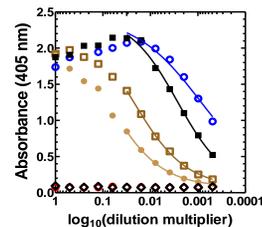
## Conjugate Preparation and Analysis



**Figure 3.** PAA-coated nanoparticles are first activated with EDCI to form the sulfo-NHS activated ester, which is purified and incubated with the antibody of choice to give the conjugate with surface densities proportional to the relative stoichiometries of the reaction.

- UCNPs, coated with polyacrylic acid (PAA), which emit green light when 980 nm light is absorbed, were provided by Intelligent Material Solutions
- The UCNPs were conjugated to an anti-MS2 coat protein antibody at varying concentrations to give conjugates with a range of surface densities

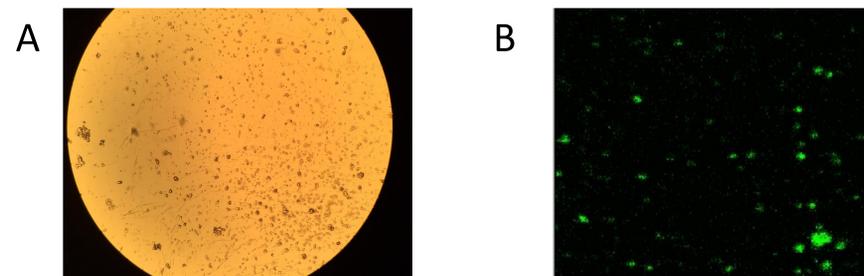
## Antibody Activity is Maintained



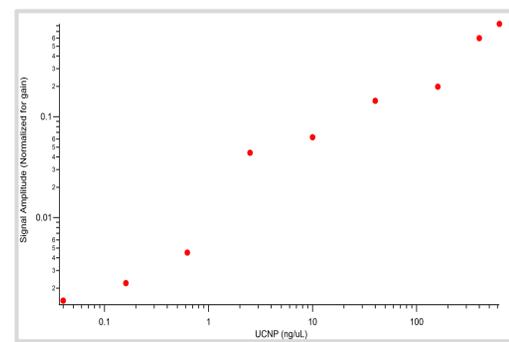
| Controls                                 | UNCP+Ab (μL Ab coupled) |
|--|-------------------------|
| • + Control (Ab only @ 40 ng/μL initial) | • UCNP+Ab50μL           |
| • - Control (no Ab)                      | • UCNP+Ab100μL          |
| • - Control UCNP only (no Ab)            | • UCNP+Ab250μL          |

**Figure 4.** Monoclonal antibodies raised against phage MS2 coat protein maintain reactivity after being coupled to UCNPs by the method described above. By ELISA, antibody reactivity with recombinant MS2 coat protein scales as the relative fraction of antibody coupled to the UCNPs increases. MS2 coat protein recognition of purified antibody (not coupled to UCNPs) is shown (open blue circles) for comparison. Nonspecific binding to uncoupled UCNPs (black open diamonds) is undetectable under these conditions.

## Visualization

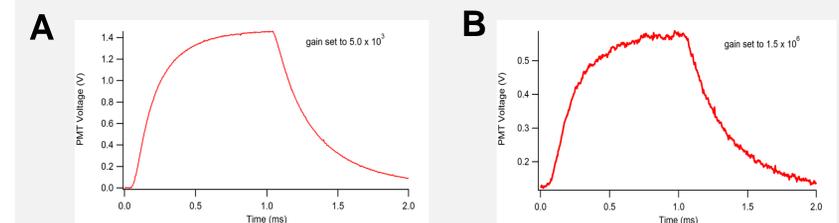


**Figure 5.** Visualization of antibody-coupled UCNPs (A) Bright field view of UCNPs-Antibody conjugates at 10x magnification. (B) UCNPs-Antibody conjugate emission under 980 nm excitation, with a 369 x 369 μm field of view (subset of bright-field image (A)).



**Figure 6.** Titration curve from LFI strips, after normalizing for additional gain on the low end (lowest 3 data points).

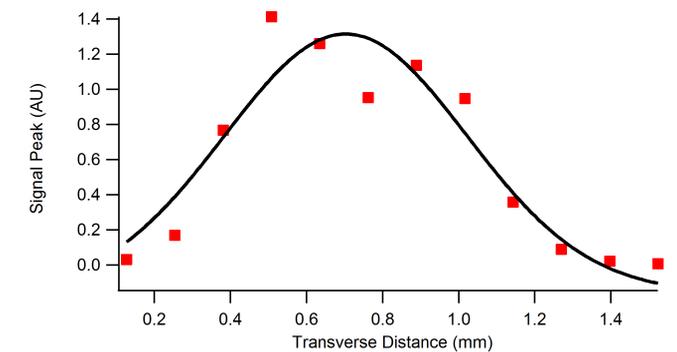
## Visualization



**Figure 8.** Time dependent emission from 625 ng/mL and 40 pg/mL. The PMT gain was initially 0.4V control voltage ( $5 \times 10^3$ ) but was adjusted upward for the low range to 0.9V control voltage ( $1.5 \times 10^6$ ). The electronic gain is  $10^5$ . No light-tight enclosure was used, so low level ambient light was present. The excitation source was a 2W 980 nm laser, and the emission was filtered with a 700nm short pass filter. The laser was turned on and off to allow for gated detection. The time-dependent emission from the 625 ng/mL strip was fit to a double-exponential, and the following parameters were found to best fit the data:

$$A_1 = 0.534 \pm 0.092 \quad A_2 = 0.805 \pm 0.084$$

$$\tau_1 = 0.158 \pm 0.011 \text{ ms} \quad \tau_2 = 0.398 \pm 0.033 \text{ ms}$$



**Figure 9.** The signal in the transverse direction (presumably the direction of flow in a real assay) was measured at optimal z-axis position in increments of 0.005" and plotted above. The signal was fit to a Gaussian, with width =  $0.451 \pm 0.093$  mm.

## Conclusions

- UCNPs offer greater sensitivity than typical performance of gold nanoparticles, with highly tunable spectral response.
- Antibodies maintain target recognition after coupling to UCNPs. UCNPs have negligible nonspecific binding in preliminary studies.
- UCNPs maintain discrete morphology in preliminary LFI visualization experiments.

## Acknowledgements

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