Rapid Quantification of SARS-COV-2 Neutralizing Antibodies Using Electronic Nanoparticle Sensors

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Infectious diseases such as COVID-19 can cause serious damage to human health and the global economy. Natural infection or vaccination triggers an immune response comprising the expression of neutralizing antibodies in the bloodstream against the Severe Accurate Respiratory Syndrome Coronavirus 2 (SARS-COV-2) virus. However, the heterogeneity in human immunity, manifested by inadequate protection or rapid decay in some individuals, as well as the constant immunity-escaping mutations of emerging variants, continue to pose a challenge. Thus, quantitative immunity screening that can be quickly adapted against virus mutations is viewed important for targeted therapeutic antibody development and individualized assessment. Conventional assays to evaluate immunity, such as Foci reduction neutralizing assay (FRNT) and enzyme-linked immunosorbent assay (ELISA) are expensive, time-consuming, systematically biased, labor intensive, and in the case of FRNT, based on live viruses requiring a hardly accessible biosafety level 3 laboratory (BSL3). In this study, we propose and demonstrate a low-cost, widely accessible, rapid, sensitive, digital neutralizing assay (Fig. 1) that can distinguish the nAb potency against various variants and quantitatively measure the neutralizing ability of human serum, without the need for live viruses. The method simulates the components of neutralizing reaction by utilizing human angiotensin-converting enzyme 2 (ACE2) functionalized gold nanoparticles (ACE2-AuNPs) as the signaling beacon receptor cell representative, SARS-COV-2 receptor binding motif (RBD) of the spike (S) protein as the virus antigen and target neutralizing antibodies (nAbs) as the immunity indicator. The competition between the ACE2-AuNPs and nAbs for binding to the virus antigen directly determines the amount of free-floating AuNP beacons. For example, with high-affinity, high-concentration nAbs targeting the binding epitopes of the variant RBD proteins, AuNPs are protected from precipitation and produce colorimetric signals (Fig. 2). Such signals can be better quantified with a portable electronic detector (PED), comprising a light-emitting diode (LED), photodiode, battery, and signal-processing circuitry, similar to our prior work [1]. NAb neutralizing potency was measured for mAbs in buffer (Fig. 2) and human serum samples (Fig. 3) demonstrated the feasibility of detecting nAb concentrations over 4 logs and quantification of the nAb through mAbs spiked in serum. The proposed methodology shows great promise in low-cost, rapid (<20 min) screening for the assessment of therapeutic antibodies and individual immunity against uprising variants.

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References:

[1] X. Chen *et al.*, Biosens. Bioelectron. **202**, (2022), 113971.

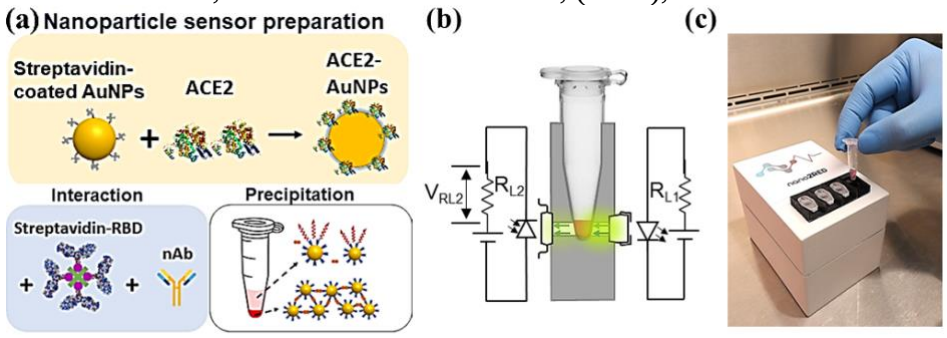

Fig. 1. Schematics and detection system overview. (a) ACE2-functionalized AuNPs (through Streptavidin-biotin reaction) is mixed with the antigen and nAbs, resulting in AuNP precipitation correlated with the level of neutralization. (b-c) Quantitative electronic readout is enabled by electronic circuitry.

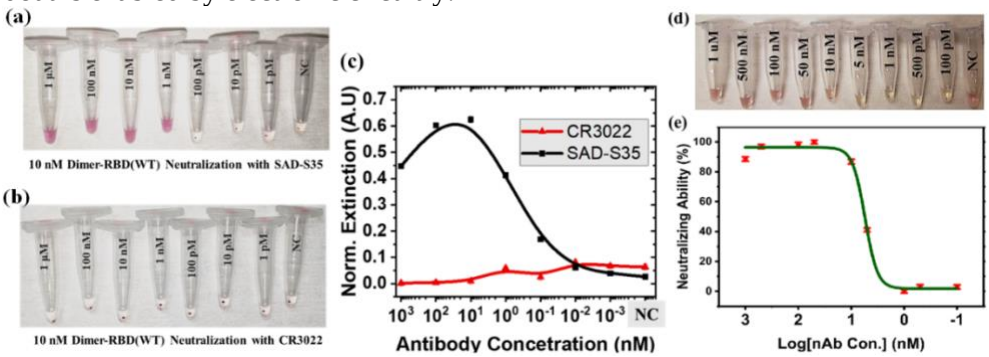

Fig. 2. Colorimetric nAb potency evaluation. (a-b-c) Visual images and neutralization curve of non-neutralizing Ab (CR-3022/red line) and nAb (SAD-S35/black line) against wild-type RBD. (d-e) Visual images and measured optical extinction of neutralizing S1(WT) with monoclonal antibody mixture treatment in serum .

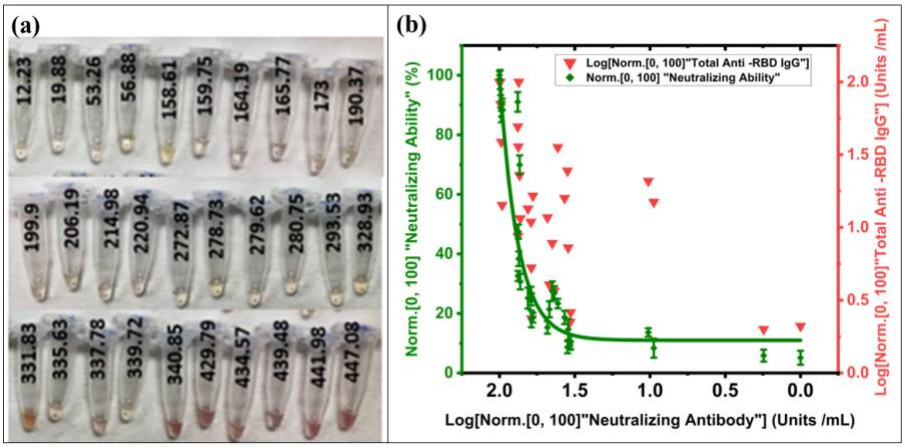

Figure 3. NAb testing of human serum samples. (a) Visual images of human serum samples (with nAb concentration in Unit/ml measured by ELISA). (b) The measured neutralizing ability of the tested samples (green squares with solid line fitting) can be used to quantify the neutralizing ability on our sensor, as plotted against the ELISA measured nAb concentration. As a reference, ELISA results of total count of IgG (red triangles) does not correlate well with the nAb measurements, showing the importance of nAb tests.