Nanoparticle-Based Assay with Optoelectronic Readout for High-Sensitivity and Rapid Detection of Infectious Diseases

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Inexpensive and rapid diagnostics of infectious diseases is crucial to timely treatment and disease prevention. Conventional diagnostic methods such as reverse-transcription polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) require skilled personnel, laboratory-hosted equipment and elaborate diagnostic protocols. Here, we present a gold nanoparticle (AuNP) based assay as a low-cost, simple and quantitative sensing platform.

Here we introduce a universal assay design procedure for rapid diagnostics of infectious diseases, consisting of three key steps (Fig. 1). First, high-specificity and high-affinity nanobodies were produced by a high-throughput protein-nanobody phage selection method from a synthetic library, which could be completed within two weeks [1]. Next, the antigen-specific biotinylated nanobodies were coated on streptavidin-bound AuNPs through biotin-streptavidin conjugation, readying the AuNP sensors to recognize target antigens. The antigen-nanobody binding triggers the AuNPs to bridge, aggregate, and precipitate. The resultant decrease of AuNP monomer concentration in assay supernatant leads to reduced extinction of the suspension, therefore changing the visual color of assays. The color change can be detected by naked eye and quantified by spectrometer or optoelectronic devices.

Using Ebola virus disease biomarker, a dimeric soluble secreted glycoprotein (sGP), as an example, we investigated the assay performance (Fig. 2). Here, high-affinity nanobodies sGP49 (17.1 kDa, K_D=4.6 nM) were selected (in Prof. Gu's lab at Univ. Washington) and surfaced functioned on 80 nm AuNPs. For rapid detection, AuNPs were mixed with sGP in fetal bovine serum and centrifuged at 1,200 g for 1 minutes, briefly incubated for 20 minutes and vortexed. The brief centrifugation greatly concentrated AuNP, and hence accelerated detection. For spectrometric quantification, the AuNPs were loaded in a 3 mm thick customized polydimethylsiloxane (PDMS) well plate (Fig. 2a and 2b). Visual inspection indicated that sGP at >1 nM concentration could be easily detected by naked eye (Fig. 2a). Quantification of the optical extinction maxima could accurately determine sGP concentrations from ~10 pM to 1 μM, achieving a broad dynamic range of 5 decades and a detection limit down to ~40 pM (Fig 2d and 2e). We further devised a highly portable, small-footprint optoelectronic readout system (Fig. 2c). The attenuation in LED transmittance was correlated with AuNP extinction, producing measurable photo-voltage (or current) on a photodiode as detector. The electronic readout system offers an accuracy in sGP quantification comparable to spectrometers but at a much lower cost (only a few dollars). It also has the potential to automate test data reporting and storage. Compared to traditional assays such as ELISA, our assay has 1-2 orders higher sensitivity (Fig. 2f), and can be developed within as short as one month compared to 3-6 months for ELISA. This makes our assay design concept and development uniquely appealing for fast deployment in massive disease screening and diseases containment.

Acknowledgement

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References

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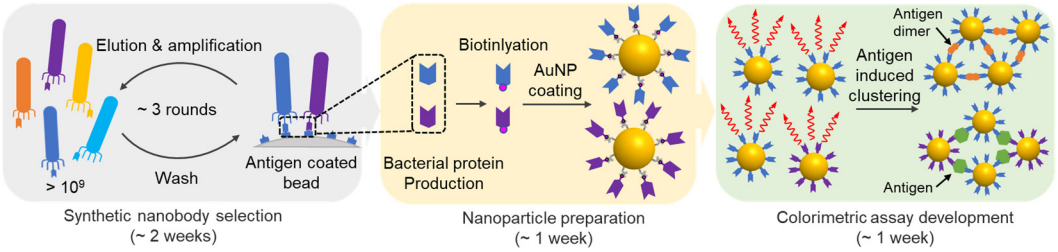

Fig. 1. Gold nanoparticle based colorimetric assay development workflow. High-affinity nanobodies are selected by phage display method with three or more cycles in the Gu lab. The selected nanobodies are biotinylated and then coated on gold nanoparticles. For multivalent antigens, a single nanobody is sufficient, while monovalent antigens require a pair of cobinding nanobodies. The presence of antigens leads to AuNP bridging, subsequently forming aggregates and precipitates. The color presentation of AuNP suspension is attributed to the total extinction of the plasmonic AuNPs, which decreases at higher antigen concentration as the AuNPs aggregate and precipitate rate increases.

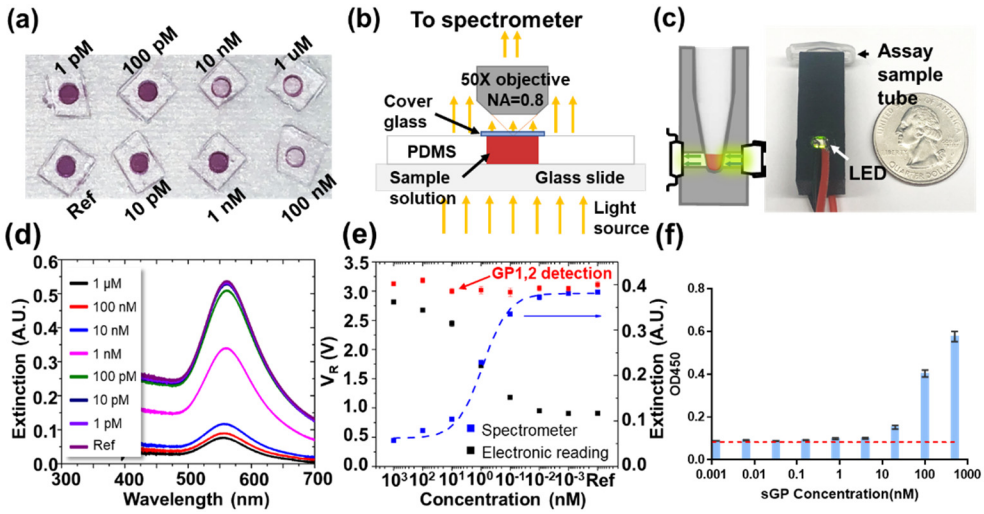

Fig. 2. Demonstrated assay performance in Ebola sGP protein sensing. (a) Assay samples in detecting sGP loaded in PMDS well plate. (b) Optical measurement using microscope-coupled spectrometer system. (c) LED-photodiode based optoelectronic readout systems. (d) Spectroscopic measurements of assay samples in (a). (e) Comparison of signals measured by lab-based spectrometer (assay extinction at 559 nm, blue) and electronic reading systems (voltage measured by a handheld multimeter, black). GP1,2 protein was used for specificity test. (f) Conventional sandwich ELISA test results of sGP.

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