Supporting information:

"Covalent Biosensing" Enables a One-Step, Reagent-Less, Low-Cost and Highly Robust Assay of SARS-CoV-2

Kai Zhang,[a, b, §] Jinlong Li, [b, §] Zhenqiang Fan,[a] Hao Li,[*d] and Jing-Juan Xu[*b]

[a]*NHC Key Laboratory of Nuclear Medicine, Jiangsu Key Laboratory of Molecular Nuclear Medicine, Jiangsu Institute of Nuclear Medicine, Wuxi, Jiangsu, China 214063*

[b]*State Key Laboratory of Analytical Chemistry for Life Science and Collaborative Innovation Center of Chemistry for Life Sciences, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023 (China)*

[c]*Department of Laboratory Medicine, The Second Hospital of Nanjing, Nanjing University of Chinese Medicine, Nanjing 210003, P.R. China*

[d]*School of Biological Science and Technology, University of Jinan, No. 106 Jiwei Road, Jinan, Shandong 250022, China*

§ These authors contributed equally to this work.

*Corresponding authors (email: xujj@nju.edu.cn (J. Xu), cardely@126.com (H. Li))

Experimental

Chemicals and Reagents

Peptidomimetic probe, and the anti-adsorption peptide (AGKGAAGAA) was manufactured by Jinan VR biotech, as lyophilized powder, purity>95%. The powder of the peptidomimetic probe was dissolved using 10 mM phosphate buffer solution (PBS, pH 7.4) to the desired concentrations. Recombinant main protease of SARS-CoV-2 was purchased from R & D Systems. Analytical-grade was attained for all of the other reagents. The serum-spiked sample of main protease was produced by reconstituting the HEPES solution of the recombinant protease with 10 mM PBS (pH 7.4) and then diluting the mixture to desired concentrations with fetal bovine serum. Redistilled water for preparation of all the solutions was produced from distilled water with a Milli-Q purification system, a specific resistance of 18 MΩ·cm was reached to guarantee purity. Body fluid samples containing SARS-CoV-2 were obtained from the Clinical Laboratory of the Second Hospital of Nanjing, after elected consent by Medical Ethics Committee of Nanjing Second Hospital. The blood samples were stored in heparin vial, before testing, the samples were centrifugated for 15 min at 2500 RPM, and then the supernatant was collected. All experiments were performed in compliance with the Guidelines for Health-related Research Involving Humans of CIOMS(Council for International Organizations of Medical Science).

Electrode Treatment

Transparent Au sprayed ITO slides (Aldrich, a layer thickness of 100 Å) were cut to fit the size of the cuvette employed for light absorbance measurement. These slides were cleaned by a mild stream of nitrogen, sonication in any solution, particularly piranha solution, would likely result in partial or even total destruction of the Au surface layer. Then, the slides were immersed in the assembly solution (2.5 μM peptidomimetic probe and 5mM Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) in 10mM PBS, pH 7.4) at 4 °C for 16 h, TCEP was used to prevent disulphide formation between the

peptidomimetic probes. The slides were then immersed in 9-mercaptononanol (MN) solution (1 mM MN in 10 mM PBS, pH 7.4) at room temperature for 3 h.

Enzyme Activity Assay

The prepared slides were immersed in serum spiked samples of recombinant protease, or body fluid samples containing the target protease and kept at 37 °C for 30 min for the target/probe recognition and covalent binding to proceed. Then, after violent rinsing with SDS detergent (10 mM), the slides were ready for measurement.

Experimental Measurements.

SPR measurements were made with an Autolab ESPRIT system (Echo Chemie B.V., Netherlands) furnished with a 670 nm monochromatic p-polarized light resource. Electrochemical measurements were carried out on a CHI660D Potentiostat (CH Instruments) with a conventional three-electrode system: the modified electrode as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum wire as the counter electrode. The experimental parameters for EIS: bias potential, 0.224 V vs. SCE; amplitude, 5 mV; frequency range, 0.1 Hz ~ 10 kHz. electrolyte solution: 5mM $Fe(CN)_6^{3-/4-}$ with 1 M KCl. The data are obtained from at least three times of repetition of independent experiment, error bars are shown in the figures.

Endogenous assay and condition optimization.

After verifying the target binding ability of our probe, the influence of interfering species in the serum spiked sample is studied: after incubation with the slide, the rinsing step is controlled, then the electrochemical responses of slides without rinsing, rinsed with double-distilled water, and rinsed with surfactant are compared, as shown in Fig. S1a. The SWV responses without rinsing and dd water rinsing are both larger than the case with surfactant rinsing, this is due to the fact that interfering proteins accumulated

on the sensing surface during the incubation step also contains tyrosine moieties that can be electrochemically oxidized to generate signal response. Therefore it is evident that thorough surfactant rinsing is all important to minimize false positive, while only the non-covalent complex between antibody and target protein is strong enough to withstand such rinsing, novel synthetic probes such as aptamer cannot face this challenge. As control, the above experiments are repeated on a sensing surface without the anti-adsorption surface, the results of which are included as red curves, and it is evident that the anti-adsorption surface plays a major part in reducing interference from nonspecific species. The time required for surfactant rinsing is then studied, as shown in Fig. S1b. The response steadily decreases with longer rinsing time, until a floor value is approached after one minute of rinsing. So this is employed for all the following experiments.

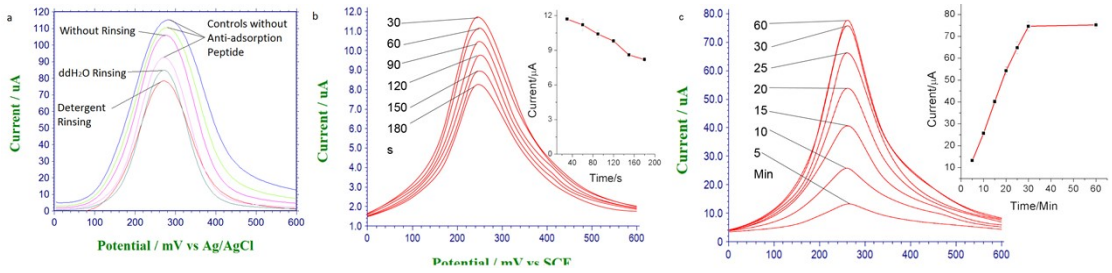

Fig S1. (a) comparison of SWV responses obtained by first incubating the conductive slide with a diluted recombinant protease sample containing 10nM target protein, and then proceed without rinsing, or with ddH$_2$O rinsing, or with surfactant rinsing, respectively. (b) Optimization of rinsing time using SWV responses, inset is peak responses plotted as a function of rinsing time, error bars represent standard deviation (n=3). (c) The optimization of incubation time for the interaction between the probe and the target viral protease, inset is the peak SWV response plotted as a function of incubation time, error bars represent standard deviation (n=3).

Specificity tests and verification of sensing processes.

The ability of the surface immobilized probes to specifically target its designated target protein is then tested using albumin, hemoglobulin, and papain protease diluted with serum. Papain protease is a protease similar to the virus main protease, both relying on

cysteine side chain thiol group to realize their catalytic activity. As can be seen in Figure S2a, all these control targets lead to only negligible response, after thorough surfactant rinsing as described above. It can then be concluded that the specificity of our probe is satisfactory. So we next study the biosensing procedure step by step via collecting electrochemical impedance spectrum (EIS) during the biosensing process. As shown in Fig. S2b, the freshly prepared conductive slide shows no evident impedance (curve 1), its impedance appears as a straight line in the Nyquist plot of EIS. After modification by the probes, a moderate semicircle appears (curve 2), representing some resistance to interface electron transfer. After incubation with recombinant protease sample, but before rinsing, a large semicircle appears (curve 3), which is corresponding to evidently increased surface impedance, showing the presence of proteins, including the target protein and various interfering proteins, on the surface. After surfactant rinsing, the semi-circle dramatically shrinks (curve 4), indicating successful removing of those interfering species. So the whole process of biosensing is validated electrochemically.

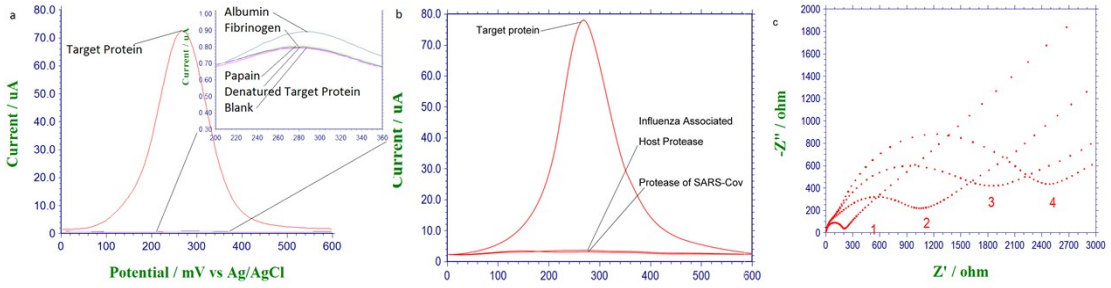

Fig. S2. (a) SWV responses showing the difference between the response of the target protein and those of the control targets, inset is an enlarged view of the responses of the control targets. (b) SWV responses showing the specificity towards the target viral protease with respect to similar proteases of other virus, names, the proteases from influenza virus and the SARS-Cov. Based on this result, false positive for SARS-CoV-2 can be excluded. (c) Nyquist plot of the electrochemical impedance spectra of the conductive slide, recorded after each step of sensing, as indicated by the numbers and detailed in the text.

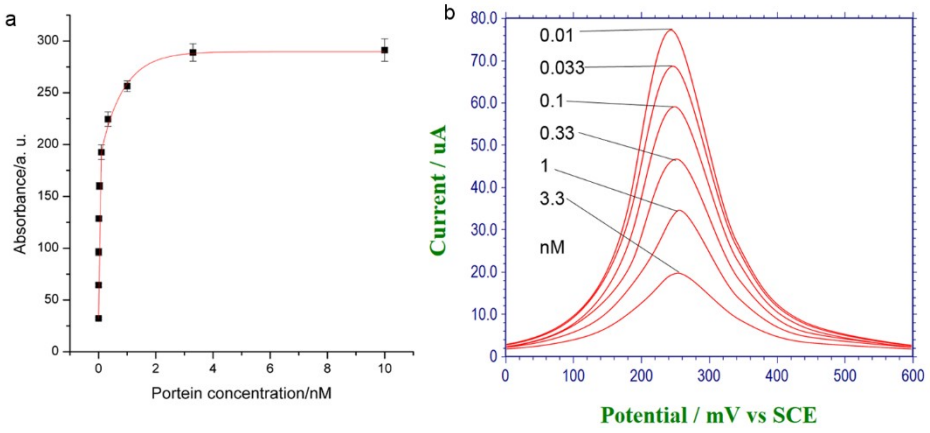

Figure S3. Comparison of the results in Figure 3 with various other detecting methods. (a) Detection of the target protein using a commericially availible ELISA kit. (b) Competetive binding with surface captured target protein molecules using peptide probes in the solution, the concentration of the peptide probe is marked on the figure. Noting that both the surface immobilized and the in-solution probe molecules are without the covalent reaction group. Or elese, the probe will irreversibly bind to the target protein

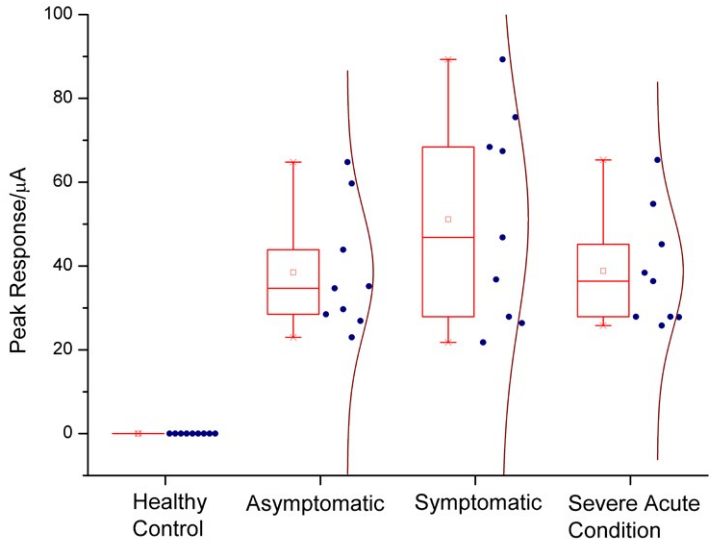

Fig. S4. Box charts representing the detected abundance of the main protease in the clinical serum samples from healthy people and SARS-CoV-2 infected patients.

Table S1

Method	Target	Limit of Detection	Reproducibility
DNA amplficaiton	mRNA	25.1 aM	4.25%
The proposed method	protein	0.1 pM	<5%

S. Yu, Y. Wang, L. Jiang, S. Bi, J. Zhu, *Anal. Chem.*, 2018, 90, 4544-4551