

Supplementary Material

Supplement to: Bin Gao, Shunyi Zhu. A Fungal Defensin Targets the SARS-CoV-2 Spike Receptor-Binding Domain

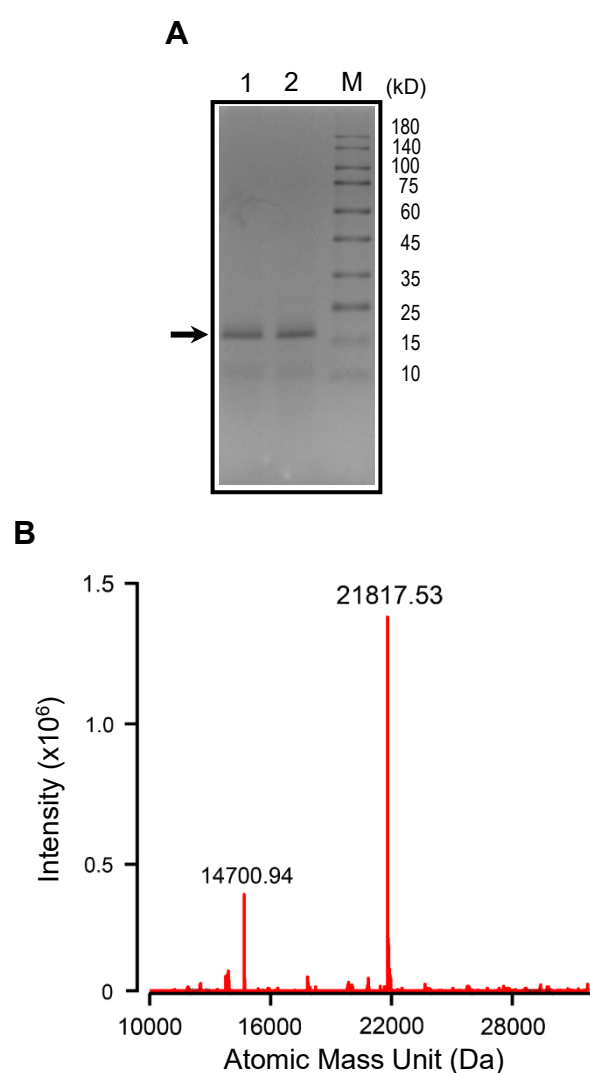
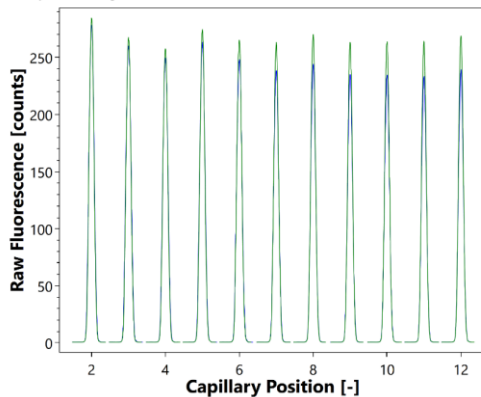


Figure S1. Characterization of soluble RBD renatured from *E. coli* inclusion bodies. (A) SDS-PAGE analysis of the renatured RBD obtained through ultra-filtered concentration with a 3 kD MWCO centrifugal device (Millipore, Bedford, MA, USA). The RBD band is marked by an arrow. (B) HPLC-Q-TOF-MS detection of the renatured RBD. Notice: 14700.94 Da was an unidentified component in the renaturation product.

A

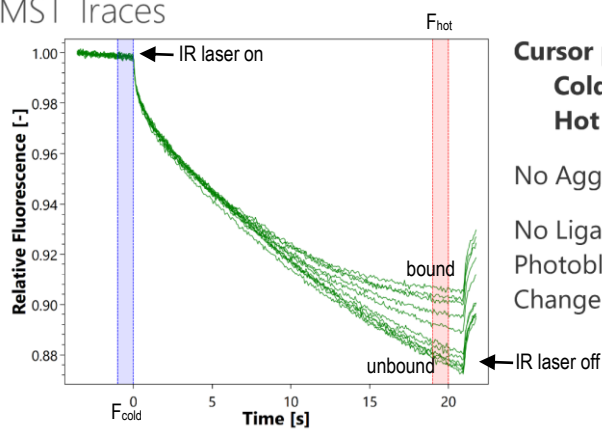
Capillary Scans

**Initial Fluorescence:****Average:** 267 counts ✓**Variation:** $\pm 6.6\%$ ✓

No adsorption ✓

No Ligand Induced
Fluorescence Change ✓**B**

MST Traces

**Cursor positions:****Cold Region:** -1s - 0s**Hot Region:** 19s - 20s

No Aggregation ✓

No Ligand Induced
Photobleaching Rate
Change ✓**C**

Dose Response

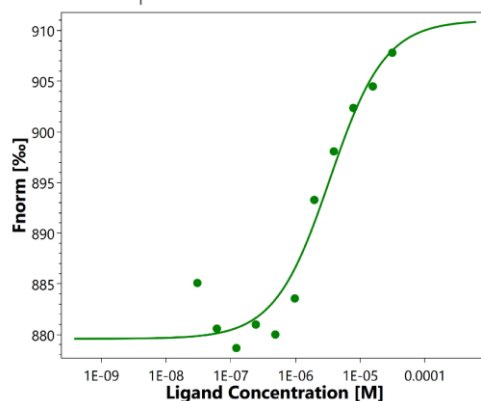
**Response Evaluation:** On Time 20s**Kd model****Unbound** 879.6**Bound** 911**Kd** 3.26 μM **TargetConc** 250 nM 🔒**Response Amplitude:** 31.4**Noise:** 2.5**Signal to Noise Ratio:** 12.6 ✓

Figure S2. Representative data collected during MST assay of protein-protein interactions. **(A)** Fluorescence capillary scans showing no ligand-induced fluorescence change across a series of capillaries that contained a constant concentration (250 nM) of RBD labelled by the BLUE-NHS fluorophore NT-495 and unlabeled rE8R at variable concentrations. **(B)** Typical MST traces for the peptide showing positive thermophoresis. **(C)** Binding of rE8R to the labelled RBD. The dose-response curve was fitted with a signal to noise ratio of 12.6.

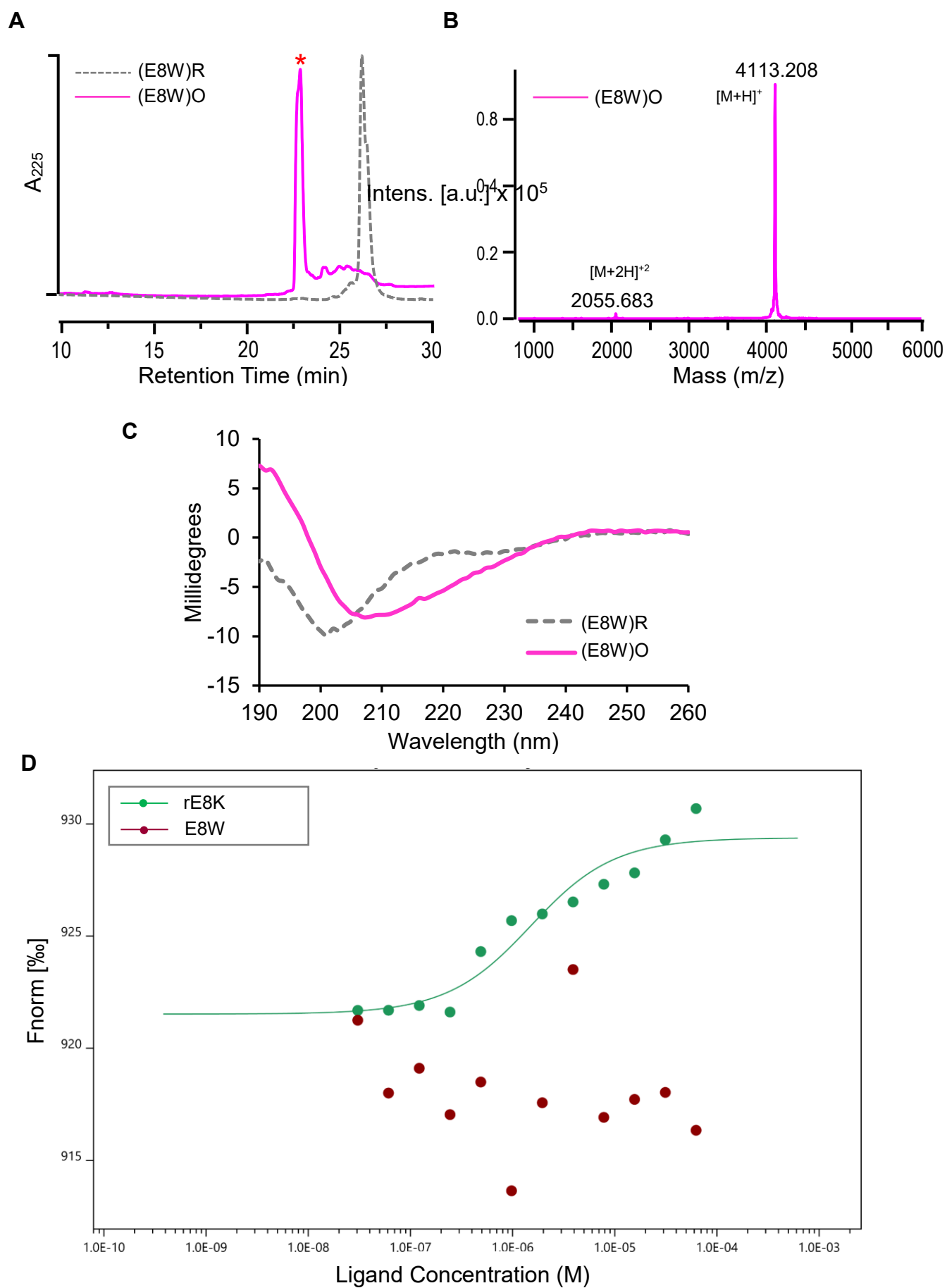


Figure S3. Oxidative fold-ing, characterization and RBD binding affinity of E8W. **(A)** RP-HPLC of the reduced and oxidized products. The latter was produced via air oxidization. **(B)** MALDI-TOF determining the molecular weight of the oxidized product showing two peaks corresponding to its singly and doubly protonated forms. **(C)** Comparison of the circular dichroism spectra between the reduced and oxidized products. **(D)** Quantification of binding affinity between RBD and E8W or rE8K by MST. Data points indicate the normalized fluorescence (%) generated by fluorescent RBD or the fluorescent RBD and peptide complex, from which curves are fitted with the K_D model.