Supplementary Information

An intramolecular FRET Biosensor for the Detection of SARS-CoV-2 in biological fluids

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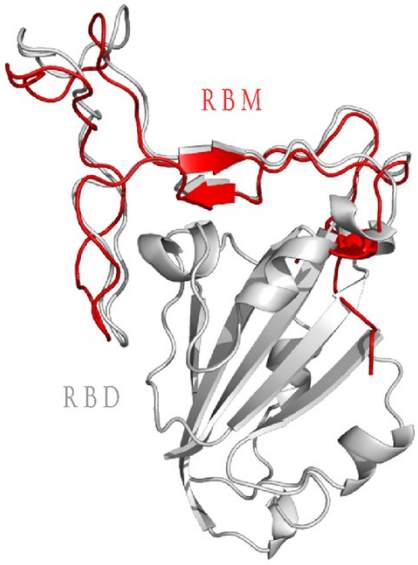


Figure S1: Comparison between the SARS-CoV-2 RBD (colored in gray) and its lower-molecular weight version RBM, formed by the RBD residues at the LCB1 interface (red).

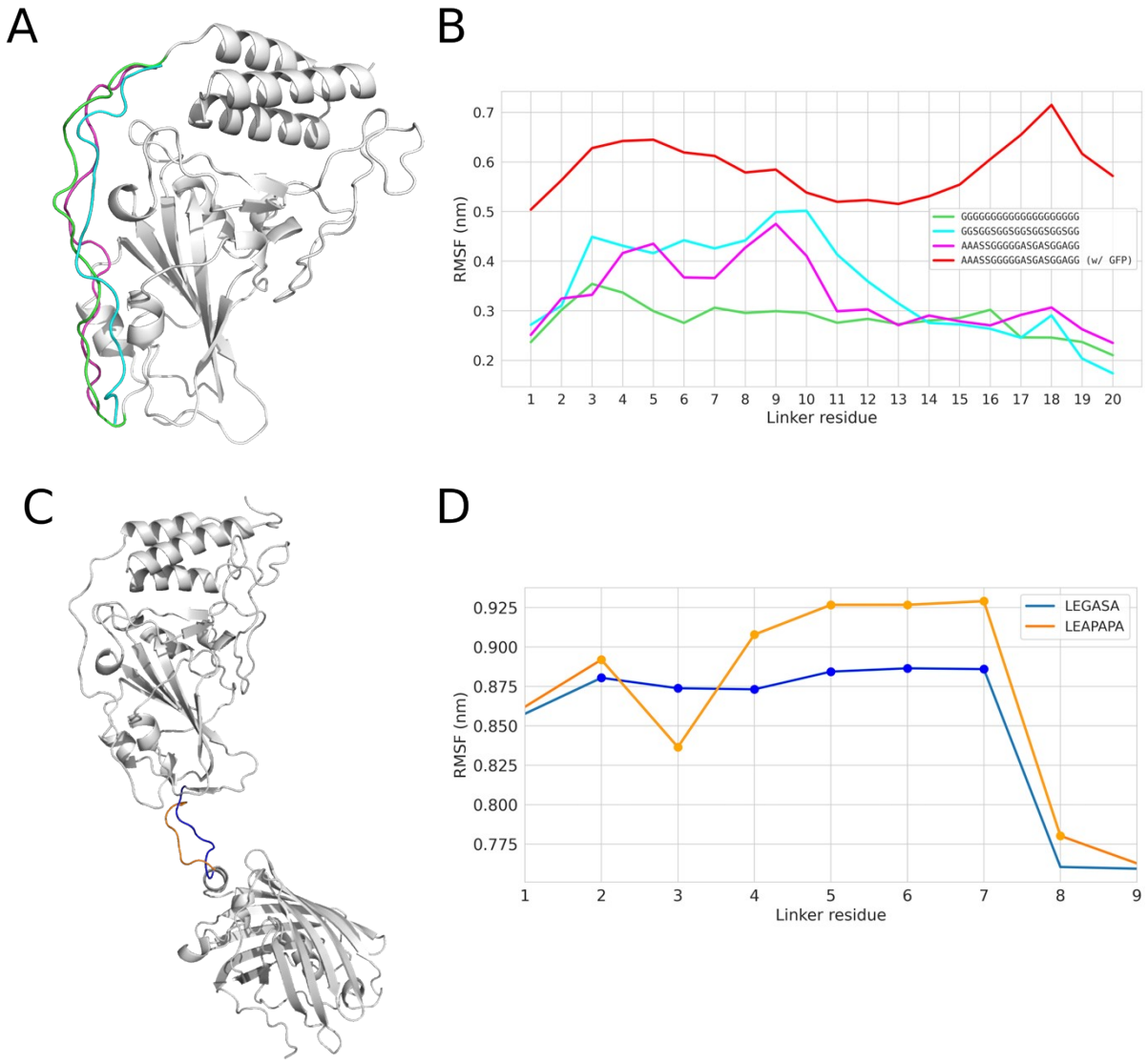


Fig. S2 A) LCB1-RBD complex with the three different linkers (Gly-Gly-Gly)7, (Gly-Gly-Ser)7 and AAASSGGGGGASGASGGAGG, colored in green, cyan, and magenta, respectively. **B)** Root Mean Square Fluctuations (RMSF) of the different linkers in the LCB1-RBD complex: (Gly-Gly- Gly)7, (Gly-Gly-Ser)7 and AAASSGGGGGASGASGGAGG, colored in green, cyan, and magenta, respectively. The RMSF of the experimental linker AAASSGGGGGASGASGGAGG when GFP is connected to RBD is colored red. **C)** LCB1-RBD-GFP complex with the two different linkers LEGASA and LEAPAPA, colored in blue and orange, respectively. **D)** RMSF of the RBD-GFP linkers (containing LEGASA or LEAPAPA, and colored in blue and orange, respectively). Residues corresponding to the amino acids LEGASA and LEAPAPA are highlighted with circles.

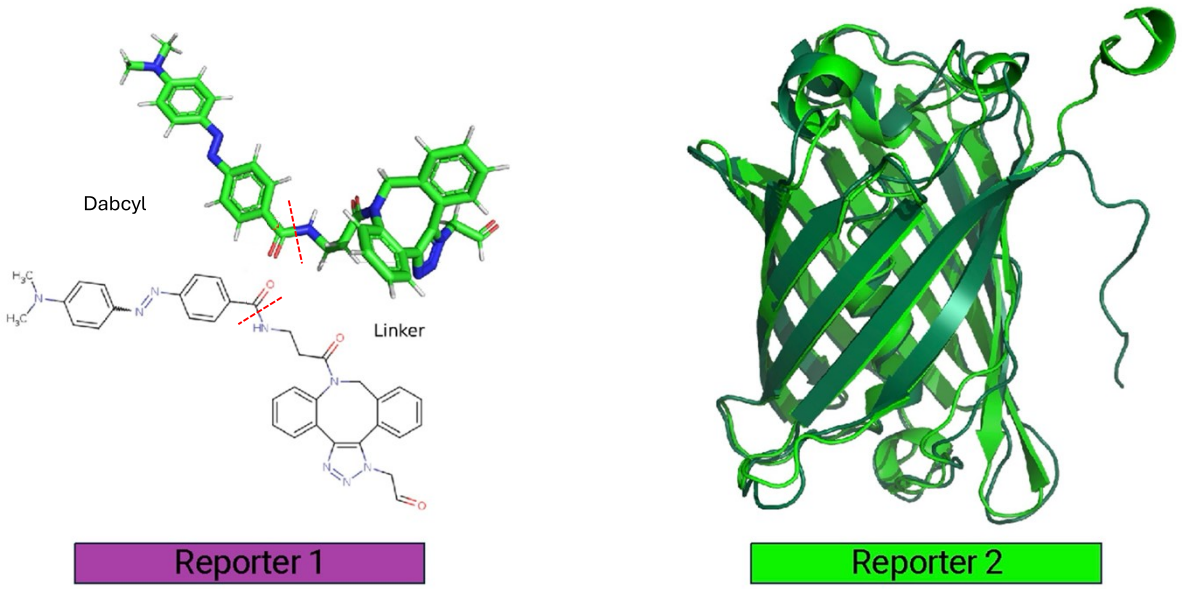


Figure S3: (Left) 2D and optimized 3D structures of the quencher covalently attached to the N-terminal portion of the LCB1 and obtained after the PyRED server calculations. It is composed by a Dabcyl fluorophore and a linker. The two parts of the probe are divided by a dashed red line. (Right) Comparison of the GFP protein structures obtained from the Homology Modeling with SWISS-MODEL (light green) and after the 50 ns MD relaxation (dark green).

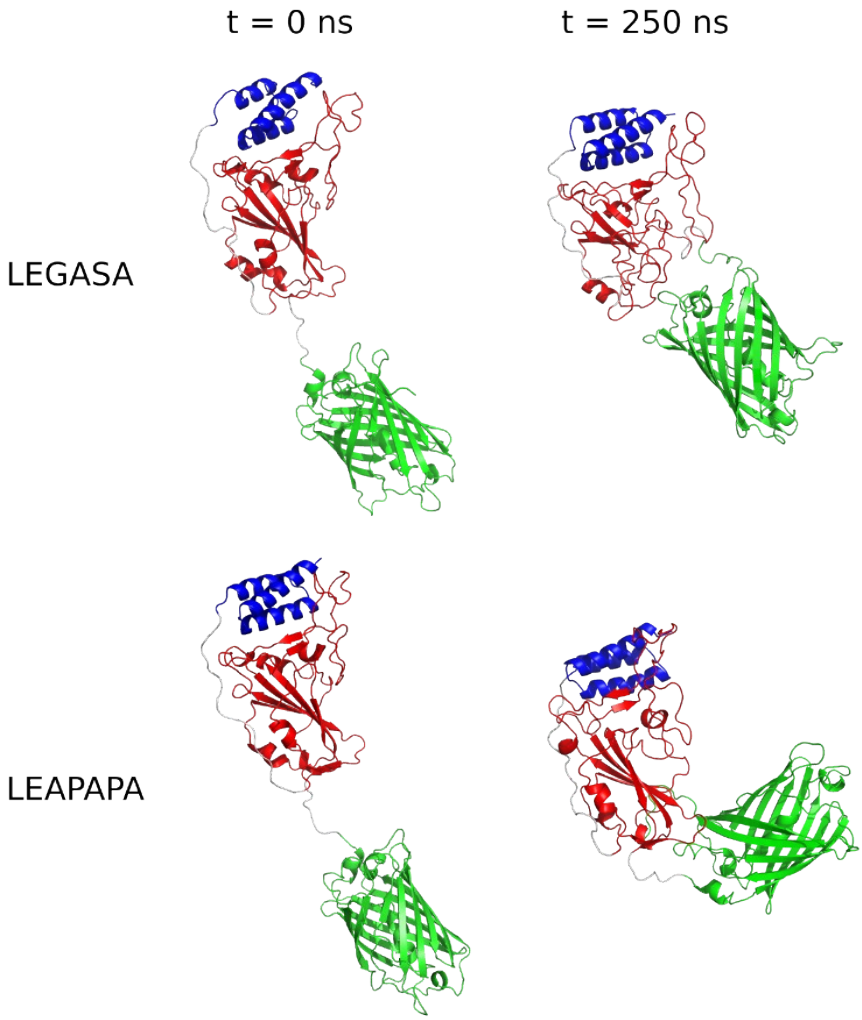


Fig. S4 Effect of the differents linkers 3 between GFP and RBD. LCB1, RBD, and GFP are colored in blue, red, and green, respectively, while the linker sequences are colored in white. With both linkers, the GFP approaches the RBD and remains close to it throughout the simulation. However, the RBD in the LEGASA sensor partially unfolds after 100 ns, while with the LEAPAPA linker, there are no dramatic structural changes in the system.

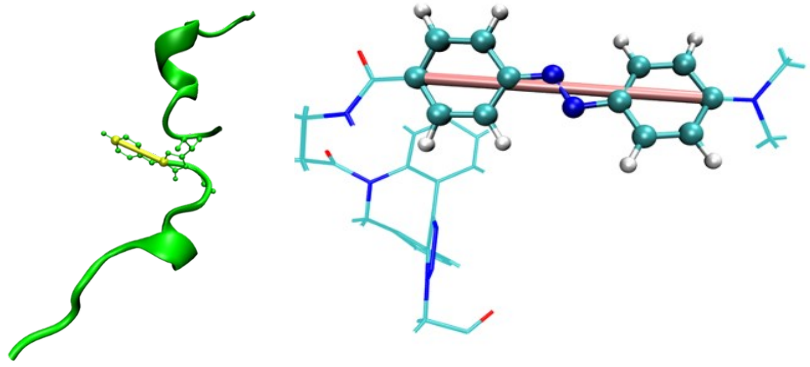
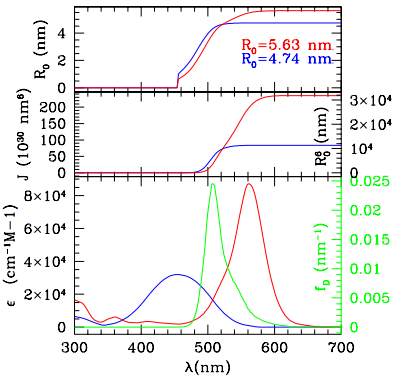


Fig. S5 (A) GFP chromophore and the orientation of its transition dipole in yellow (B) quencher and the orientation of its transition dipole (in pink).

A

$$J = \frac{\int f_D(\lambda)\,\varepsilon_A(\lambda)\,\lambda^4 d\lambda}{\int f_D(\lambda)\,d\lambda}$$

$$R_0^6 = \frac{2.07}{128\pi^5 N_{Av} n^4} Q_D \kappa^2 J$$



B

$$\kappa = \hat{\mu}_D \cdot \hat{\mu}_A - 3\,(\hat{R} \cdot \hat{\mu}_A)(\hat{\mu}_D \cdot \hat{R})$$

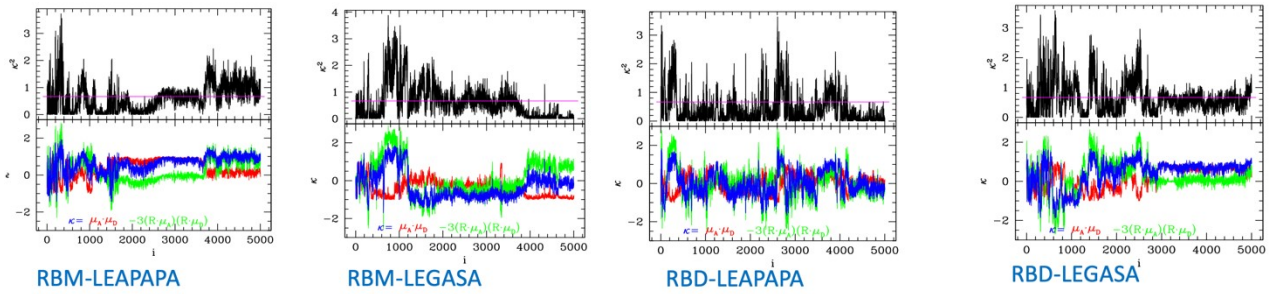


Fig. S6 (A) Calculation of the R_0 the superposition integral J of the GFP adsorption spectrum (green in the bottom plot) and two different quenchers, the Dabcyl (blue used in simulations) and the QSY9 (red). The J is reported in the middle plot along λ and the values of R_0 in the two cases (reported) are obtained from its asymptotic value. (B) Evaluationf of the geometric factor K along different trajectories: (A) RBM-LEAPAPA, (B) RBM-LEGASA, (C) RBD-LEAPAPA, (D) RBD-LEGASA. The dipole directions are taken as in Fig S5 and the scalar products involved in the formula are evaluated in each frame of the trajectories.

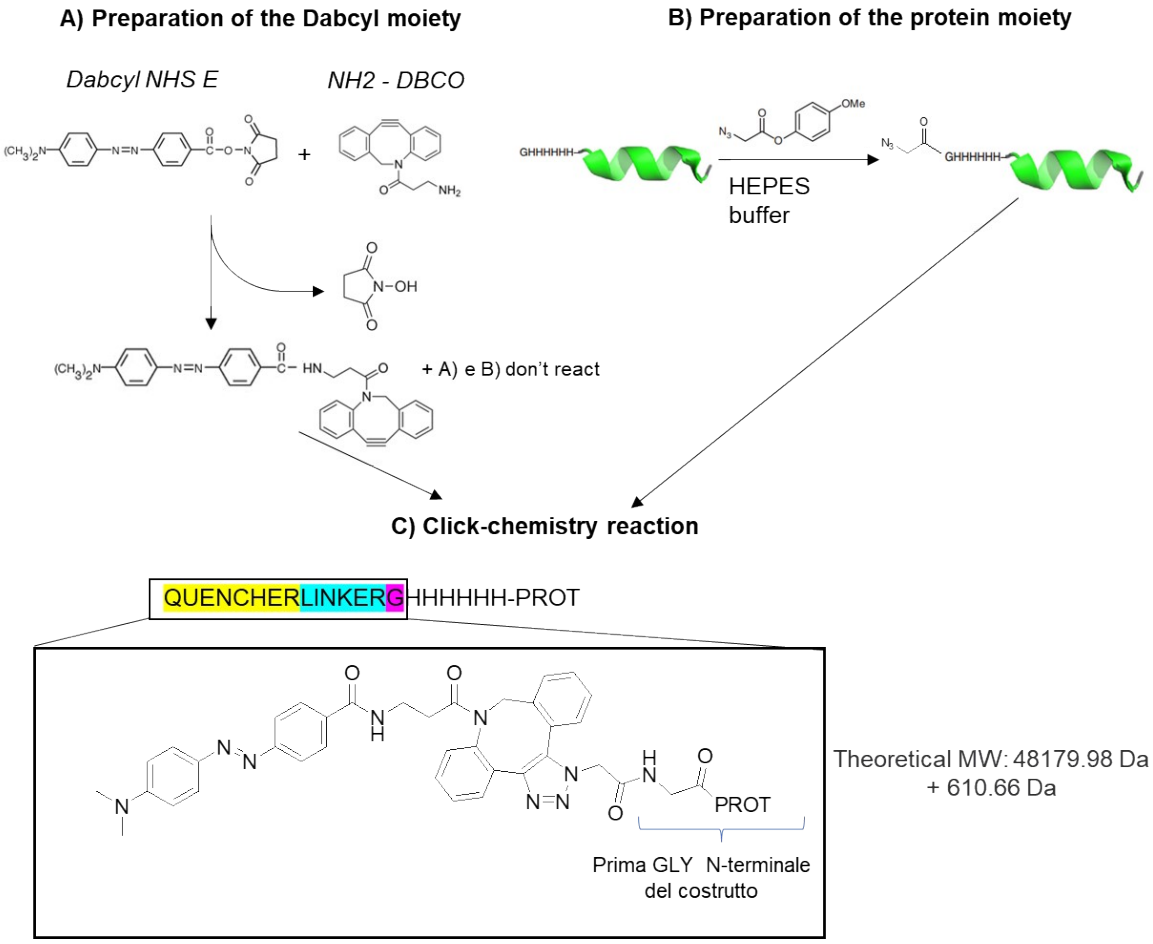


Figure S7: A) Schematic representation of the conjugation of the Dabcyl-NHS to the NH2-DBCO, leading to the introduction of alkyne reactive element in the quencher structure. B) Schematic representation of the bio-conjugation reaction necessary to introduce the azide element at the N-term position of the protein of interest. C) Schematic representation of the click-chemistry reaction leading to the covalent conjugation of the quencher to the protein sequence.

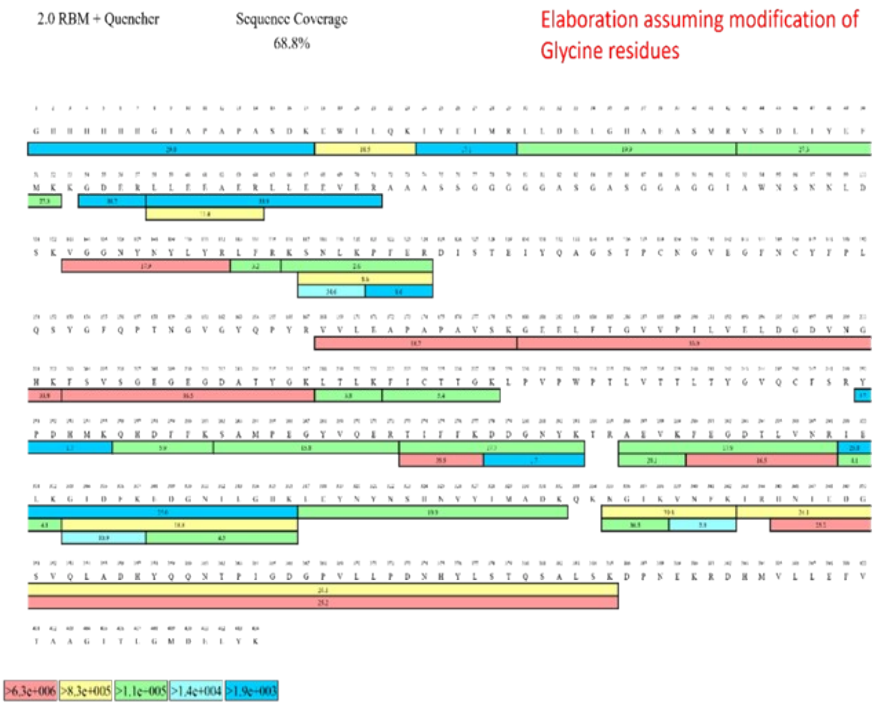


Figure S8: To better understand the discrepancies between the theoretical MW and the one obtained with RP-HPLC, a trypsin digestion of the proteins was performed, and obtained fragments were mapped to the theoretical sequence. Data revealed the loss of the last residues of the EGFP protein.

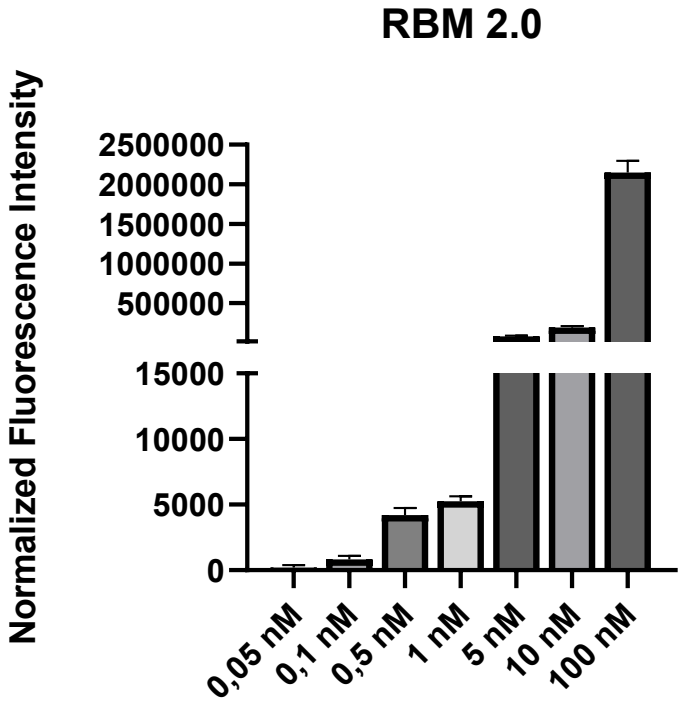


Figure S9. Graphs report the fluorescence intensity in the saliva biological fluid of RBM-S in a multi-well plate at 0.05, 0.1, 0.5, 1, 5, 10, 100 nM. Data are reported as mean±SEM of the values pooled from 4 different biological fluids performed in triplicate. The plotted fluorescence intensity has been subtracted from the contribution of PBS buffer.

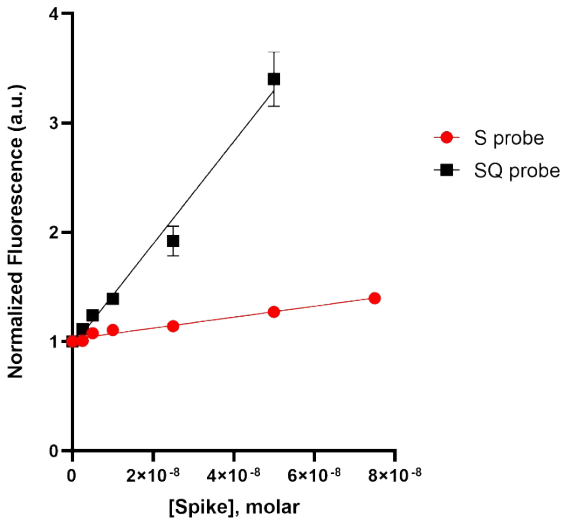


Figure S10. Graph reporting the normalized fluorescence intensity of 10 nM S and SQ incubated with increasing concentrations of Spike protein. Data are reported as mean±SEM of the values pooled from 5 different measures. The plotted fluorescence intensity has been subtracted from the contribution of buffer and Spike, before normalization. The linear trend of the working range is superimposed on both curves.