Plasmonic Fiberoptic Absorbance Biosensor (P-FAB) for Rapid Detection of SARS-CoV-2 Nucleocapsid protein

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Supporting information

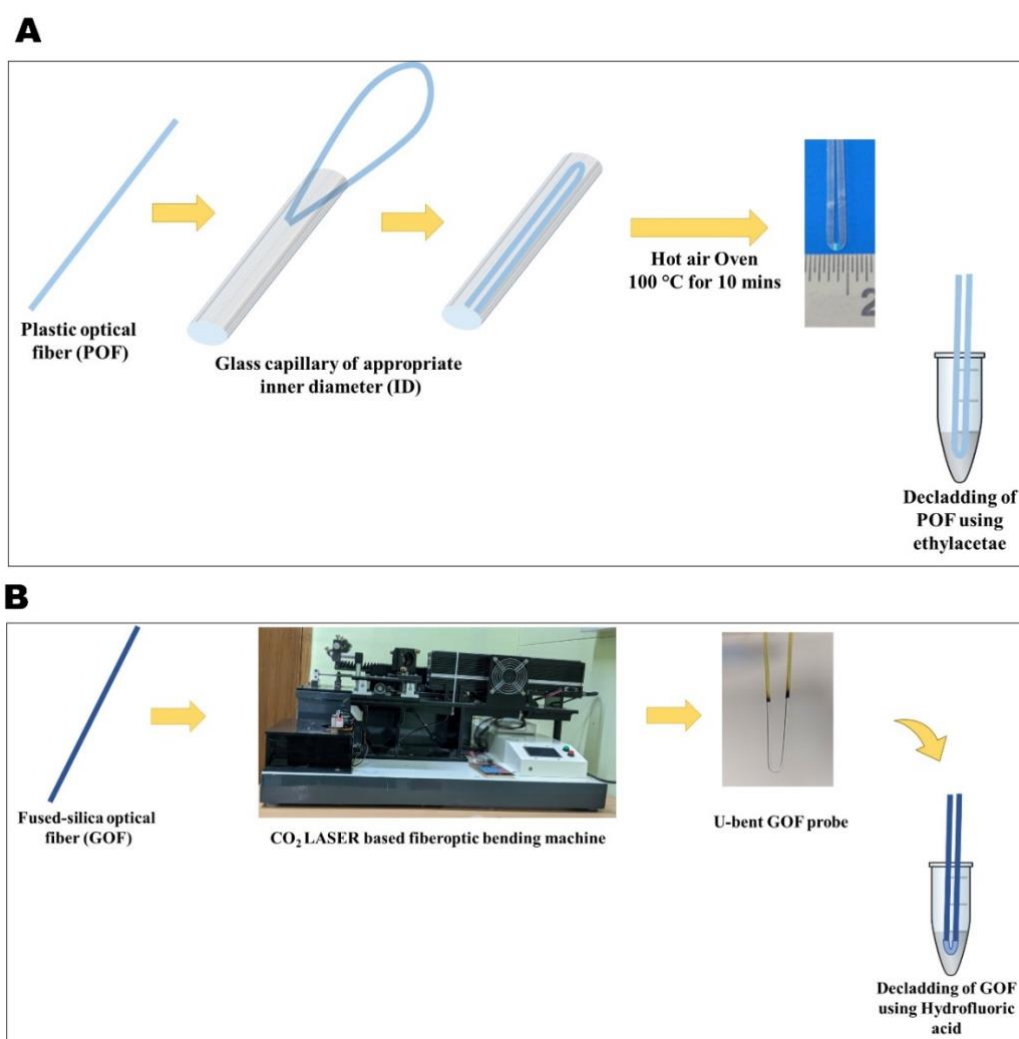## Fabrication of U-bent fiber optic sensor probes

Figure S1: Schematic representation of fabrication of U-bent (A) POF and (B) GOF sensor probes.

Figure S2: In-house built CO_2 laser based fiber optic bending machine

Section S1: Determination of the total number of AuNPs and number of antibody molecules per AuNP

Calculating the number of AuNPs in synthesized solution:

Nanoparticle diameter (TEM image analysis)   : 40 nm

Volume of single AuNP is (assuming sphere)   : 33493×10^{-21} cm^3.

Using Au density (19.30 g/cc),

One AuNP Mass (Volume × Density)   : 6.464×10^{-16} g

1 mL (12.7 mM) solution is diluted to 40 mL hence the concentration is 0.3175 mM which gives 0.06254 g/L (Considering 1mol Au weight is 197g)

Hence number of AuNP in 1 mL of liquid is given by $\{ \frac{0.06254 \text{ g/L}}{6.464 \times 10^{-16} \text{ g}} \} = 9.6 \times 10^{10}$ nanoparticles

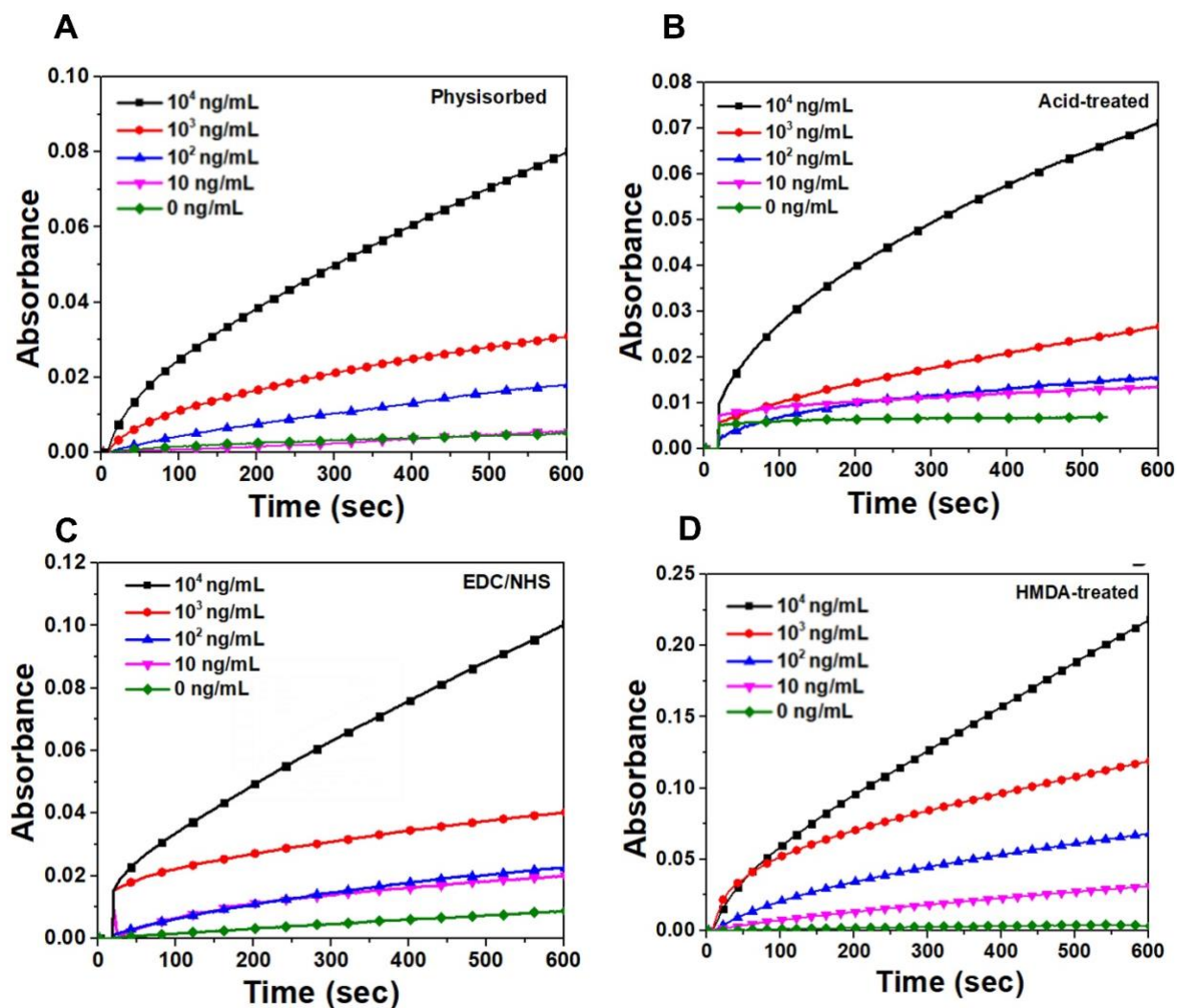**U-bent POF probes and surface modification strategies:**

Figure S3: Temporal absorbance response obtained from U-bent POF probes immobilized with capture antibodies by means of (A) physisorption, (B) acid hydrolysis, (C) acid hydrolysis followed by EDC/NHS treatment and acid-hydrolysis followed by HMDA treatment-treatment.
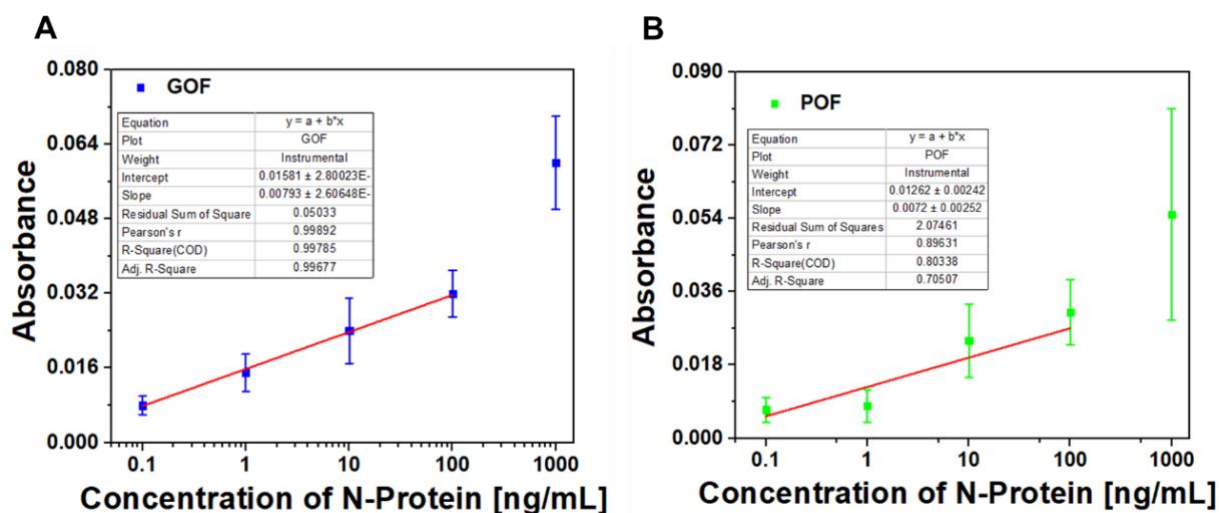
Figure S4: Calibrated absorbance response obtained for a range of SARS-CoV-2 N-protein concentrations from POF and GOF sensor probes chemisorbed with anti-SARS-CoV-2 -N-protein IgG1 with the help of a green LED-photodetector setup due to binding of immunocomplex with AuNP labels (40 nm, 10x) conjugated with anti-SARS CoV-2 -N-protein IgG2 (n=3). Inset: Linear fit parameters.

Table S1: Theoretical estimation for antibody conjugation to AuNP

Size (nm)	No. of AuNP ($\times 10^9$)/100 µL	AuNP surface area/footprint area of IgG	No. of Abs ($\times 10^9$) to saturate 100 µL of AuNPs	Approximate quantity of Ab required (µg)
20	70	14	980	0.245
40	9	56	504	0.126
60	2.6	125	325	0.081
40 nm*	9.6	56	538	0.135

*Synthesized citrate-capped AuNPs 40 nm. The number 20, 40 and 60 nm AuNPs were obtained from the manufacturer (BBI solutions, UK), while for the synthesized particles it is estimated as shown in section S1. Based on the estimated values, 100 µL of conjugates were prepared using 0.125, 0.25, 0.5 µg were conducted using anti-SARS-CoV-2-N-protein IgG2 and subjected to NaCl salt stability test.
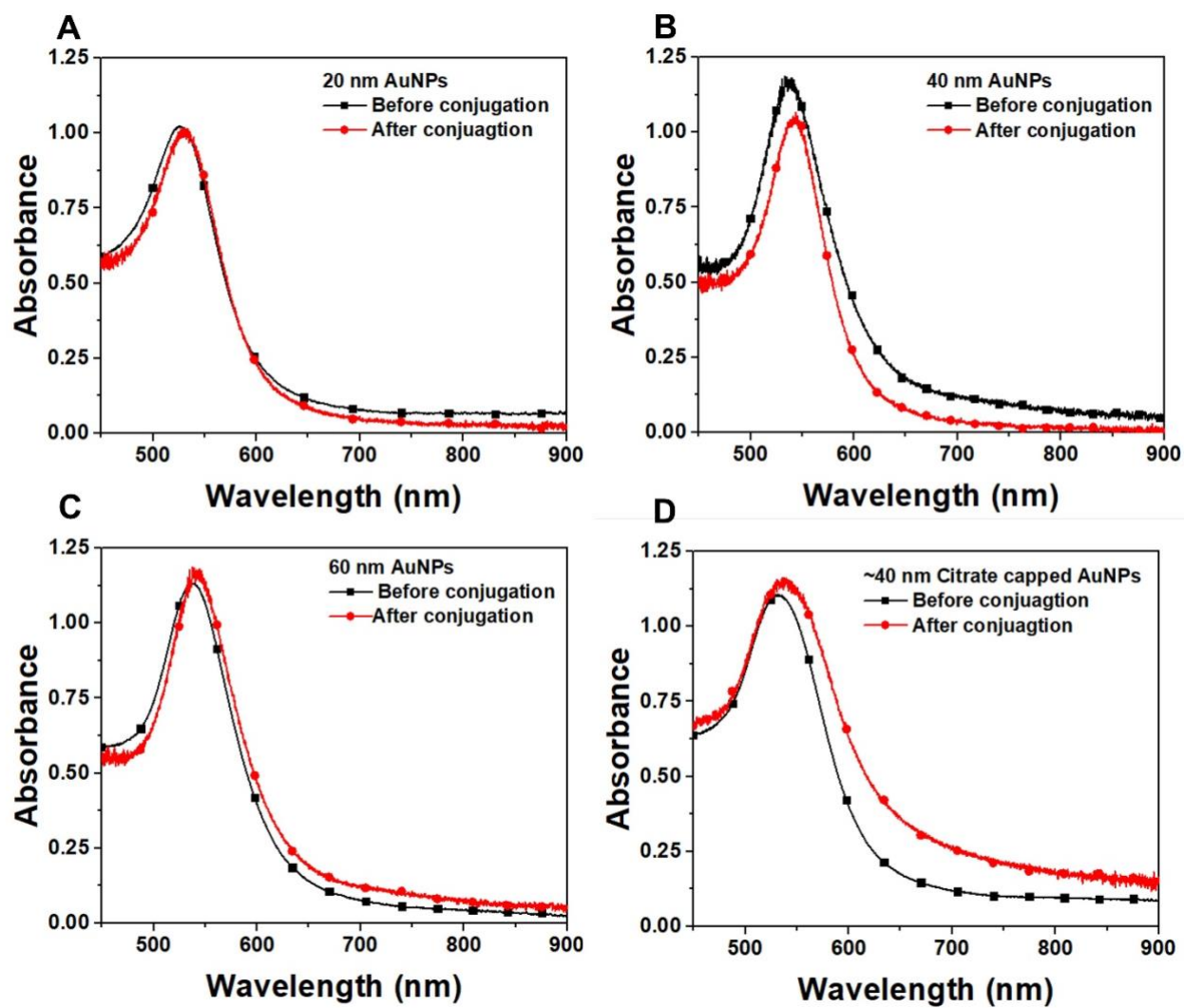
Figure S5: Absorbance spectra of AuNPs of different sizes obtained before and after conjugation with anti-SARS-CoV-2 N-protein IgG2.
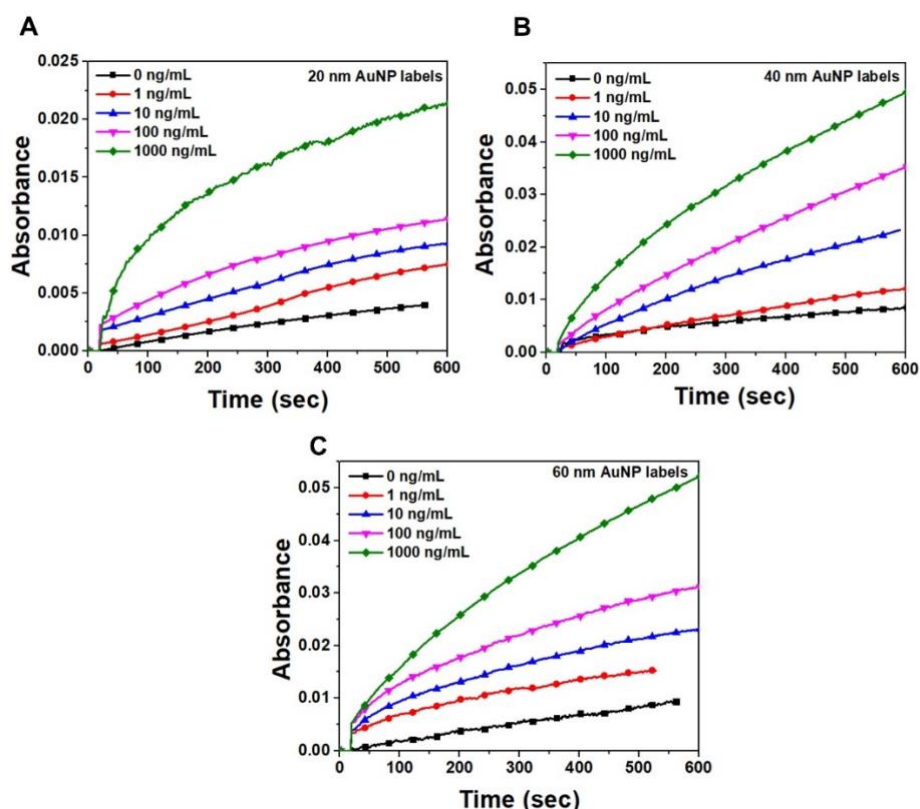
Figure S6: (A) Temporal absorbance response obtained for a range of SARS-CoV-2 N-protein concentrations from GOF sensor probes chemisorbed with anti-SARS-CoV-2-N-protein IgG**1** with the help of the optical experimental setup due to binding of immunocomplex with AuNP labels varying sizes and 10x concentration conjugated with anti-SARS CoV-2 -N-protein IgG2.
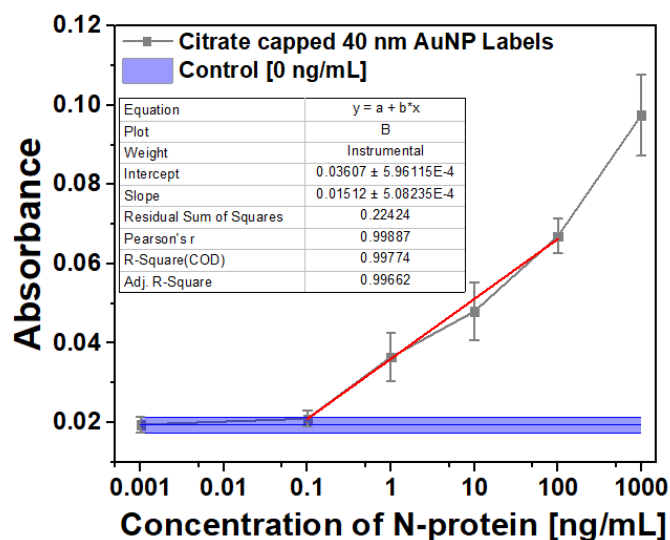

Figure S7: Calibrated absorbance response for a range of SARS-CoV-2 N-protein concentrations from GOF sensor probes chemisorbed with anti-SARS-CoV-2-N-protein IgG1 with the help of the optical experimental setup due to binding of immunocomplex with citrate capped 40 nm AuNP labels varying sizes and 10x concentration conjugated with anti-SARS CoV-2 -N-protein IgG2 (n=3). Inset: Linear fit parameters. The LOD was calculated using $3\sigma/S$, $[(3(0.002\ Abs)/0.01512\ Abs/\log[C]) = \log(0.396)$, Which is 2.48 (~2.5) ng/mL]
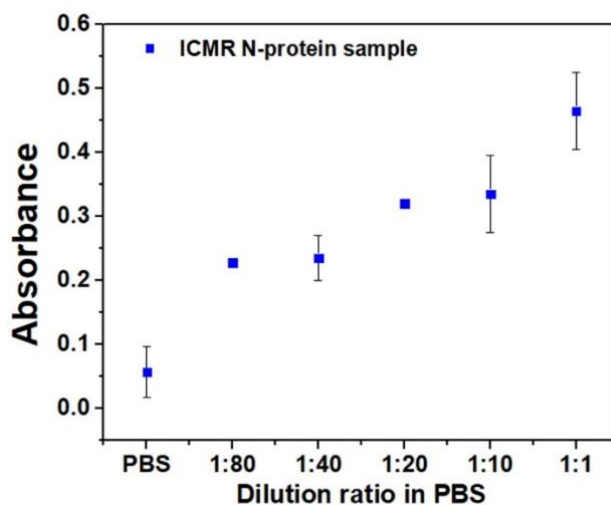
Figure S8: ICMR reference sample

P-FAB response for standard reference sample:

In comparison to PBS solution without N-protein, a distinguishable response was observed for N-protein dilutions as low as 80×. While the absolute concentration of N-protein in the standard reference is not known, the conventional AuNP label based lateral flow assay antigen test kits were sensitive to a dilution down to 1:40 (Courtesy: VoxturBio Ltd). On the other hand, the P-FAB response demonstrates its ability to detect analyte concentrations below 1:80 dilution. The sensitivity of the GOF sensor probes for the detection of SARS-COV-2-N-protein within the dynamic range of 1:80 to 1:1 was found to be 0.15 A_{530nm}/(log SARS-CoV-2 N-protein percentage (%)) with an R-square value of 0.90.
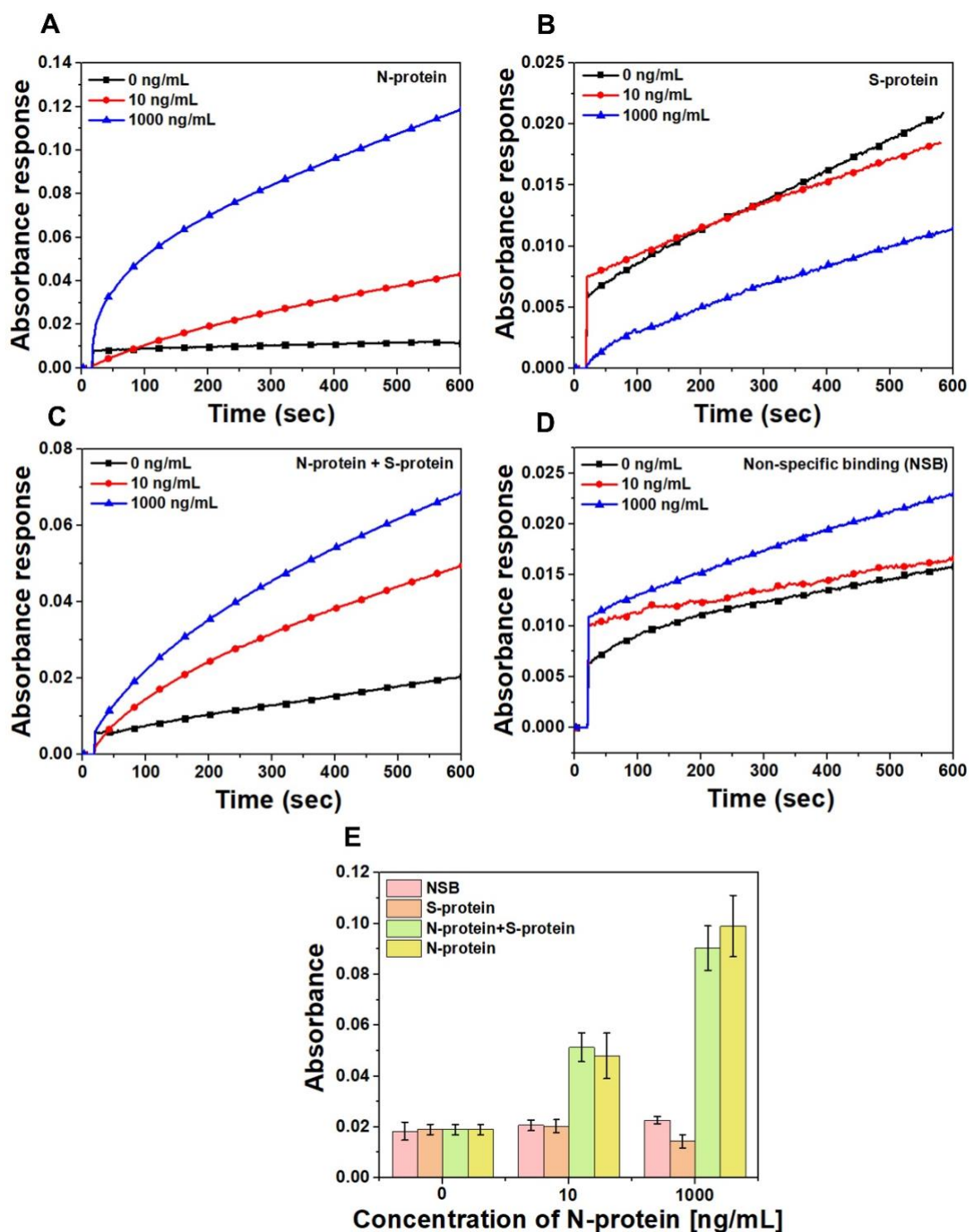
Figure S9: Temporal absorbance response obtained from U-bent GOF sensor probes immobilized with anti-SARS CoV-2 IgG1 due to binding of anti-SARS CoV-2 N-protein IgG2-AuNP labels in presence of (A) N-protein alone, (B) S-protein alone and (C) a mixture of S-protein and N-protein as analyte. (D) Temporal absorbance response obtained from U-bent GOF sensor probes immobilized with goat anti-human IgG due to non-specific adsorption of anti-SARS CoV-2 N-protein IgG2-AuNP labels in presence of N-protein at different concentrations. (E) Absorbance response obtained U-bent GOF sensor probes due to specific and non-specific binding (NSB) of anti-SARS CoV-2 N-protein IgG2-AuNP labels at varying concentrations (n=3).