Supplementary Information

A Portable and Disposable Electrochemical Sensor Utilizing Laser-Scribed Graphene for Rapid SARS-CoV-2 Detection

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1. Chemicals and materials for EsterLigase-VHH E production pyrene-E-Tag synthesis

Fmoc-amino acids were purchased from CS Bio Ltd (Shanghai, China). Hydrazine hydrate and 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) were purchased from (AK Scientific, Union City, CA, USA). N,N-dimethylformamide (DMF; AR grade) and acetonitrile (MeCN, HPLC grade) were purchased from Thermo Scientific (Hampshire, NH, USA). Diethyl ether (Et2O) was purchased from Avantor Performance Materials (Center Valley, USA) and dichloromethane were purchased from EP Ltd. (Auckland, New Zealand). Analytical reverse phase high-performance liquid chromatography (RP-HPLC) was performed on a Waters (Waltham, MA, USA) Alliance analytical HPLC equipped with a Phenomenex (Torrance, CA, USA) Luna C18 column (100 A, 5 μm, 4.6 mm x 250 mm) operated at room temperature, with chromatograms recorded at 214 nm and 254 nm. Semi-preparative RP-HPLC was performed on a Waters 1525 Binary HPLC pump equipped

with a Waters 2489 UV/visible detector (214 nm) using a Phenomenex Luna C18 semi-prep column (100 Å, 5 μm, 250 mm x 10 mm). For both analytical and semi-preparative RP-HPLC, solvents used were as follows: solvent A = 0.1% TFA in water (MQ H2O) and solvent B = 0.1% TFA in MeCN.

2. The calculation of the limit of detection

The calculation of the limit of detection (LOD) is based on the Signal-to-Noise Approach [1], where three times the sensor response from a blank buffer solution (PBS) was utilised. After measurements of the blank signal trice, the average value of $\Delta R_{ct}/R_{ct}^0$ was 0.0151 for LSGE, and the calibration equation for the LSG-based sensor was $\Delta R_{ct}/R_{ct}^0 = 0.0866 \times \lg[\text{SP-RBD}]+1.0078$. Therefore, $\text{LoD}=10^{(3x\Delta Rct/Rct0-1.0078)/(0.0866)}=10^{(3x0.0151+1.0078)/(0.0866)}=7.68$ pM. The calculated LOD of the developed sensor was 7.68 pM.

A)

MSYYHHHHHHDYDIPTTENLYFQGASQVQLVETGGGFVQPGGSLRLSCAASGVTLDYYAIGWFRQAP

GKEREGVSCIGSSDGRTYYSDSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCALTVGTYYSG

NYHYTCSDDMDYWGKGTQVTVSSGAGSGSGSGRVTNKKIVSSLQTTVEADGQSSTAEKSAEVTEN

KDGVNVVDTIHYKGLIPKQKYEVVGILYEVKDGKLVDPNKPITISNGTGEYTVSDSGEGEWKLNF

GKIDGVEARKSYVVYEEVTSVENLVDTDNDGNTS

B)

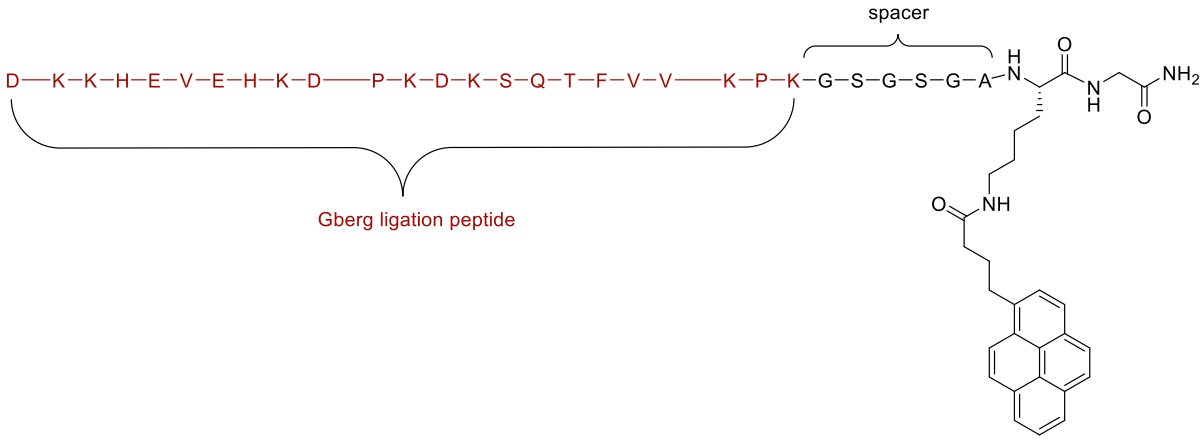

Figure S1. (**A**) Sequence of VHH E nanobody fused with EsterLigase. The N-terminal His^{6} IMAC tag and rTEV protease recognition sequence are underlined and removed from the purified VHH E EsterLigase recombinant protein. The VHH E nanobody sequence (grey) is linked to the *Gemella bergeriae* (Gberg) EsterLigase domain (yellow) via a flexible linker (red). (**B**) Structure of pyrene-modified E-Tag. The E-Tag is composed of a Gberg ligation peptide with a C-terminal spacer that is conjugated to pyrene via the lysine N$^{\varepsilon}$ amino group.

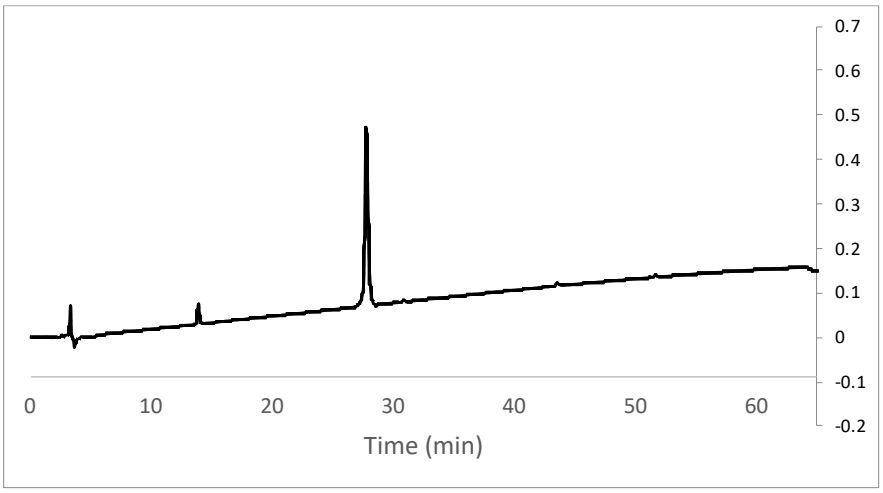

Figure S2. Analytical RP-HPLC for pyrene-modified E-Tag. RP-HPLC profile (214 nm) of pyrene modified E-Tag peptide. t_R 27.8 min, Phenomenex Luna C18 (100Å, 5 µm, 4.6 mm x 250 mm), linear gradient 5%B to 65%B (*ca.* 1%B/min) at 1 mL/min.

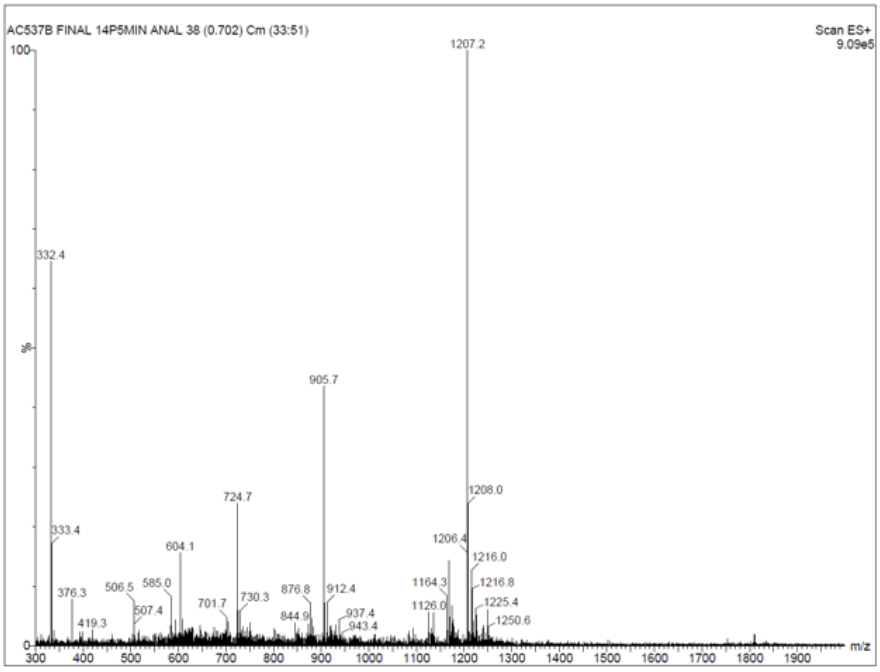

Figure S3. ESI-MS data for pyrene-modified E-Tag. Pyrene modified synthetic peptide ESI-MS (+ve) data. *m/z* calculated for [$C_{165}H_{253}N_{45}O_{47}$] 3617.9; mass observed deconv: 3618.6 ± 0.13. Charge states; 604.1 $[M+6H]^{6+}$, 724.7 $[M+5H]^{5+}$, 905.7 $[M+4H]^{4+}$, 1207.2 $[M+3H]^{3+}$.

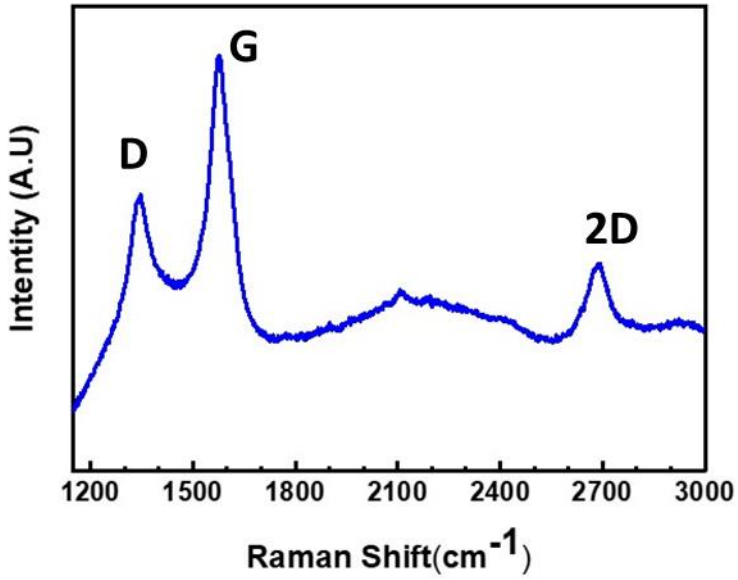

Figure S4. The Raman spectrum of the LSG. Excitation wavelength: 523 nm.

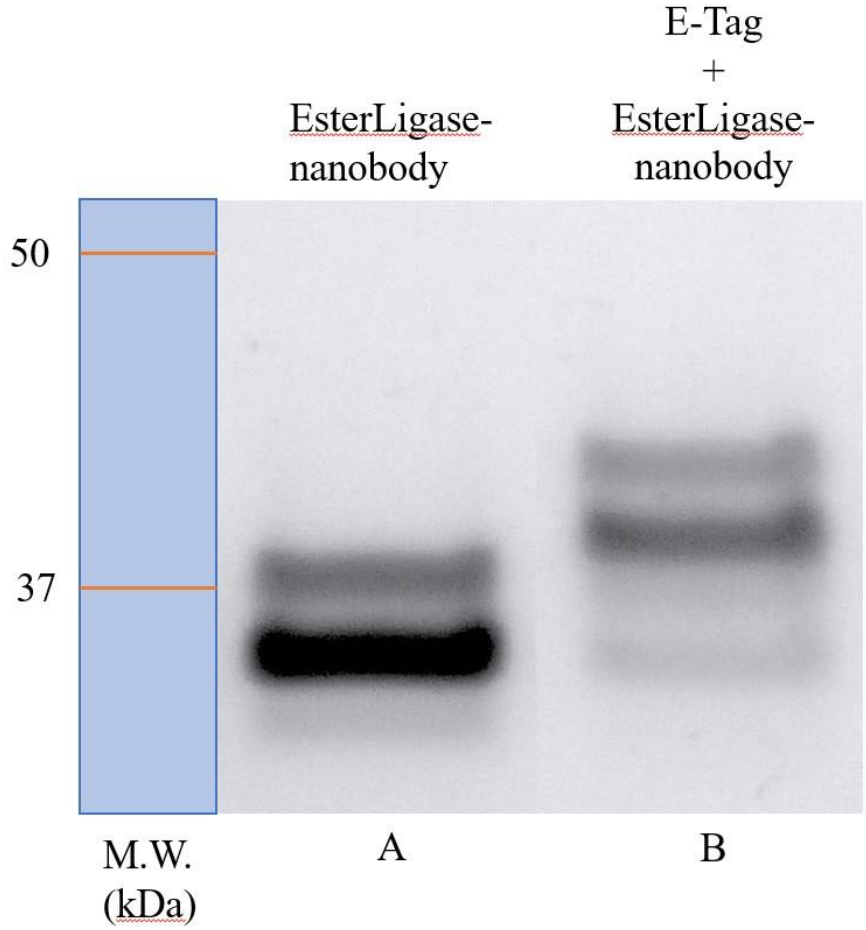

Figure S5. SDS-PAGE analysis (12% acrylamide gel) for ester bond formation. **Lane A**: EsterLigase-nanobody. **Lane B**: E-Tag and EsterLigase-nanobody allowed for overnight ligation reaction. Two species are visible from SDS-PAGE as the nanobody

is not fully reduced.

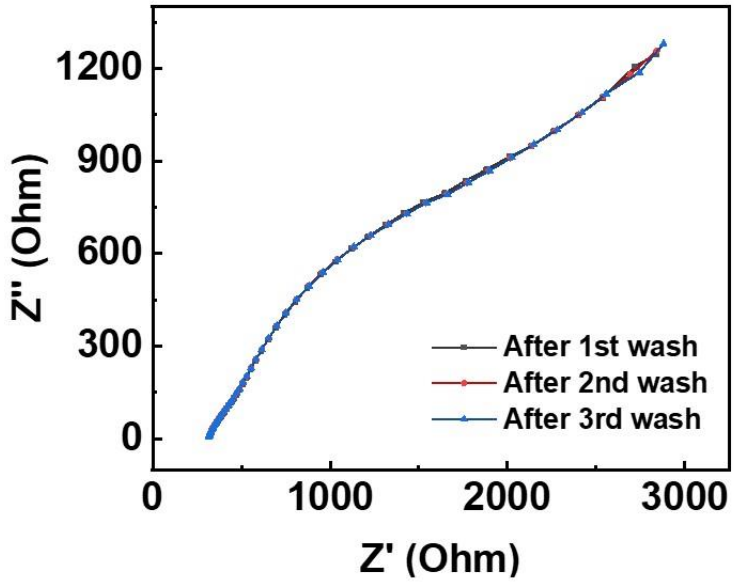

Figure S6. EIS spectra of LSG/PBA-pyrene-E-Tag linker/EsterLigase-nanobody after 1, 2 and 3 times washes with PBS buffer.

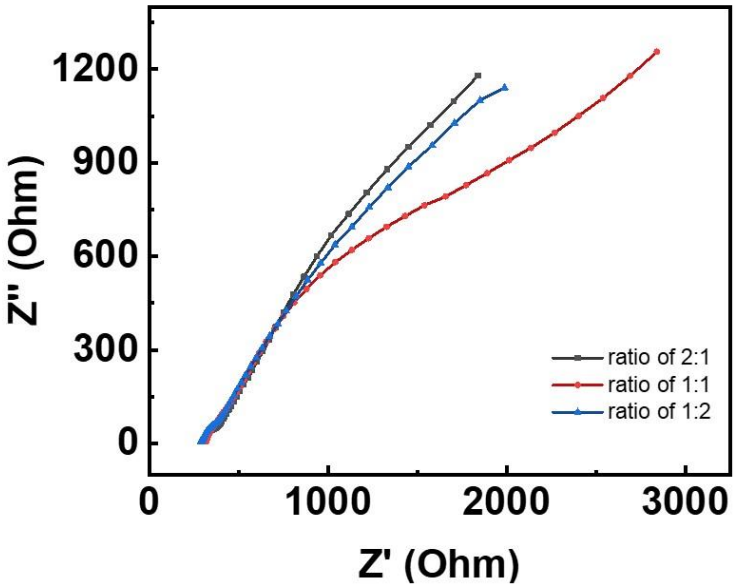

Figure S7. EIS spectra from the sensors with three different molar ratios of PBA to pyrene-E-Tag linker.

Reference

1. Armbruster, D.A.; Pry, T. Limit of blank, limit of detection and limit of quantitation. *Clin. Biochem. Rev.* **2008**, *29*, S49–S52.