Supporting Information

Using Liquid Crystal as a Readout System in Urinary Albumin Assays

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AHSA Surface Density Calculations

To obtain a calibration curve of fluorescence intensities at different AHSA surface densities, we spotted standard FITC-AHSA solutions of various concentrations (***C*** = 0, 1, 5, 10, 25, 50, 65, 80 and 100μg/mL) on a DMOAP-coated slide in an array format.[1] The array was left to dry for 2 h without any further rinsing to ensure that AHSA in each droplet is fully adsorbed on the surface. The equivalent AHSA surface density (Γ_{AHSA}), nm^{-2}, in each dried spot can be calculated as follows:

$$\Gamma_{AHSA}\left(\frac{molecules}{nm^2}\right) = \frac{amount\ of\ AHSA\ adsorbed}{surface\ area}$$

$$\Gamma_{AHSA}\left(\frac{molecules}{nm^2}\right) = \frac{AHSA\ concentration\left(\frac{g}{mL}\right)\times Volume(mL)}{Surface\ area\left(nm^2\right)} * \frac{1}{MW\left(\frac{g}{mol}\right)} * N_A\left(\frac{molecules}{mol}\right)$$

$$\Gamma_{AHSA}\left(nm^{-2}\right) = \frac{C\left(\frac{\mu g}{mL}\right)\left(\frac{1g}{10^6\,\mu g}\right)\times V(uL)\left(\frac{1mL}{10^3\,\mu L}\right)}{\frac{\pi D^2(um^2)}{4}\left(\frac{10^3\,nm}{1\mu m}\right)^2} * \frac{1}{150{,}000\left(\frac{g}{mol}\right)} * 6.023\times10^{23}\left(\frac{molecules}{mol}\right)$$

$$\Gamma_{AHSA}\left(nm^{-2}\right) = \frac{\left(1\times10^{-9} * 6.023\times10^{23}\right) * C\left(\frac{\mu g}{mL}\right)\times V(uL)}{\frac{150{,}000}{4} * 1\times10^6 * \pi D^2(um^2)}$$

$$\Gamma_{AHSA}\left(nm^{-2}\right) = \frac{\left(1\times10^{-9} * 6.023\times10^{23}\right) * C\left(\frac{\mu g}{mL}\right)\times V(uL)}{\frac{150{,}000}{4} * 1\times10^6 * \pi D^2(um^2)}$$

$$\Gamma_{AHSA}\left(nm^{-2}\right) = 5.11\times10^3\,\frac{C\left(\frac{\mu g}{mL}\right)\times V(uL)}{D^2(um^2)} \qquad \textbf{[1]}$$

where ***C*** is the concentration (μg/mL), ***V*** is the volume (μL), ***MW*** is the molecular weight of AHSA (MW_{AHSA} = 150 000 g/mol), $\boldsymbol{N_A}$ is the Avogadros number (N_A = 6.023×10^{23} molecules/mol) and ***D*** is the diameter of the spot (μm). Based on the

experimental setup, each FITC-AHSA spot has a volume (***V***) of 0.1μL, and a diameter (***D***) of 800μm.

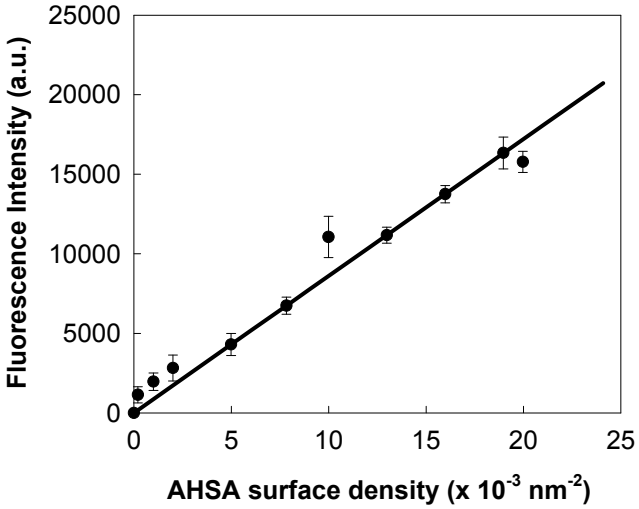

Fig.S1 Calibration curve of fluorescence intensity as a function of AHSA surface density.

FITC-AHSA solutions used in the experiment (***C*** = 0, 10, 15, 20, 25, 30, 40, 50, and 100μg/mL) were then spotted to the DMOAP-coated slides and then rinsed after 2 h incubation. Fig. S2 shows the fluorescence images of each spots and their corresponding AHSA surface density estimated from Fig. S1.

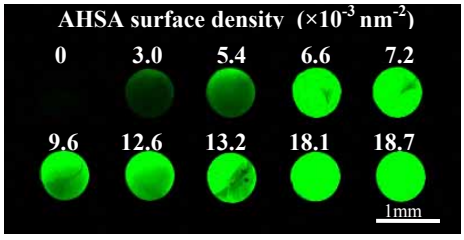

Fig.S2 Fluorescence images of the AHSA solutions used in the experiment.

Next, we studied the relationship between the AHSA concentration in the solution and the surface density of immobilized AHSA on the surface. When the concentration of AHSA in the solution is increased from 1 μg/mL to 100 μg/mL, the fluorescence intensity also increases from 1,100 a.u. to 18,300 a.u. (see Fig. S2). This suggests that the concentration of AHSA in the solution affects the amount of AHSA adsorbed on the surface. On the basis of the fluorescence results, the maximum AHSA surface density is $18.1 \pm 0.6 \times 10^{-3}$ nm^{-2} (see Fig.S3).

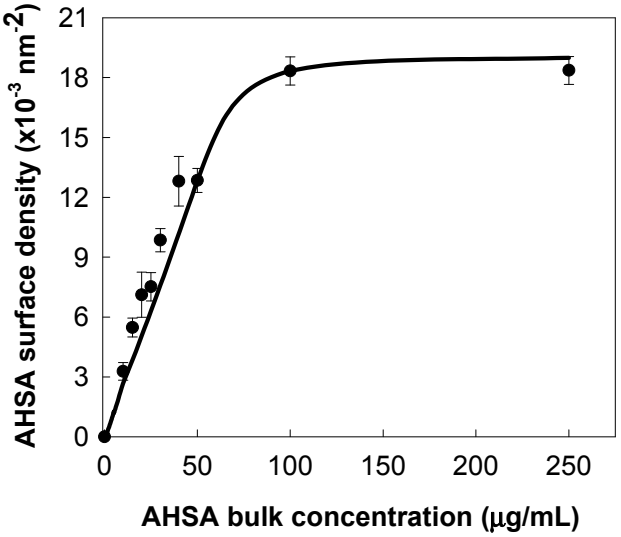

Fig.S3 AHSA surface density as a function of AHSA concentration in solution. (Plotted line is a graph taken from the calibration curve. Points plotted are the actual data points taken from spotted AHSA solutions rinsed with PBS buffer.)

Effect of incubation time and HSA concentrations

To find the optimum time required for the binding of HSA to surface immobilized AHSA, we spotted solutions containing different HSA concentrations (10, 25, 50, and 100 μg/mL of FITC-HSA) on AHSA-decorated slides and then varied the incubation time from 1, 5, 15, 30, 60 to 120 min. Fig. S4 shows that the amount of HSA that binds to the AHSA-decorated slide increases with time. However, this result also reveals that HSA binds very quickly onto the surface in the beginning (especially at higher concentrations), but the binding rate slows down after 30 min, probably because the surface is saturated with HSA. Since the amount of HSA bound to the surface from 30 min to 60 min is minimal, we use 30 min as the incubation time in the following experiments.

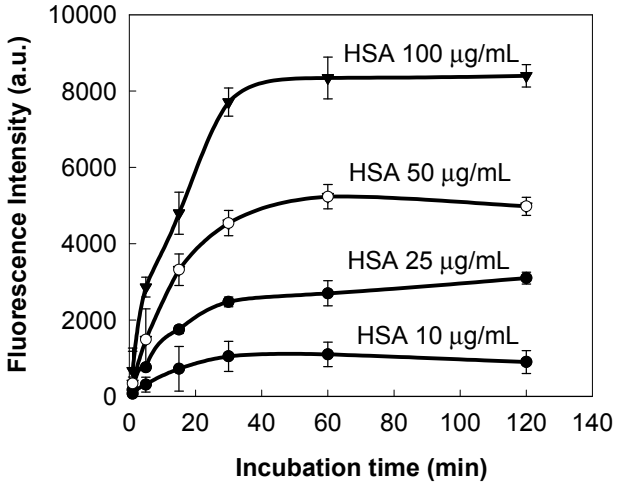

Fig. S4 Effect of incubation time on the binding of HSA to surface immobilized AHSA.

Detection of HSA using Chip Electrophoresis

Fig. S5 shows the runs made using several standard HSA solutions in order to determine the HSA limit of detection (LOD) of the chip electrophoresis. The figure exhibits a band near 63 kDa of the ladder which corresponds to the molecular weight of HSA. In addition, Fig. S5 (columns 1-5) shows decreasing band intensity as the amount of HSA in the solution decreases. Further decreased of the amount of HSA in the solution was done until it can no longer be detected (LOD = 20 μg/mL).

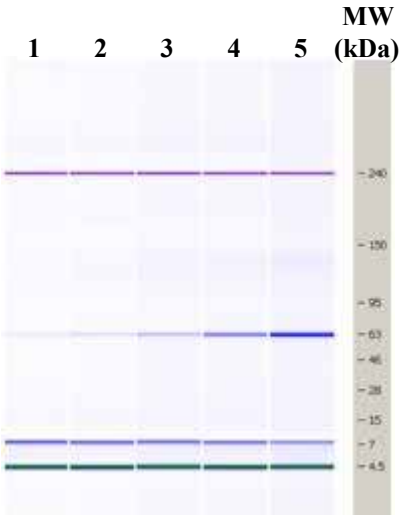

Fig.S5 Detecting HSA by using chip electrophoresis. Columns 1-5 represent standard HSA solutions whose concentrations are 20, 100, 200, 500, and 1000μg/mL, respectively.

Fig. S6 shows a representative electropherogram for samples collected from both groups. The urine sample from the group of CKD patients, as shown in Fig. S6B, exhibits a band near 63 kDa which confirms the presence of HSA in the urine samples of CKD patients. In contrast, no band can be seen when the urine sample from the control group were used (Fig. S6A).

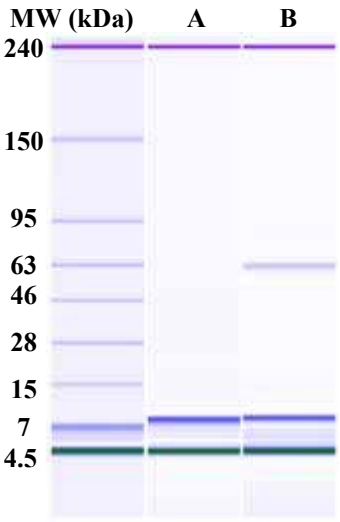

Fig. S6 Analysis of two different urine samples using chip electrophoresis. (A) sample from the control group and (B) sample from a CKD patient. The presence of HSA in the sample collected from the CKD patient is confirmed by using the protein ladder on the left.

Reference:

1. X. Bi, S. L. Lai and K.-L. Yang, *Anal. Chem.*, 2009, **81**, 5503-5509.