Nanoparticle-Supported, Rapid, Electronic Detecting System for Accessible Infectious Disease Diagnosis

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Highly sensitive infectious disease detection typically relies on Nucleic Acid Amplification Tests (NAAT), which, however, require enzymatic amplifications and are time-consuming. High-sensitivity antigen detection assays, such as enzyme-linked immunosorbent assay (ELISA), require multistep and complex workflow in laboratory settings and are not ideal for rapid and portable use. Lateral flow immunoassay (LFI) are portable for field applications, but typically have a lower sensitivity and thus poorer accuracy. Indeed, there is often a tradeoff between sensitivity and the assay cost/complexity. To bridge the technology gap for accessible, rapid, sensitive yet inexpensive tests, our lab is developing a new protein and antibody detection method and system [1], i.e. nanoparticle-supported, rapid electronic detection (NasRED), as a modular sensing platform with demonstrated limit of detection (LoD) orders of magnitude better than ELISA. In NasRED, gold nanoparticles (AuNPs) are covalently coated with high-density ligands, such as streptavidin proteins, and then functionalized with high-affinity binders, such as monoclonal antibodies (mAbs), nanobodies, or peptides (Fig. 1 a-b). Fundamentally different from conventional ELISA assays, Nano2RED is an in-solution assay without a stationary reaction surface, but relying on AuNPs to actively bind target molecules upon mixing and to form clusters that subsequently precipitate from the solution (Fig. 1 c-d). Without any amplifications, the AuNPs serve to improve multivalent protein binding, create a concentration-boosting effect after centrifugation to further promote detection at ultra-low target molecule concentrations (down to attomolar), accelerate signal readout within 15 to 30 min from sample mixing, and also act as an effective optoelectronic beacon to scatter light and help produce electronic signals from a simple portable electronic detection (PED) system rather than sophisticated microscopy and spectrometers (Fig. 1 e-f).

I will present our most recent progress of engineering the NasRED systems with high analytical sensitivity as low as atto-molar range for a variety of infectious antigens, including Ebola virus, SARS-CoV-2, African swine fever, Lyme disease, etc. Uniquely, NasRED employs low-cost electronic circuitry for readout and digital signal transmission, supporting future automated operation. Because NasRED requires no labeling/washing or enzymatic reaction, it significantly reduces the processing complexity and minimizes sample volume, i.e. 4 to 8 μL of biological fluids, such as serum, saliva, nasal fluidis, urine, and whole blood. The reagent cost is estimated ~$0.1 per μL, or ~$2 per test, and large-scale production of AuNP sensors may further lower the material cost to <~$0.01 per test.

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References: [1] X. Chen *et al.*, Biosens. Bioelectron. **202**, (2022).

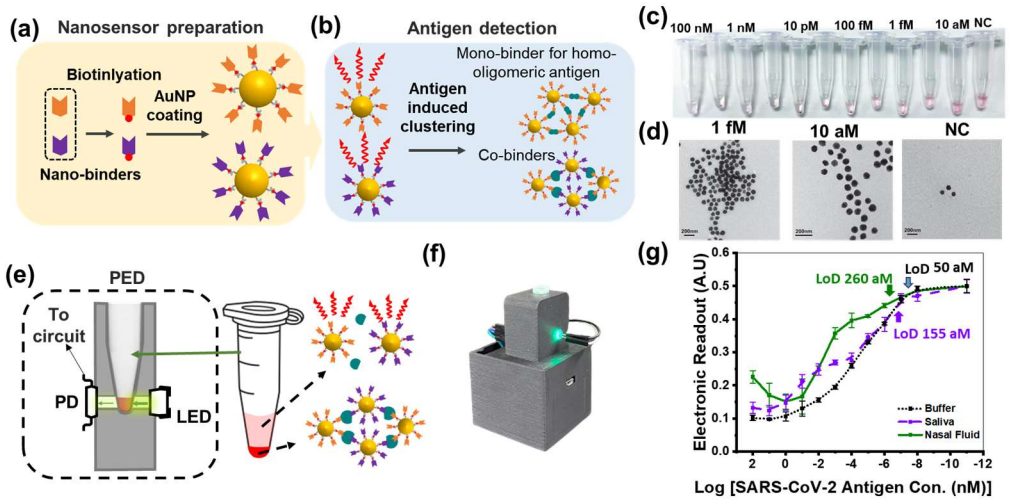

Fig. 1. NasRED process flow in antigen tests. **(a-b)** Modular nanosensor preparation and sensing scheme. The streptavidin-coated AuNPs can be functionalized with biotinylated monobinders to detect homo-oligomer antigens or non-competing co-binders to detect any antigens. **(c)** Optical images of SARS-CoV-2 N-protein sensing from 100 nM to 10 aM in buffer (1×PBS, 20% v/v glycerol and 1 wt% BSA). NC: negative control. **(d)** Exemplary transmission electron microscopy (TEM) images showing AuNP clustering at different antigen concentrations. **(e)** Schematic showing PED system that detects floating AuNPs in the supernatant after reaction. **(f)** An optical image of PED system, where the LED and photodetectors are mounted on 3D-printed tube holder and circuit elements are built on a PCB board. **(g)** Measured sensing curves for SARS-CoV-2 N-protein in PBS buffer (black, LoD 50 aM), saliva (purple, LoD 155 aM), and nasal fluids (green, LoD 260 aM).