

Supplementary for

## **Revealing Protein Binding Affinity on Metal Surfaces: An Electrochemistry Approach**

Danya Lyu<sup>a</sup>, Pingshi Wang<sup>a</sup>, Shuo Zhang<sup>a</sup>, Guokun Liu<sup>b, c, \*</sup>, Bin Ren<sup>a, \*</sup>

*a. State Key Laboratory of Physical Chemistry of Solid Surfaces, College of Chemistry and  
Chemical Engineering, Xiamen University, Xiamen 361005, China.*

*b. State Key Laboratory of Marine Environmental Science, College of the Environment and  
Ecology, Xiamen University, Xiamen 361005, China.*

*c. Fujian Provincial Key Laboratory for Coastal Ecology and Environmental Studies, Center for  
Marine Environmental Chemistry & Toxicology, Xiamen University, Xiamen 361102, China*

\*Corresponding author

Guokun Liu

Email: [guokunliu@xmu.edu.cn](mailto:guokunliu@xmu.edu.cn)

Tel: (86)-0592-2186532

Fax: (86)-0592-2181906

Bin Ren

Email: [bren@xmu.edu.cn](mailto:bren@xmu.edu.cn)

Tel: (86)-0592-2186532

Fax: (86)-0592-2181906

## Chemicals and instruments

SARS-CoV-2 spike protein was obtained from Sino Biological and dissolved in 100 mM PBS (pH = 7.0). Water with 18.2 M $\Omega$ ·cm (Milli-Q system, Merck) was used throughout the study. The gold, AISI 304 stainless steel (consisting of Fe: 67-71.5%, Cr: 17.5-19.5% and Ni: 8-10.5%) and copper disks with diameters of 2 mm were used as working electrodes.

Electrode treatment and chronoamperometry measurement were performed on a potentiostat (CHI660e, CH Instruments). The capacitance was determined by the double-potential-step measurement, where a potentiostatic step of 10 mV was applied on the electrode in 100 mM PBS (pH = 7.0) solution. The current was obtained at a sampling frequency of 10 kHz with 4 s rest period by applying a square wave. The potential was referenced to Hg|Hg<sub>2</sub>SO<sub>4</sub> electrode.

Raman spectra were recorded on a confocal Raman microscope (Renishaw inVia) coupled with a 633 nm laser, an 1800 grooves/mm grating, and a 50 $\times$  (NA 0.55) objective (Olympus). The laser power on the sample was 0.034 mW.

## Electrode preparation

Electrodes were polished with alumina slurry (Buehler, Ltd.) of 1.0, 0.3 and 0.05  $\mu$ m successively and further degreased with ethanol and water in an ultrasonic bath for 3 min each. Gold electrodes were further cleaned in 0.5 M H<sub>2</sub>SO<sub>4</sub> via cyclic voltammetry from -0.6 V to 1.2 V for 100 cycles. In the 100 mM PBS (pH = 7.0), cyclic voltammetry was carried out on copper electrode between -1.0 V and -0.3 V and on stainless steel electrode between -1.4 to 0.6 V until the curve becomes stable. All potentials were referenced to Hg|Hg<sub>2</sub>SO<sub>4</sub> electrode.

## Preparation of SERS substrate, sample and SERS measurement

Roughened polycrystalline Au and Cu electrode were used as SERS substrates and prepared according to the reference.<sup>1</sup> In brief, the Au electrode was roughened in 0.1 M KCl solution by performing potential-sweep oxidation-reduction cycles (ORCs). The potential was referenced to saturated calomel electrode (SCE). The potential was first held at -0.3 V for 30 s then swept to 1.2 V at 1 V/s and held at 1.2 V for 2 s. Finally, the potential was swept back to -0.3 V at 0.5 V/s and held for 30s. This ORC was repeated for 25 cycles. After washing the electrode with water, a drop of sample solution (6  $\mu$ L) was placed on the Au electrode surface for 30 min before detection. The Cu electrode was treated by a square wave potential in 0.1 M Na<sub>2</sub>SO<sub>4</sub>. The potential was hold at -0.8 V (vs Hg/Hg<sub>2</sub>SO<sub>4</sub> reference electrode to avoid the influence of Cl<sup>-</sup>) for 30 s and then stepped to -0.3 V for 2 s and returned to -0.8 V. The ORC process was repeated for two times. A drop of sample solution (6  $\mu$ L) was kept on the Cu electrode surface for 10 min before detection. A 100  $\mu$ M dithiothreitol (DTT) solution was added to the S<sub>RBD</sub> solution (18.8  $\mu$ M) and kept for 1 hour. Then DTT was removed by ultrafiltration. The pretreated S<sub>RBD</sub> was used immediately after the ultrafiltration.

## Calculation the capacitance

Following the theory of the electrical double layer, water and solvated ions constitute the compact Helmholtz plane or the Stern layer. When protein is adsorbed on the electrode surface, the solvated ions and water molecules were replaced by protein, leading to the change of the capacitance. Such a change gives the chance to reveal the adsorption kinetics in real time. We may be able to obtain adsorption ability of protein on different electrode materials by calculating the surface coverage. Assuming the adsorption process of protein follows the Langmuir adsorption isotherm, the surface coverage ( $\theta$ ) can be expressed as equation (S1) where N represents the active site and S represent the electrochemically active surface area.

$$\theta = \frac{N - N_{unoccupied}}{N} = \frac{S - S_{unoccupied}}{S} \quad (S1)$$

The electrochemically active surface area (S) is obtained as the geometry surface area of the electrode times the roughness factor of electrode shown in equation (S2). The roughness factor is obtained by normalizing the double layer capacitance of the electrode ( $C_{dl}$ ) with the double layer capacitance of a flat reference surface ( $C_{ref}$ ).<sup>2, 3</sup>

$$S = S_{Geom} \frac{C_{dl}}{C_{ref}} \quad (S2)$$

Thus, the surface coverage can be calculated by combining equation (S1) and (S2) as equation (S3):

$$\theta = 1 - \frac{C_{dl\infty}}{C_{dl0}} \quad (S3)$$

where  $C_{dl0}$  and  $C_{dl\infty}$  are the capacitances at initial and equilibrium states, respectively.

The Langmuir adsorption model is expressed by equation (S4)<sup>4</sup>

$$\theta(t) = 1 - \exp\left(\frac{-kC_0t}{RT}\right) \quad (S4)$$

Combining equation (S4) and Arrhenius formula,

$$\ln kC_0 = \ln A - \frac{E_a}{RT} \quad (S5)$$

we can obtain the activation energy of adsorption process ( $E_a$ )

### Calculation of force constant

The calculation of force constant K is based on the equation (S6).

$$v = \frac{1}{2\pi} \sqrt{\frac{K}{\mu}} \quad (S6)$$

$$\mu = \frac{m_A m_B}{m_A + m_B} \quad (S7)$$

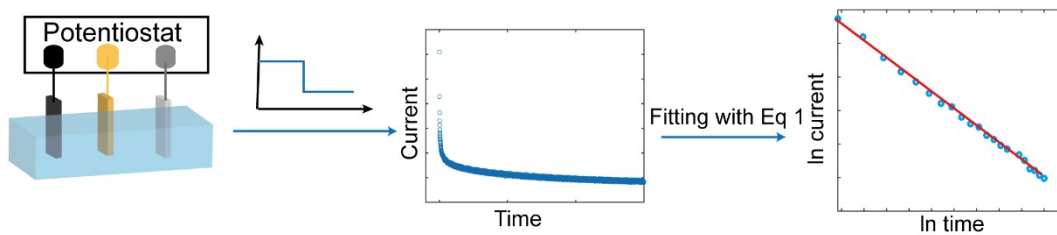


Figure S1 Schematic diagram of data acquisition and analysis processes.

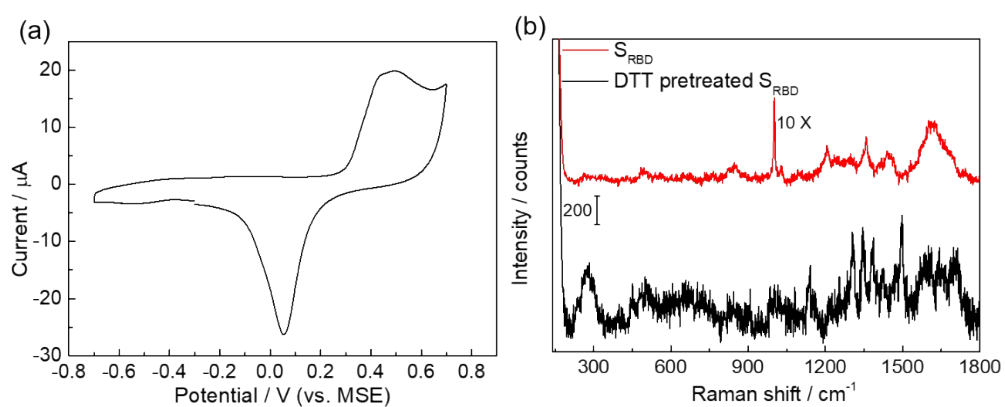


Figure S2 (a) CV curve of an Au electrode in 100 mM PBS (pH = 7.0) (b) SERS of  $S_{\text{RBD}}$  and DTT pretreated  $S_{\text{RBD}}$  on a roughened gold electrode.

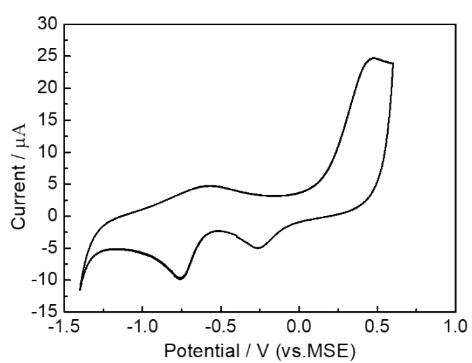


Figure S3 CV curve of a stainless steel electrode in 100 mM PBS (pH = 7.0).

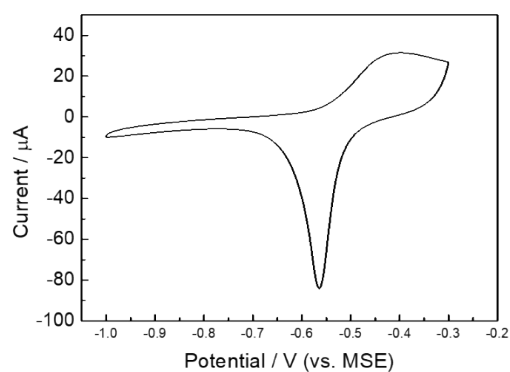


Figure S4 CV curve of a Cu electrode in 100 mM PBS (pH = 7.0).

**Table S1.** Adsorption behavior of S<sub>RBD</sub> on three metal surfaces.

Metal	Surface coverage	Adsorption activation energy	Adsorption type
Au	99.3%	63 KJ/mol	Chemical
Cu	37.8%	17.5 KJ/mol	Chemical
Stainless steel	7.7%	0.3 KJ/mol	Physical

### Supplementary references

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