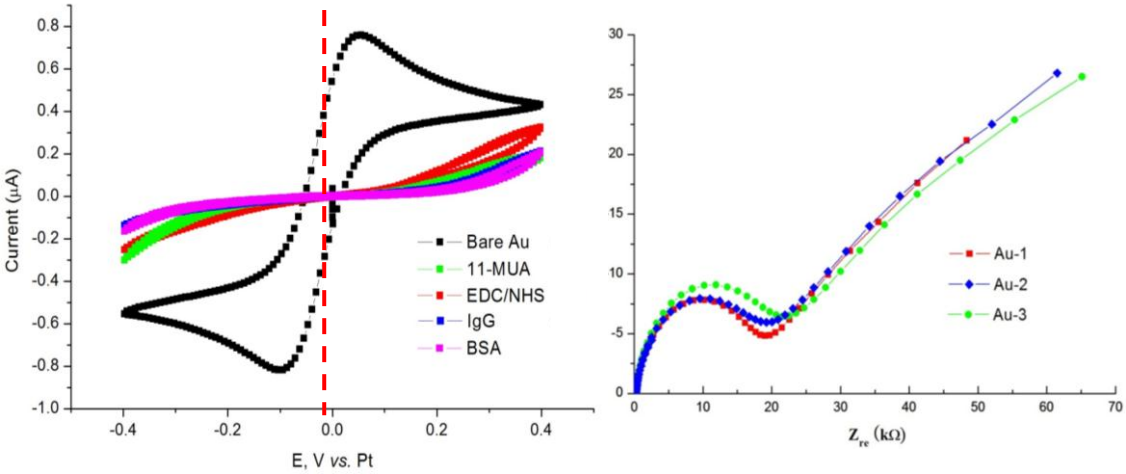
Supplementary Materials

1. CV responses to each immobilized layers

A scan rate of 100 mV/s was used in a 20 mM 2-(*N*-morpholino) ethanesulfonic acid (MES, pH= 5) solution containing $[Fe(CN)_6]^{3-/4-}$ (2.5 mM, 1:1) and using 100 mM KCl as an oxidation/reduction probe. For $[Fe(CN)_6]^{3-/4-}$, the standard potential of a bare Au electrode versus a Pt reference electrode was measured to be approximately -0.02 V, and the peak-to-peak separation was approximately 120 mV. First, the micro-fabricated bare Au electrode was pretreated with 11-MUA to form sulphide-based SAM on the Au surface, and then the COOH group on the Au-SAM surface was activated by EDC/NHS. After EDC/NHS activation, the IgG antibody (0.1 mg/ml) and BSA were stepwise incubated to functionalize the Au electrode and to block the un-immobilized sites, respectively. The resulting CV curves are shown in Fig. S1(a), which shows that the positive and negative current peaks are attributed to the reaction of the $[Fe(CN)_6]^{4-/3-}$ redox pair on the bare gold electrode surface, respectively. After 11-MUA modification in the bare Au electrode, the oxidation/reduction peaks disappeared because the long chain thiol linked to the interface of the electrode formed a dense structure that created a blocking effect. Therefore, with an increase in the modified layers, the immobilization of IgG and BSA on the 11-MUA-coated electrode resulted in a descending current response.

Fig. S1(b) shows the Nyquist plots of impedance spectra measured from three different bare Au electrodes, which were cleaned in piranha solution for 2 min. The buffer solution is an MES (20 mM, pH=5) solution containing $[Fe(CN)_6]^{3-/4-}$ (2.5 mM, 1:1) with 100 mM KCl. The applied frequency range for measurement was from 100 kHz to 0.1 Hz at 25 °C (AC: 5 mV, DC: -0.02 V *vs.* Pt). The Nyquist plot of the bare

Au electrode presents as a half-circle resistance at high frequency, and a linear part that appears at low frequencies is attributed to diffusion phenomena. The results show a low variation in the impedance spectrum for each fabricated bare Au electrode after cleaning the surface with piranha solution.



stepwise immobilization of 11-MUA, EDC/NHS, IgG and BSA. (b) Nyquist plots of impedance spectra measured from three different Au electrodes that were cleaned in piranha solution. The results show a low variation in the impedance spectrum for each fabricated bare Au electrode.

Table S1 Impedance changes at different concentrations of protein A versus different electrokinetic concentration times.

	ΔRet (MΩ) mean ± S.D.			
Concentration	30s	60s	90s	120s
20 ng/ml	24.49 ± 3.94	35.1 ± 2.45	57.51 ± 6.25	57.61 ± 5.75
2 ng/ml	13.1 ± 2.21	14.04 ± 2.92	14 ± 2.53	
0.2 mg/ml	2.39 ± 1.08	2.31 ± 1.12	2.38 ± 0.84	

2. Ion concentration affected antibody-antigen binding

According to equations (1) and (2), a low ion concentration buffer is needed to induce a sufficiently high ACEO convection. Therefore, a diluted PBS buffer (0.01 x PBS, σ ~ 149 μS/cm) was used to induce a sufficiently high ACEO for the purpose of rapidly concentrating protein into the detection area. The reaction buffers were individually controlled at two different PBS concentrations (stock PBS and 0.01x PBS) to investigate the buffer effect on antibody-antigen binding without ACEO concentration. The results show that the affinity reaction using an incubation time (without electrokinetic concentration) of 1 hr in 1x PBS and 0.01x PBS buffers achieved the ΔR_{et} of 12.8 MΩ and 2.51 MΩ, respectively (Fig. S2). This result is attributed to the fact that the thick electric double layer (EDL) generated a high zeta potential that produced a higher electrostatic repulsion force at a low ionic strength and thus a reduction in antibody-antigen binding (Cheng et al. 2012). In contrast, ACEO provided a long-ranged molecular convection to concentrate a large number of proteins/molecules to the EIS working electrode to increase the number of molecular bindings on the antibody-modified surface, thus the ΔR_{et} could become larger than 50 MΩ (Fig. S2), and the reaction time was also shortened from one hour to 90 sec. We suspect that this scenario could be a result of the AC field-induced ACEO drag and electrokinetic forces (EP and DEP) that are capable of overcoming the electrostatic repulsion force from the surface of the antibodies and antigens. Therefore, the proteins could still effectively bind to the antibodies in a buffer of lower ion concentrations. Using the buffer in lower ion concentrations could also assist in the removal of nonspecific bound objects during the wash step, thus promoting its specificity.

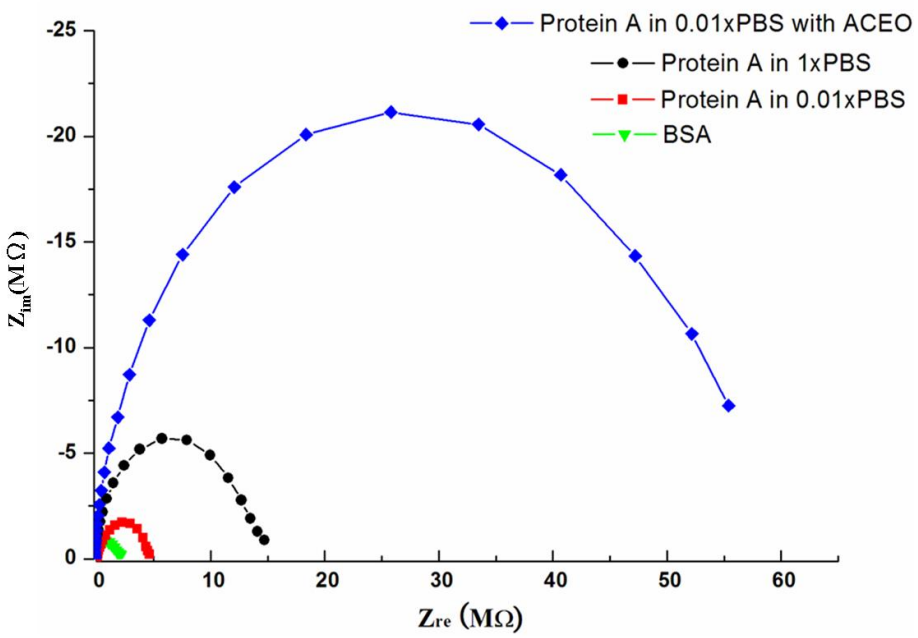


Fig. S2 The investigation of ionic strength affecting antibody-antigen binding with/without electrokinetic enhancement.