Nanoparticle-assisted, Portable Detection of African Swine Fever Infection

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African swine fever (ASF) is a highly contagious and often lethal infectious swine disease without effective treatment or vaccine. The current commercial methods in ASF diagnostics involve high-cost and complex operations such as Polymerase Chain Reaction (PCR) or Enzyme-Linked Immunosorbent Assay (ELISA), which are not ideal for high-frequency and accessible deployment of tests. There is a critical need for portable, low-cost, yet accurate ASF tests to detect both antigens and antibodies for disease detection at different infection stages [1].

We propose and demonstrate a novel nanoparticle-based, rapid, in-solution assay for ASF detection (Figure 1). This assay does not require washing, amplification, or labeling, thereby lowering reagent consumption and test costs. Our design concept introduces a novel signal transduction for antigen- and antibody-sensing, based on antigen-antibody binding induced metal nanoparticle aggregation and subsequent plasmonic color change. Gold nanoparticle (AuNP) precipitation accelerated by centrifugation followed by vortexing, resulting in resuspension of non-reacting floating AuNPs. For rapid and accurate readout, we design a portable electronic detector (PED), comprising a light-emitting diode (LED), photodiode, battery, and signal-processing circuitry, towards pen-side diagnostics [2,3].

First, we evaluated the limit of detection (LoD) of our sensors by spiking ASF antibodies and antigens in buffers and swine sera. The LoDs were found ~2 fM for ASF antibody in physiologic buffer and ~8 fM in swine sera with a broad (7 logs) dynamic range. The LoDs for ASF antigens were ~330 fM in physiological buffer and ~1.7 pM in swine sera with >5 logs of dynamic range (Figure 2). Further, we tested swine sera samples from infected pigs, and demonstrated positive detections of antibodies and antigens with 4 and 8 titration logs, respectively, in agreement with positive sample titration ELISA (1:10000) [4]. Further, our assay selectively detected the P72 antigen among other ASF antigens, indicating its specificity. The costs for the test and the portable readout system were estimated less than $2 and $20, respectively. The time from sample mixing to signal readout was within 20 minutes. Our sensing platform presents a significant advancement for ASF diagnostics on the pen side to contain the worldwide spread of ASFV.

References:

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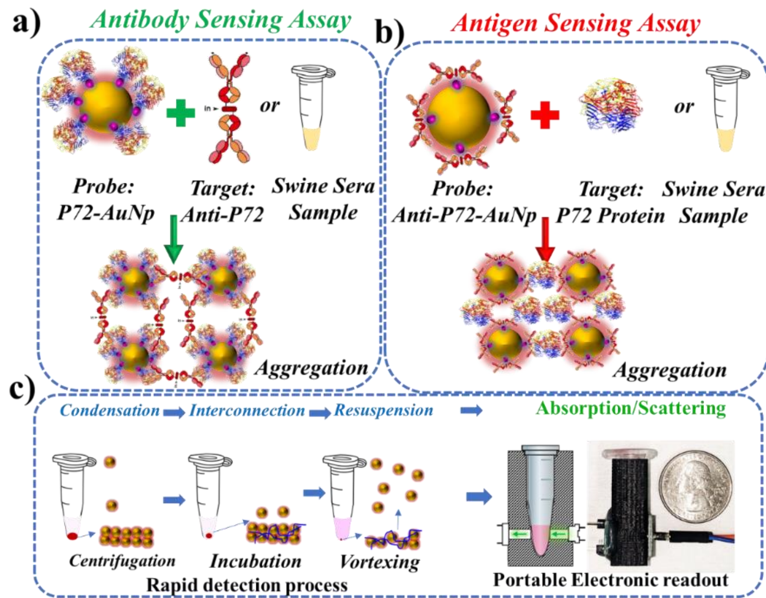

Figure 1. Schematics of Design concept. a) Antibody/(b)antigen sensing using antigen/antibody coated AuNps mixed with spiked antibody/antigen or swine sera sample leading to aggregation of AuNps. c) Rapid assay including centrifugation condensation of probes, incubation for linkage formation, vortexing to resuspend non-reacting probes, and subsequent electronic readout of plasmonic color changes.

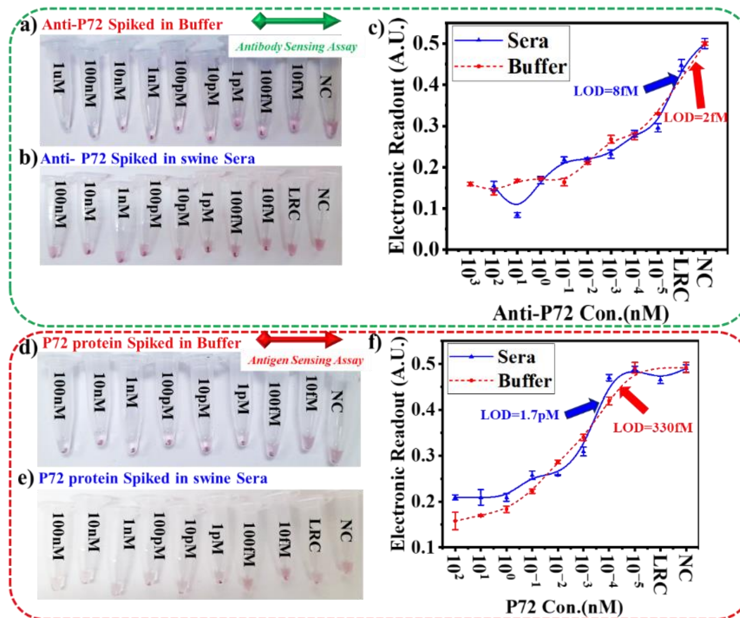

Figure 2. Detection of ASF p72 antigen and antibody spiked in swine sera. *(a-b,d,e) Optical pictures of the testing tube samples ready for PED readout. The p72 antigens(red panel)/antibodies(green panel) were spiked in (a,d) PBS buffer and (b,e) negative ASF swine sera, mixed with AuNPs functionalized with p-72 antibodies(red panel)/antigens(green panel), centrifuged, incubated, and vortex-agitated. c) Collected electronic signals for antibody assay and f) antigen assay samples when tested in serum (blue lines) and buffer (red dashes).*