

## Supplementary Information

### SARS-CoV-2 detection using a nanobody-functionalized voltammetric device

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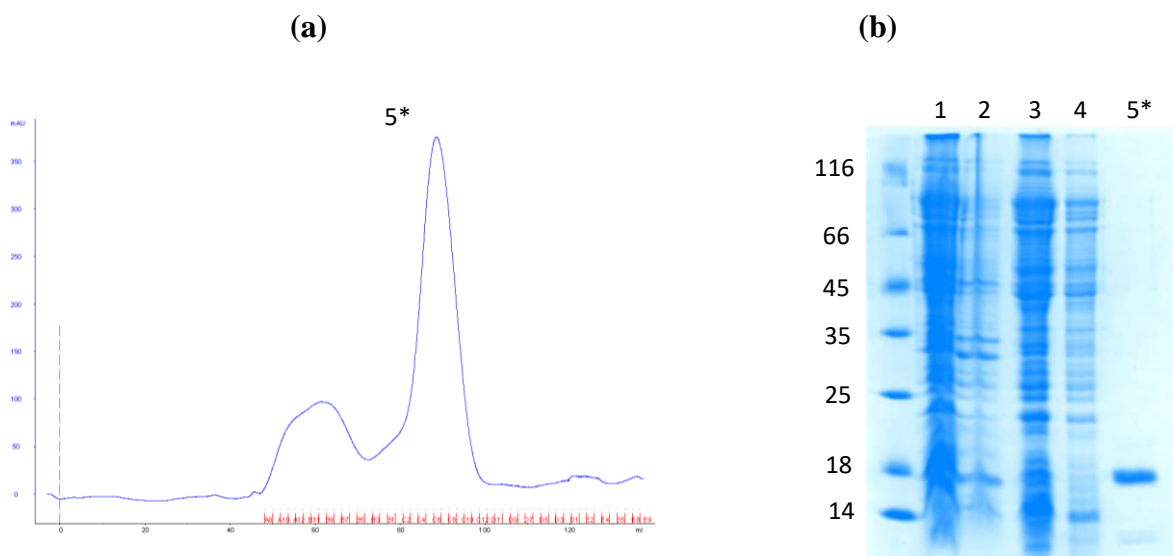
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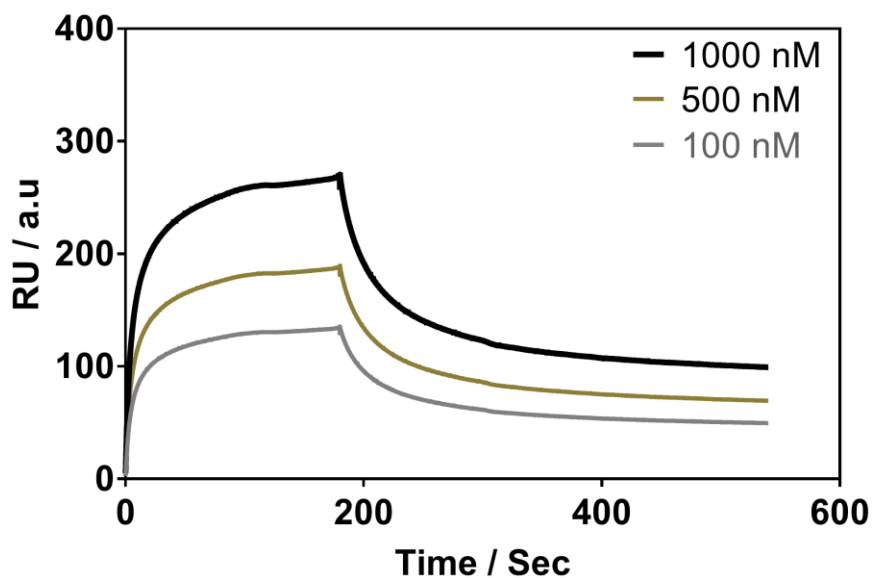
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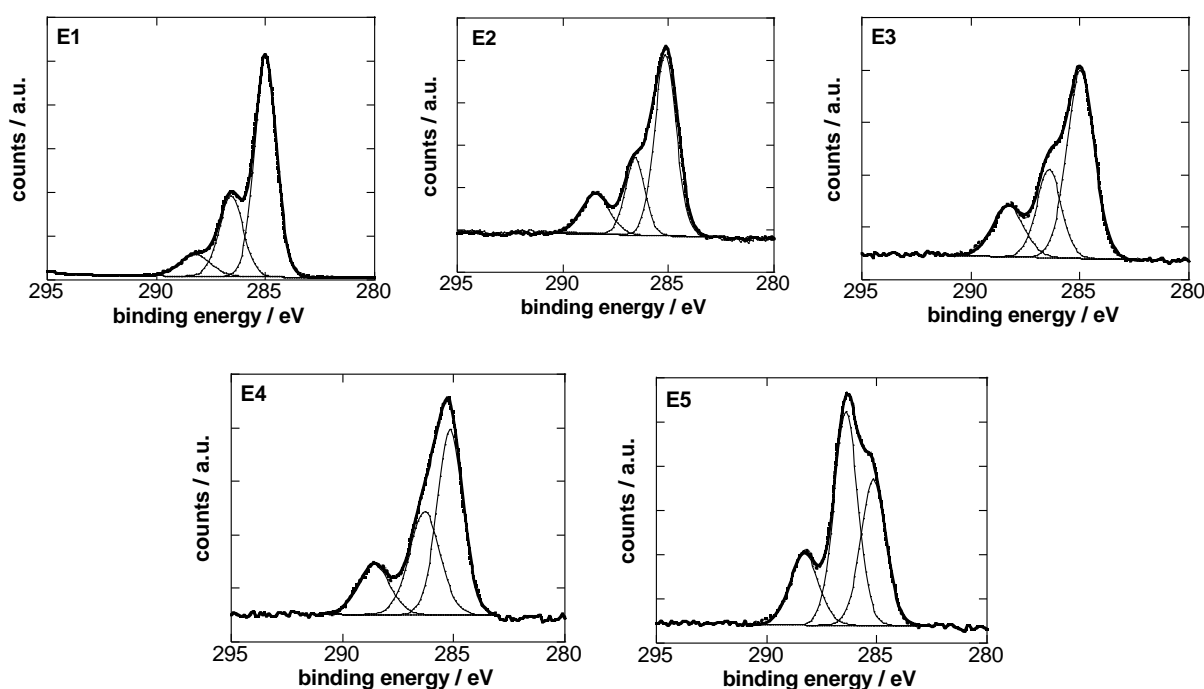
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**Figure S1.** (a) Affinity and size exclusion chromatography: (b) SDS-PAGE, acrylamide 15 %, Coomassie blue staining: 1: Total. 2: Pellet. 3: Soluble fraction. 4: Nickel / Flow-through. 5: Gel filtration / Elution



**Figure S2.** SPR binding curve of RBD to VHH-72 modified gold SPR chip (recorded on Biacore T200). The SPR binding curves were recorded on a Biocore T200 (Cytiva Life Science, using as running buffer HBS-P+ 1× (containing 0.1 M HEPES, 1.5 M NaCl and 0.5% v/v Surfactant P20). A CM5 SPR chip was employed and after activation using NHS/EDC (15 mM in HBS-P+ 1×) for 30 sec (flow rate=  $5\mu\text{L min}^{-1}$ , VHH-72 ( $100\mu\text{g mL}^{-1}$  in HBS-P+ 1×) was flown over surface for 30 sec at  $5\mu\text{L min}^{-1}$  to reach a modification response of 200 RU (equal to  $0.2\text{ ng mm}^{-2}$ ). The interaction with RBD (in HBS-P+) was at a flow of  $30\mu\text{L min}^{-1}$ .



**Figure S3.** High resolution of the C1s region of interfaces E1-E5 (Table 1). The gold surface was modified with 3-mercaptopropionic acid before (E1) and after (E2) VHH-72 immobilization. (E3) Direct linkage of VHH-72. Gold electrode modified with 3-mercaptopropionic acid, followed by NH<sub>2</sub>-PEG-maleimide linkage before (E4) and after (E5) VHH-72-13C as immobilization.

**Table S1.** Element percentage obtained from XPS analysis for electrodes E1-E5

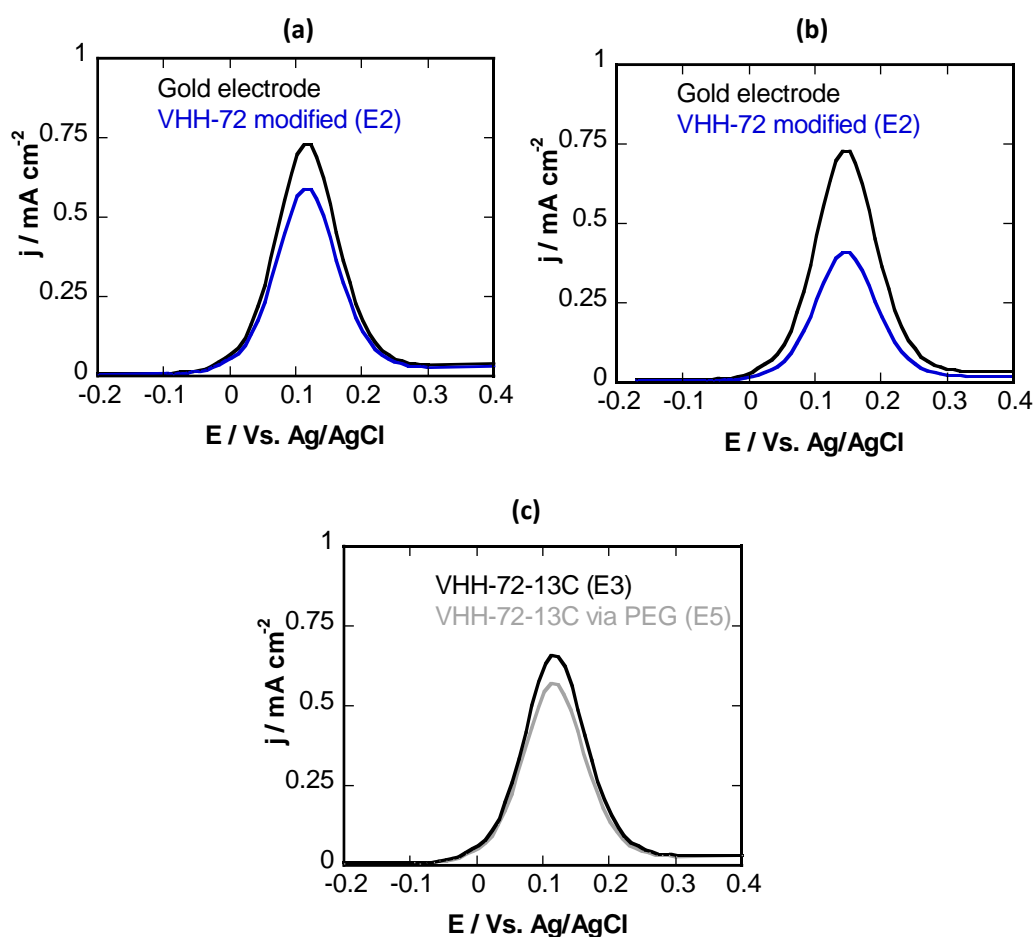
Electrode	Modification strategy	Atomic percentage (%)			
		C1s	O1s	N1s	S2p
E1	3-Mercaptopropionic acid (MPA)	75.3±0.3	20.6±0.2	-	4.1±0.5
E2	1. MPA 2. VHH-72	69.8±0.5	18.9±0.4	8.9±0.2	2.4±0.5
E3	1. VHH-72-13C	69.9±0.4	20.2±0.2	8.8±0.4	1.1±0.2
E4	1. MPA 2. NH <sub>2</sub> -PEG <sub>6</sub> -maleimide	69.5±0.4	20.1±0.8	6.2±0.5	4.2±0.5
E5	1. 3-Mercaptopropionic acid 2. NH <sub>2</sub> -PEG <sub>6</sub> -maleimide 3. VHH-72-13C (ou VHH11D4-13C)	65.3±0.5	21.4±0.6	9.2±0.2	4.1±0.7

The success of the integration of VHH-72 was validated by XPS analysis. The C1s high resolution spectra of the 5 interfaces are seen in **Figure S3**. The C1s spectrum of E1 can be deconvoluted into bands at 285.0 eV (C-C/C-H), 286.7 eV (C-S/C-O) and 288.2 (O-C=O) eV in accordance with the chemical composition of the electrode surface. The C1s high resolution spectrum of the surface E2, prepared by direct linking of VHH-72 *via* amide bond formation,

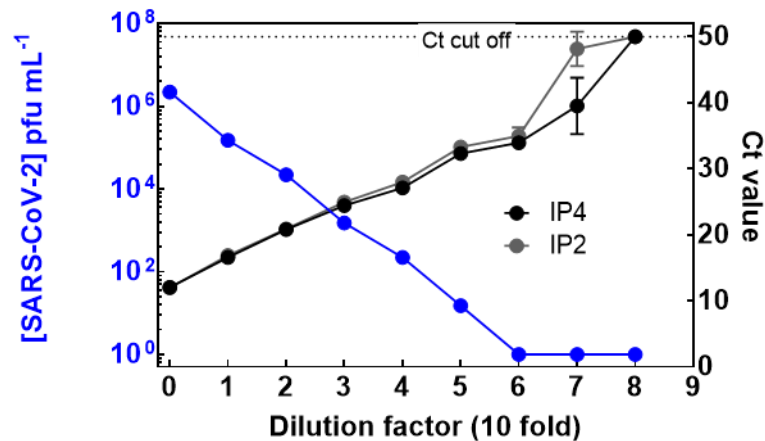
can be curve-fitted with several bands at 285.0 eV (C-C/C-H), 286.7 eV (C-N/C-S/C-O) and 288.6 eV (O-C=O/ N-C=O).

The C1s spectrum of interface **E3**, obtained through the direct linkage of VHH-C13 to gold surface, shows comparable features as E2, with bands at 285.0 eV (C-C/C-H), 286.7 eV (C-S/C-O/C-N) and 288.6 eV (O-C=O/N-C=O).

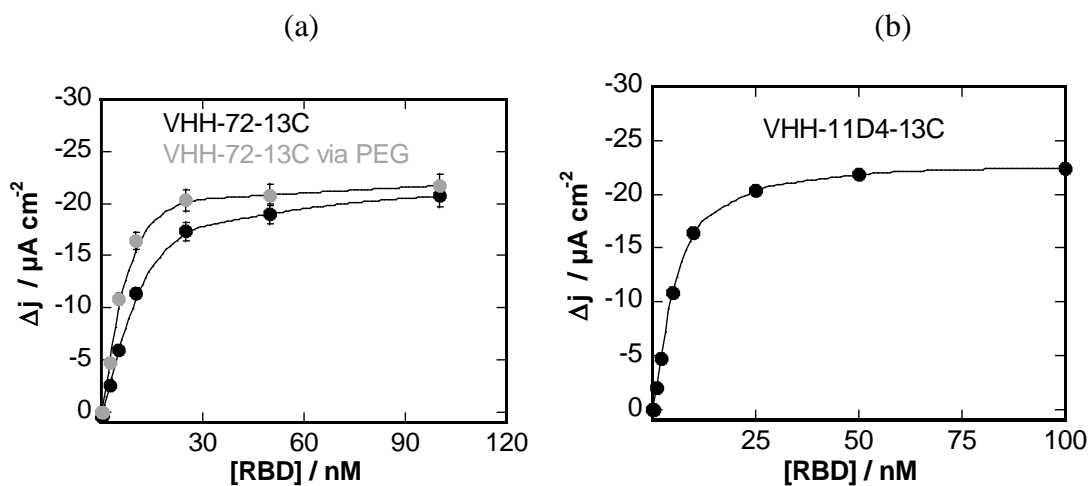
The core level XPS spectrum of the C1s of the pegylated gold interface functionalised with maleimide terminal group (**E4**) can be fitted with several components at 285.0 eV (C-C/C-H), 286.3 eV (C-O) and 288.7 eV (C=O and N-C=O), in full agreement with the chemical composition of the surface. Incorporation of VHH-72-C13 or VHH-11D4-13C *via* thiol-maleimide bond formation (**E5**) induces slight changes in the C1s band with contributions at 285.0 eV (C-C/C-H), 286.3 eV (C-O, C-N, C-S) and 288.2 eV (C=O). The increase of the band at 286.3 eV is due to the introduction of additional C-N and C-S bonds brought by the VHH-72-C13 or VHH-11D4-13C.



**Figure S4. Differential pulse voltammograms of different electrodes:** (a) Gold electrode before (black) and after (blue) VHH-72 immobilization (surface **E2**) in ferrocenemethanol (1 mM in 0.1 M PBS, pH 7.4). (b) Gold electrode before (black) and after (blue) VHH-72 (surface **E2**) immobilization in  $\text{Fe}(\text{CN})_6^{4-/3-}$  (1 mM in 0.1 M PBS, pH 7.4). (c) Gold electrode modified with VHH-72-13C (surface **E3**) and via maleimide-modified PEG linker (surface **E5**) immobilization in ferrocenemethanol (1 mM in 0.1 M PBS, pH 7.4).



**Figure S5: Correlation of Ct values with SARS-CoV-2 infectivity:** Vero E6 cells ( $2.5 \times 10^5$  cells/well) were infected with 10-fold dilutions of a SARS-CoV-2 isolate. The plates were incubated for 6 days in 5% CO<sub>2</sub> atmosphere at 37 °C and examined daily using an inverted microscope (ZEISS Primovert) to evaluate the extent of the virus-induced cytopathic effect in cell culture. Calculation of estimated virus concentration was carried out by the Spearman and Karber method<sup>1,2</sup> and expressed as TCID<sub>50</sub>/mL (50% tissue culture infectious dose). TCID<sub>50</sub>/mL values were transformed to PFU mL<sup>-1</sup> by using the formula  $\text{PFU mL}^{-1} = \text{TCID}_{50}/\text{mL} \times 0.7$  RNA extraction.<sup>3</sup> Negative RT-PCR results were set to Ct= 50. The results are expressed as the mean  $\pm$  SEM of at least 4 independent samples for each group.



**Figure S6: Calibration curves for RBD on VHH-72-13C and VHH11D4-13C modified interfaces.** Change in current density responses towards RBD standard addition on gold electrodes modified with (a) VHH-72-C13 *via* direct linkage (surface **E3**) with a PEG linker (surface **E5**), and (b) CHH11D4-13C using ferrocenemethanol (1 mM in 0.1 M PBS, pH 7.4) as redox probe. The results are expressed as the mean  $\pm$  SEM of at least 3 independent samples for each group.

### Supplementary References

- 1 Spearman, C., The Method of "Right and Wrong Cases" (Constant Stimuli) without Gauss's Formula. *Br. J. Psychol.* 1908, 2, 227-242.
- 2 Kärber, G., Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. . *Arch. exp. Pathol. U. Pharmacol.* 1931, 162, 480-483.
- 3 <https://www.lgcstandards-atcc.org/support/faqs/48802/Converting%20TCID50%20to%20plaque%20forming%20units%20PFU-124.aspx>.