Quantifying Biomolecular Binding Constants using Video Paper Analytical Devices

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Abstract: A novel ultra-low-cost biochemical analysis platform to quantify protein dissociation binding constants and kinetics using paper microfluidics is reported. This approach marries video imaging with one of humankind’s oldest materials: paper, requiring no large, expensive laboratory equipment, complex microfluidics or external power. Temporal measurements of nanoparticle–antibody conjugates binding on paper is found to follow the Langmuir Adsorption Model. This is exploited to measure a series of antibody–antigen dissociation constants on paper, showing excellent agreement with a gold-standard benchtop interferometer. The concept is demonstrated with a camera and low-end smartphone, 500-fold cheaper than the reference method, and can be multiplexed to measure ten reactions in parallel. These findings will help to widen access to quantitative analytical biochemistry, for diverse applications spanning disease diagnostics, drug discovery, and environmental analysis in resource-limited settings.

Today, a new generation of low cost consumer electronic-based biosensors is emerging[1–2] with the potential to dramatically widen access to analytical chemistry capabilities in resource-limited settings.[3–5] This emerging field seeks to harness: mass manufactured sensors found within smartphones, such as cameras, to electronically capture test results; phone battery to power external devices; processing power to analyze results; screens to display results; and connectivity to transmit geo-located results to central databases. There is increasing interest in the use of smartphones to detect results from lateral flow tests. Lateral flow tests, also known as microfluidic paper-based analytical devices (μPADs), including 2D[6] and 3D structures[7–9] and paper origami,[10–13] are opening up new capabilities for multiplexed analysis with small sample volumes and on-test sample handling. The merits of μPADs are their compatibility with a broad range of chemical and biological molecules, low non-specific interactions, low manufacturing cost (as little as $0.001[14]), portability, low sample volumes, safe disposal and power-free fluid pumping, exploiting the natural capillarity of paper.[14]

To date, the use of smartphone cameras to interpret lateral flow tests has focused on individual still image end-point readings to interpret paper-based tests for diagnostics,[15–21] chemical sensing,[22, 24] and drug monitoring.[26, 27] Cameras and smartphones have also been used with other microfluidic techniques to quantify biological reactions, such as the detection of nucleic acid sequences[28] and E. coli detection using quantum dots.[29] Video tracking of biological interactions has also been used for real-time recording polymerase chain reaction amplification using a digital single lens reflex camera,[30] glucose sensing with a mobile phone,[25] and the use of a complementary metal-oxide semiconductor image sensor to track the motion of sperm cells.[31] Temporal surface plasmon resonance protein detection has also been demonstrated with a smartphone[32] using a polydimethylsiloxane microfluidic device.

Here, for the first time in the literature, we progress beyond still images of lateral flow tests to video analysis in order to investigate whether dynamic ligand–receptor binding on μPADs follows the Langmuir Adsorption Isotherm Model,[33] and whether μPADs could quantify fundamental biomolecular parameters, namely, the thermodynamic equilibrium dissociation constant, $K_D$ and kinetic $K_{on}$ and $k_{off}$ rates. We overcome potential barriers associated with quantitative analysis on lateral flow tests cited in previous work, including sample volume limitations,[34] color inhomogeneity,[21] reproducibility issues,[33] such as surface flow and inconsistent membranes,[26] porous 3D surface, protein dissociation over long periods, and possible reaction-limiting local sample depletion due to flow rate.

The ability to measure such fundamental chemical binding constants and kinetic reaction rates lies at the heart of chemistry and traditionally relies on access to sophisticated laboratory instrumentation, such as surface plasmon resonance[34] and interferometry, used here as a gold-standard reference method, typically using instruments costing in excess of £100 000. Other label-free methods that also require specific instrumentation...
are dynamic light scattering and isothermal titration calorimetry. There are a variety of fluorescence-labeling techniques such as fluorescence polarization, fluorescence correlation spectroscopy, total internal reflection fluorescence microscopy, and Förster resonance energy transfer. These methods all require fluorescence readout, such as a fluorescence microscope or spectrometer. In contrast, the method presented here is ultra-low cost, simply requiring a digital camera/smartphone, giving equipment costs of just £500/£214 respectively, and per assay paper microfluidic strip and consumables costs of approximately £1.20 (Supporting Information (SI), Table S1).

Our low-cost technique uses a simple set-up consisting of a consumer camera or smartphone, a series of direct-detection μPADs, and a 96-well plate. Lines of antigen are immobilized on nitrocellulose paper strips. When the antibody-functionalized gold nanoparticles (Ab-AuNP) flow along the membrane, they bind to the test line (Scheme 1a), generating a red color, the intensity of which is proportional to the number of gold nanoparticles (SI, Figure S1), and therefore the number of bound antibody-antigens. The nitrocellulose strips are mounted together, with a large absorbent pad to prevent saturation, and dipped into a 96-well plate, where each well contains a different concentration of Ab-AuNP solution (Scheme 1b).

Figure 1a shows an example set of video-μPAD time-intensity plots to track the binding of a monoclonal antibody to the influenza hemagglutinin H5 antigen test line. A series of eight different Ab-AuNP concentrations are measured (0.9 pM to 1000 pM). Figure 1b shows the fitted Langmuir binding plots for different concentrations of anti-H5 Ab-AuNPs binding to μPAD test lines functionalized with H5N1 hemagglutinin antigen. Raw data are shown as dots and exponential line fits (Equation (1)) are shown as solid lines. (b) Langmuir plot of extracted infinite test line intensity values, \( I_1 \), from (a). Experiment performed in triplicate and results shown as the mean with error-bars representing the standard deviation. The data are fitted to the Langmuir model (Equation (2)) (solid line). Inset is a graph of the observed reaction rate, \( k_{\text{on}} \), plotted against the concentration of Ab-AuNP solution. The gradient of the linear fit (solid line) corresponds to the reaction on-rate, \( k_{\text{on}} \), and the y-intercept, the reaction off-rate, \( k_{\text{off}} \) (Equation (3)).
1.9 nm). The number of antibodies per AuNP, and antigen-on-strip concentration, are held constant. Time-intensity plots are fitted to the exponential equation, shown below as Equation (1):

\[ I = I^e \left(1 - e^{-k_{on}t}\right) \]

(1)

where \( I \) is intensity (test line peak height), \( I^e \) is the equilibrium intensity value, \( k_{on} \) is the fitted observed binding rate, and \( t \) is time. As \( t \to \infty \), \( I \to I^e \). The \( I^e \) values are determined for each concentration of analyte, and fitted to a Langmuir model shown below as Equation (2)[33] (Figure 1 b):

\[ I^e = a \cdot C / (K_0 + C) \]

(2)

where \( a \) is the saturation intensity when all available binding sites are occupied, and \( C \) is Ab-AuNP concentration.

To determine the \( k_{on} \) and \( k_{off} \) rates, the values of \( k_{obs} \) are extracted from the fits of Equation (1) and plotted against \( C \). They are then fitted to following the relationship in order to extract \( k_{on} \) and \( k_{off} \) shown below as Equation (3):

\[ k_{obs} = k_{off} + k_{on} \cdot C \]

(3)

An example of this is shown in Figure 1 b (inset).

Our results show strong agreement between the binding kinetics measured by video analysis and the Langmuir model. We then apply video analysis to five different antibody-antigen pairs (SI, Table S2). In parallel, benchmarking studies are performed with the same proteins using a FortéBio Octet RED96 benchtop interferometer. The \( K_0 \) fits shown in Figure 2a demonstrate strong agreement between video-μPADs (solid lines) and interferometry (dotted lines). This is further demonstrated in Figure 2b, where the \( K_0 \) values measured by video-μPADs and interferometry show a linear relationship with a gradient of 1.1 (standard error 0.032), and an adjusted \( R^2 \) value of 0.996. For all raw data and fits, see SI, Figures S6, S7, and Table S3. The estimated concentration of antibodies in video-μPADs assays assumes that all antibodies bind to AuNPs in an active, available conformation, equating to \( \approx 15 \) antibodies per AuNP. We note that the measured \( k_{on} \) and \( k_{off} \) values differ from those measured by interferometry, although the relationship \( K_0 = k_{off}/k_{on} \) still holds (SI, Figures S8 and S9). This interesting result highlights the value of quantifying antibody-antigen reaction kinetics on μPADs in order to optimize diagnostic test performance to achieve fast kinetics and a strong \( K_0 \), giving a sensitive, rapid test with low-volume sample.

A proof-of-principle of video-μPADs using a smartphone is shown with an LG Nexus 5 (Figure 3a). The performance is compared to the Canon Powershot G15 camera for a model anti-human IgG Fc-human IgG interaction (Figure 3b and SI, Figure S10). No significant difference is found between the resulting \( K_0 \) values (two-tailed t-test gives \( p \)-value of 0.22, \( t \)-value of 1.2, degrees of freedom = 52), confirming the LG Nexus 5 smartphone can be used for \( K_0 \) measurements.

Building on our work with single antibody–antigen pairs, we then sought to investigate whether video-μPADs could measure multiple antibody–antigen interactions simultaneously. This could be useful for antibody and drug screening to quantify multiple antibodies’ binding affinities to a single target. Therefore, in contrast to the above, the AuNPs are functionalized with the antigen, and the antibody is spotted on the μPADs. The proof-of-concept is shown in Figure 4. An array of ten antibody spots is deposited on each μPAD. Figure 4a shows a filmstrip of a multiplex video-μPAD developing over time, with a heat map of pixel values shown below. This is translated into the time-intensity graph in Figure 4b showing nine multiplex video-μPADs—eight different antigen-AuNP concentrations and a buffer control. Each μPAD’s ten spots are plotted overlaid for each concentration. The low variances between spots illustrates that the kinetics are independent of spot position. Here, identical antibody–antigen combinations are used as a proof-of-concept. In future, each spot could be a different capture ligand. We show that the \( K_0 \) measured from singleplex and multiplex video-μPADs are not significantly different (two-tailed t-test gives \( p \)-value = 0.25, \( t \)-value = 1.1, degrees of freedom = 96), validating this reversed orientation (see SI, Figure S11).
Herein we harness consumer electronic video imaging for low-cost μPADs, creating an accurate platform for measurement of antibody–antigen dissociation constants. Our approach does not require expensive, complex laboratory-based equipment, simply using a camera or smartphone. The measured video-μPAD $K_D$ values for five antibody–antigen pairs show excellent agreement with a reference benchtop interferometer (Figure 2b; adjusted $R^2$ = 0.996). The assumptions and justifications for using the Langmuir model are discussed in SI Discussion and Table S4. We demonstrate that a smartphone can measure $K_D$ values, and create a multiplex platform to detect multiple ligand–receptor interactions in parallel. Although AuNP labeling is used here, future assays could employ a label-free competitive inhibition format.

The low-cost of video-μPADs is a major advantage over expensive benchtop instrumentation. SI Table S1 lists the cost, size, and weight of video-μPAD instrumentation compared to the FortéBio Octet RED96, showing that video-μPAD equipment costs are around 250–580 times cheaper, making it much more accessible to academic laboratories and resource-limited settings. Low-end smartphones are amenable to this application, since neither high camera resolutions nor large processing power are needed. Moreover, video-μPADs require 64-fold lower amounts of capture reagents (SI Table S1), advantageous for early stage discovery projects requiring biophysical characterization of reagents, where the quantities of material may be limited.

In our study, video analysis is performed off-device; however, this could be performed in real-time, even on a low-cost smartphone, with a capture rate of 1 Hz. In future, automatic strip detection by traditional image processing or machine learning would make the method more user-friendly.

To close, this technique allows low-cost, quantitative, multiplexed analysis and is generalizable to a wide range of biological and chemical ligand–receptor interactions, with many potential applications in analytical chemistry, biomedicine, forensics, and environmental analysis.

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Conflict of interest

The authors declare no conflict of interest.
