Sensitive Optoelectronic Detection of Small Molecules Using Portable Metal Nanoparticle Readers

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The universal presence of small molecules in our surroundings, from toxins, vitamins, and hormones to drugs, makes it highly desirable to develop inexpensive, sensitive, and simple-to-use systems that can specifically detect and quantify these molecules. Mass Spectrometry (MS) and enzyme-linked immunosorbent assay (ELISA) are the two platforms most commonly used for small molecule detection but costly and time-consuming [1]. Here, we report a more sensitive and rapid detecting system with an inexpensive and portable optoelectronic readout that greatly improves the detection limit (by 8 times) and reduces the detection time (to <2 hours) compared to ELISA [1]. Specifically, multivalent small molecule sensors were designed by conjugating nano-binders targeting non-competing epitopes on the same molecules onto gold nanoparticles (AuNP). Such AuNP sensors aggregate through specific biochemical binding at the presence of the target molecules, and accordingly precipitate, resulting in increased solution transparency. The resultant color change is therefore strongly correlated with the target molecule concentration, and can be further quantified using a spectrometer or a customized portable electronic readout system comprising light-emitting diode (LED), photodiode, and battery. Using Cannabidiol (CBD) as the target, we demonstrate sensitive detection of <100 pM in urine and saliva with a high specificity (distinguishable from closely related Tetrahydrocannabinol (THC)) and a large dynamic range (5 logs).

Our molecule detection scheme (Fig. 1) included two simple steps. First, nanobody heterodimers, i.e. capture binder (CA1, ~17 kDa, KD ~6 μM) and dimerization binder (DN1, ~17 kDa, KD ~56 μM), were functionalized onto 80 nm AuNPs through biotin-streptavidin conjugation (Fig 1a). The multivalent binding nature of such sensors served to promote higher functional affinity, beneficial to improving the detection sensitivity. Then during detection, we pre-incubated the CBD molecules with CA1-AuNPs for 1 hour and briefly centrifuged (1200g for 2 mins). The pre-incubation step enabled us to use CA1-AuNPs as a carrier for the CBD, which otherwise would not precipitate (Fig 1b). The centrifugation steps confined CBD-CA1-AuNPs and DN1-AuNPs at the bottom of the tube, creating a spatially and temporally high-concentration reaction zone (Fig 1b). We estimated that this reaction zone is only ~2% of the total solution volume. This small volume and high concentration greatly improve the AuNP aggregation rate and reduce the sedimentation time. As a result, 20 mins incubation after mixing was sufficient for AuNP aggregation and subsequent precipitation for CBD signal readout. This new sensing scheme significantly simplifies the detection by both reducing the readout time and eliminating washing, dye labelling, or amplification.

For signal readout, the solution transparency (Fig 2a) can be quantified by spectrometric or optoelectronic readout. For spectrometric detection, we extracted the top liquid from the tube (~5 μL) and loaded it into a custom-made PDMS plate (Fig 2b), and took UV-VIS spectral measurement (Fig 2c). The plotted extinction peak values vs CBD concentration showed a limit of detection (LOD) of ~165 pM

-in urine and ~198 pM for saliva sample with a large dynamic range (5 logs) and a high specificity against THC (Fig 2d). Next, for optoelectronic detection, we directly used the assay tubes inside our portable readout system, consisting of a LED as a light source, a photodiode as a detector, and a 3D printed sample tube holder (Fig 2e) [2]. Transmitted light through assay colloids was converted to voltages and measured by a handheld multimeter and demonstrated reliable quantification with the LOD (~88.5 and ~97.5 pM for urine and saliva), ~8 times better (Fig 2f) than ELISA [1]. This enhanced performance can be attributed to the reduced signal deviation in the electron reading. Our low-cost optoelectronic small molecule detection system holds great promise in the fields of drug development, controlled substance detection as well as therapeutic and personalized drug dosage monitoring.

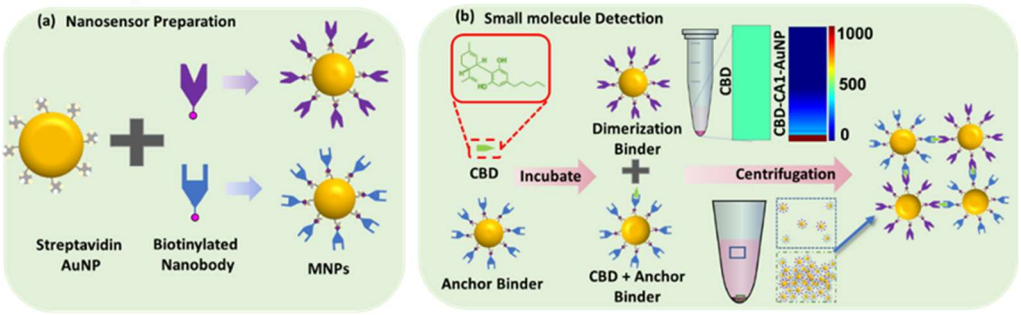

Fig. 1. Overview of small molecule detection assay development. (a) Streptavidin-coated AuNPs were functionalized with biotinylated nanobodies. (b) Detection phase schematic with Pre-incubation and Centrifugation step. Top particle density map shows relative CBD location with and without Pre-incubation. Tube schematic showing AuNP aggregate precipitation and clear upper liquid after crosslinking.

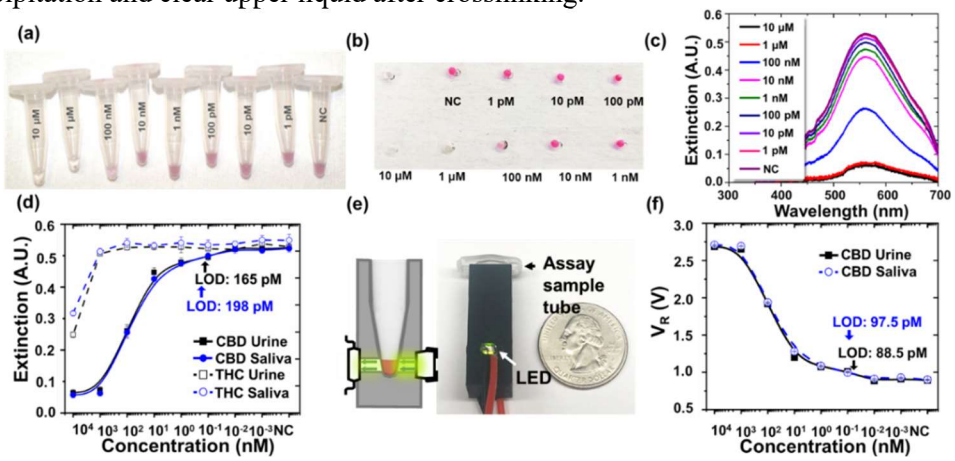

Fig. 2. Demonstrated assay performance in CBD detection. (a) Visual images of samples in microcentrifuge tubes, right after vortexing. (b) The upper-level liquid samples loaded in PDMS well plate. (c) Extinction spectra measured from PDMS well plate. (d) Extinction peak (559 nm) values plotted against THC (Dashed) and CBD (Solid) concentration in Urine (Black Square) and Saliva (Blue Circle) (e)LED-Photodiode electronic readout systems [2]. (f) Voltage reading plotted against CBD concentration in Urine (Black Solid square), Saliva (Blue Dashed Circle).

References:

[1] S. Kang et al., Journal of the American Chemical Society, vol. 141, no. 28, pp. 10948-10952, 2019.

[2] X. Chen et al., Biosensors and Bioelectronics, vol. 202, p. 113971, 2022.