

Supplementary Information

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

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S1. 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 Å, 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.

A)

MSYYHHHHHDYDIPTTENLYFQGASQVQLVETGGGFVQPGGSLRLSCAASGVTLDYAIGWFRQAP
 GKEREGVSCIGSSDGRITYSDSVKGRFTISRDNKNTVYLQMNLSLKPEDTAVYYCALTVGTYYSG
 NYHYTCSDDMDYWGKGTQVTVSSGAGSGSGSGRVTNKKIVSSLQTTVEADGQSSTA EKSAEVTEN
KDGVNVVDTIHYKGLIPKQKYEVVGI LYEVDGKGLVDPNKPITISNGTGEYTVSDSGEGEWKLN
GKIDGVEARKSYVVYEEVTSVENLVDTDNDGNTS

B)

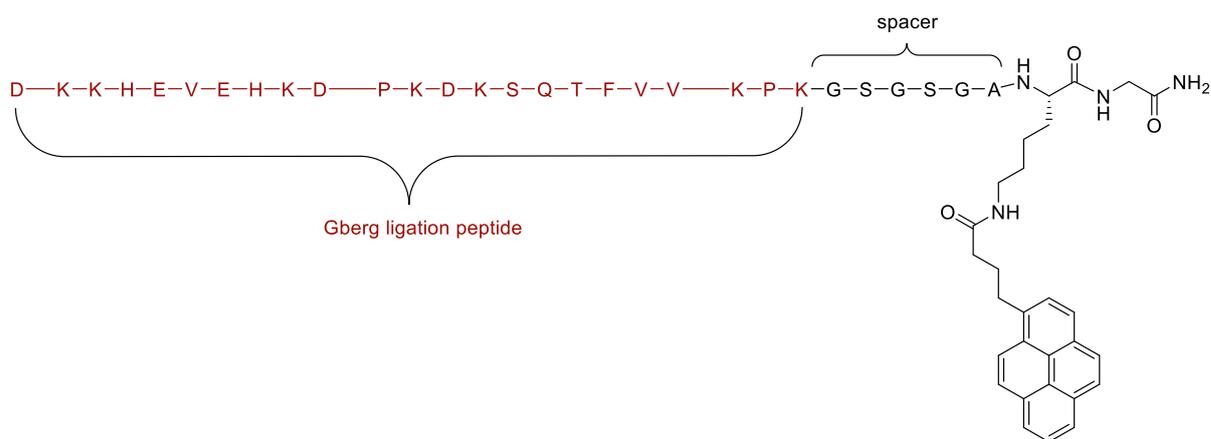


Figure S1. (A) Sequence of VHH E nanobody fused with EsterLigase. The N-terminal His⁶ IMAC tag and rTEV protease recognition sequence are underlined and removed from the purified VHH E EsterLigase recombinant protein. The VHH E nanobody sequence (grey) is linked to the *Gemella bergeriae* (Gberg) EsterLigase 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^ε amino group.

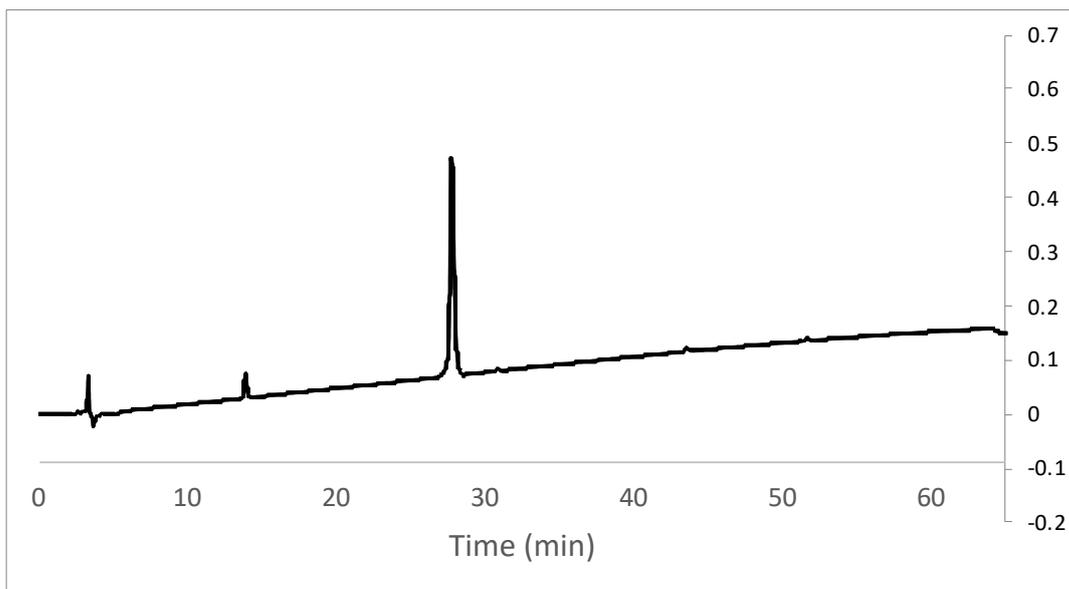


Figure S2. Analytical RP-HPLC for pyrene-modified E-Tag. RP-HPLC profile (214 nm) of pyrene modified E-Tag peptide. t_R 27.8 min, Phenomenex Luna C18 (100Å, 5 μ m, 4.6 mm x 250 mm), linear gradient 5%B to 65%B (ca. 1%B/min) at 1 mL/min.

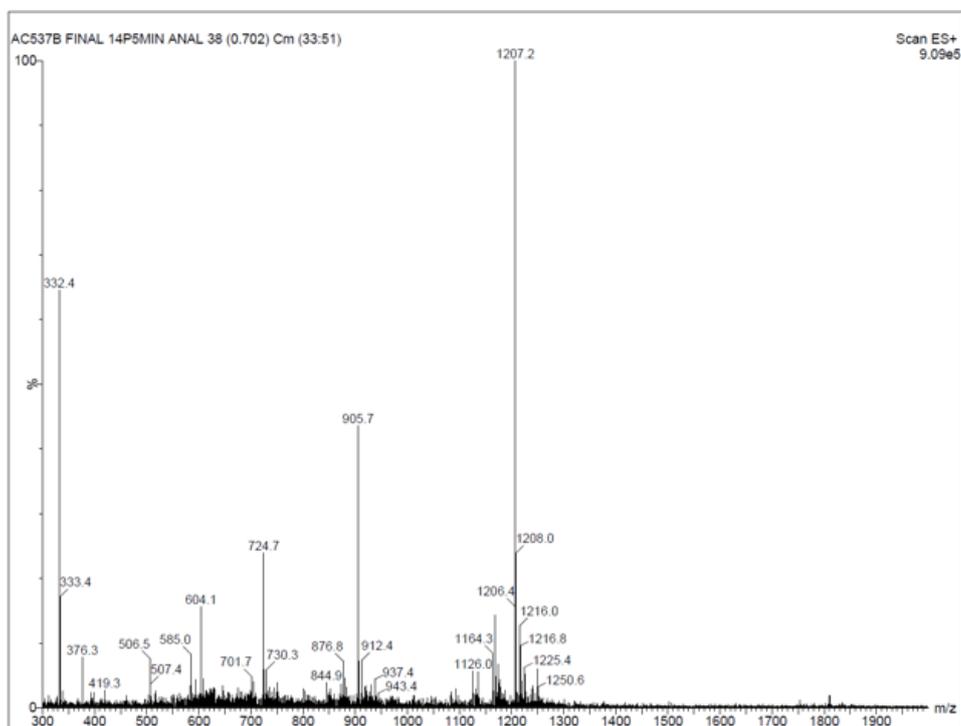


Figure S3. ESI-MS data for pyrene-modified E-Tag. Pyrene modified synthetic peptide ESI-MS (+ve) data. m/z calculated for $[C_{165}H_{253}N_{45}O_{47}]$ 3617.9; mass observed deconv: 3618.6 ± 0.13 . Charge states; 604.1 $[M+6H]^{6+}$, 724.7 $[M+5H]^{5+}$, 905.7 $[M+4H]^{4+}$, 1207.2 $[M+3H]^{3+}$.

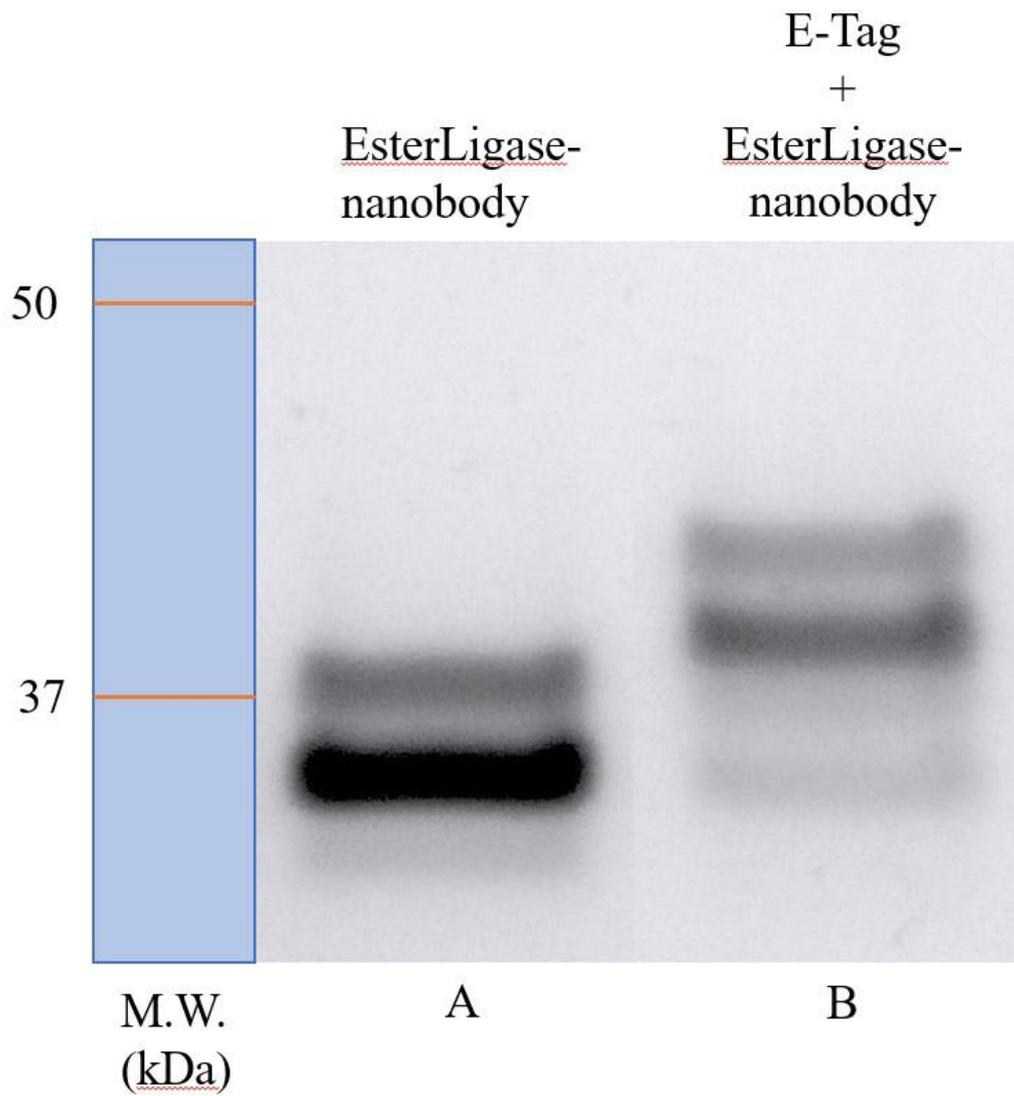


Figure S4. SDS-PAGE analysis (12% acrylamide gel) for ester bond formation. **Lane A:** EsterLigase-nanobody. **Lane B:** E-Tag and EsterLigase-nanobody allowed for overnight ligation reaction. Two species are visible from SDS-PAGE as the nanobody is not fully reduced