Rapid TNF-Alpha Quantification Using Gold Nanoparticles towards Cytokine Monitoring in Inflammatory Diseases

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Tumor Necrosis Factor alpha (TNF-α) is an inflammatory cytokine produced by macrophages/monocytes during acute inflammation, playing a critical role in a wide range of autoimmune and inflammatory diseases, such as Rheumatoid Arthritis, Crohn's disease, etc. The diseases are usually treated with anti-TNF-α antibodies (e.g. Adalimumab); however, the disease management is challenged by the elicitation of immunogenic responses, including anti-drug antibodies (ADA)[1]. Common methods of TNF-α assays such as enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and bioassays are sensitive but costly and time-consuming. In this study, we propose an innovative approach making use of plasmonic gold nanoparticles (AuNPs) to rapidly and accurately quantify TNF-α at the presence of anti-TNF-α Abs and ADA to improve disease treatment efficacy. Our in-solution assay employs Tumor Necrosis Factor Receptor 2 (TNFR2) functionalized gold nanoparticles (TNFR2-AuNPs) as signaling beacon receptors, TNF-α as the antigen, Adalimumab as the therapeutic agent, and ADA as the immune system indicator. The competition between TNFR2-AuNPs and Adalimumab for TNF-α binding directly influences the AuNPs clustering and therefore the amount of free-floating AuNP beacons. With an ample amount of Adalimumab binding to TNF-α, AuNPs are shielded from clustering, generating colorimetric signals due to plasmonic extinction (refer to Fig. 1). Conversely, the presence of ADA blocks Adalimumab epitopes, allowing free TNF-α to bind with TNFR2-AuNPs and form AuNP aggregates. These AuNP extinction signals can be accurately quantified using our portable electronic detector (PED), which includes a light-emitting diode (LED), photodiode, battery, and signal processing circuitry, similar to our prior works[2]. To prove the concept, we have tested various concentrations of TNF-α, Adalimumab, and ADA in PBS buffer and serum (see Fig.2), demonstrating the feasibility of detecting TNF-α concentrations over a range of 3 logs. Our methodology proves to be highly effective in swiftly, economically, and sensitively detecting TNF-α using a portable optoelectronic system with a detection time of 30 minutes and a low limit of detection in the pM range across different stages of clinical treatment.

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[1] M Ogric, et al. Immunol Res. 2017 Feb;65(1):172-185.

[2] MDA Ikbal, et al. ACS Sensor. 2023, 8, 12, 4696–4706

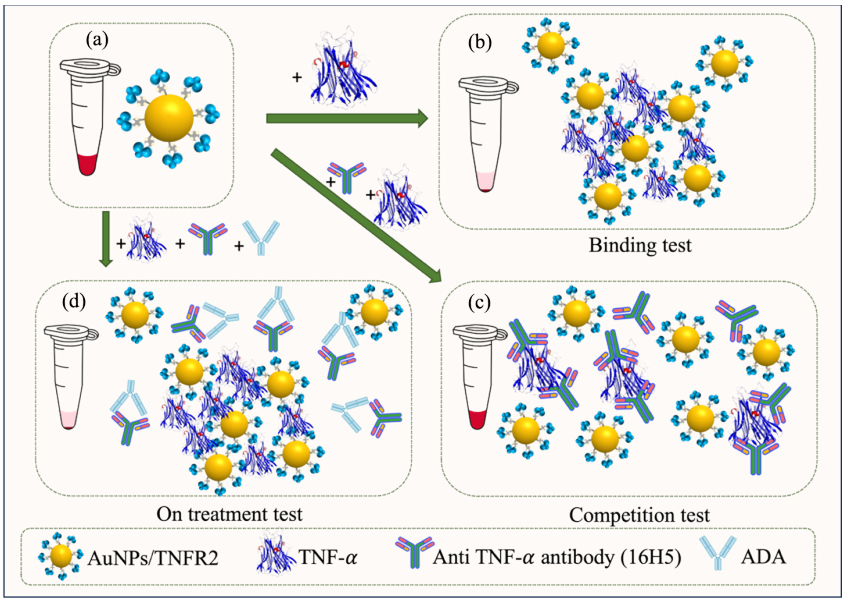

Figure 1: Schematic of assay design in therapeutic monitoring of TNF-α (a) TNFR2-AuNPs, (b) mixing TNF-α with the TNFR2-AuNPS solutions, where AuNP aggregation forms at a high TNF-α concentration,(c) detection in a hypothetical on-treatment scenario where anti TNF-α antibody is present, and (d) detection in a hypothetical scenario that a patient is under treatment with anti-TNF-α and ADA is also present.

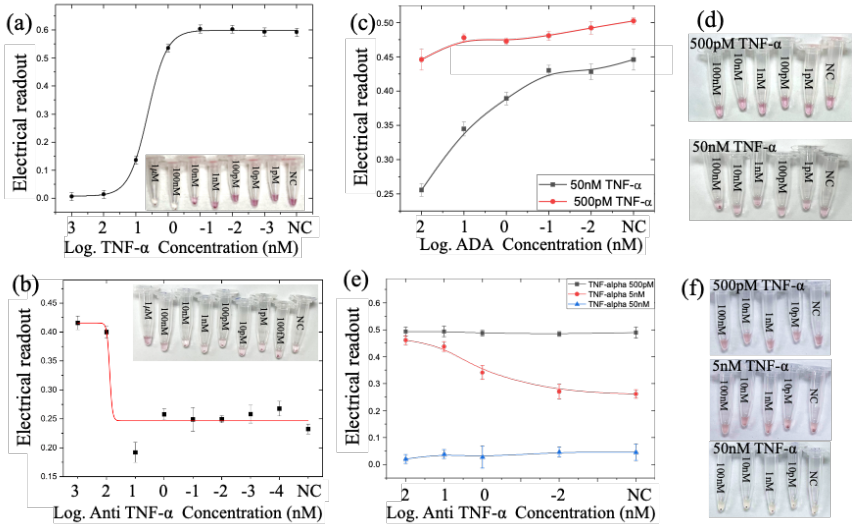

Figure 2: Demonstrated assays in TNF-α antigen sensing (a) TNF-α detection in PBS using TNFR2-AuNPs, showing a high concentration of TNF-α can aggregate AuNP probes. (b) Impact of anti-TNF-α concentration in serum in the presence of 50nM TNF-α, showing a high concentration of anti-TNF-α can neutralize TNF-α and free AuNPs. (c-d) Impact of ADA concentration in PBS in the presence of 75nM anti-TNF-α in high (50nM) and low (500pM) concentrations of TNF-α, showing neutralization of ADA and anti-TNF-α, and visual images of the test (e-f) Impact of anti-TNF-α concentration needed to neutralize 50nM, 5nM, 500pM TNF-α in presence of 10nM ADA in serum sample, and visual images of the tests. If the concentration of TNF-α is high, despite of concentration of anti-TNF-α and ADA, all probes will aggregate and vice versa. In medium amount of TNF-α, effect of Anti-TNF-α in presence of ADA is shown.