Electronic Supplementary information for

Rapid and sensitive SARS-CoV-2 detection using a fluorescent immunosensor Quenchbody with crowding agents

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Supplementary Methods

Cloning, expression, and purification of recombinant N protein. An *E. coli* codon-optimized nucleocapsid (N) protein gene of SARS-CoV-2 was obtained from Addgene (#149330, pDONR223 SARS-CoV-2 N). The N protein gene was amplified by PCR and cloned into pET28a vector (Merck) between NdeI and HindIII sites, which was designed for adding a hexahistidine tag at the N-terminus of the expressed protein. The *E. coli* BL21 (DE3) harboring the expression vector was pre-cultured at 37 °C in LB medium supplemented with 30 μg/ml kanamycin. Protein expression was induced by an autoinduction system[1] at 18 °C for 24 h, 1/20 volume of the pre-culture was transferred into Terrific Broth (23.6 g/L Yeast Extract, 11.8 g/L Tryptone, 9.4 g/L K_2HPO_4, 2.2 g/L KH_2PO_4) supplemented with 0.05 % (w/v) glucose, 0.2% (w/v) lactose, 0.6 % (v/v) glycerol and 30 μg/ml kanamycin. The harvested cell was resuspended in 10 times the volume of buffer A (50 mM Tris–HCl pH 7.4, 5 mM $MgCl_2$, 10 % glycerol, 1 M NaCl, and 5 mM imidazole) and disrupted by sonication for 5 min on ice. The cell debris was removed by centrifugation (20,000 *g*, 30 min). The clear supernatant of the lysate was applied to the HisTrap FF crude 5 mL (Cytiva) column which was equilibrated with buffer B (25 mM Tris–HCl pH 7.4, 5 mM $MgCl_2$, 300 mM NaCl, and 5 mM imidazole) followed by washing the column with the same buffer. The N protein was eluted by a 20 mM to 500 mM linear gradient of imidazole with buffer B. The eluted protein was pooled and exchanged to buffer C (25 mM Tris–HCl pH 7.4, 5 mM $MgCl_2$, and 100 mM NaCl) by dialysis. The dialyzed sample was applied on HiTrap SP HP 5mL (Cytiva) equilibrated with buffer C followed by washing the column with the same buffer. The N protein was eluted by a 0.1 M to 1 M linear gradient of NaCl with the same buffer. The peak fractions of eluted N protein were used for further experiments. All the protein purification was performed at 4 °C. The protein was analyzed on a 12.5% SDS-PAGE and quantified according to the bovine serum albumin (BSA) standards on the gel after Coomassie brilliant blue staining.

Biotinylation of N protein. The purified N protein was transferred into a G-25 spin column (Cytiva) to exchange the buffer for the reaction buffer (20 mM sodium phosphate, 500 mM NaCl, 0.005 % Tween 20, pH 7.4). Afterwards, EZ-Link™ NHS-PEG4-Biotin (Thermo Scientific™) was added to the solution at about thirty-times molar excess of the N protein, followed by the incubation at 25 ℃ for 30 min and at 4 ℃ overnight. The buffer was replaced again using a G-25 spin column with storage buffer (25 mM Tris–HCl, 5 mM $MgCl_2$, 1 M NaCl, 0.005 % Tween 20, pH 7.4) and the biotinylated N protein was analyzed by SDS-PAGE as above.

Supplementary Table

Table S1. Oligonucleotides and gene fragments used in this study.

Name	**Sequence** (5' to 3')
BZ-nCoV396-VHtoUQ-F	CTCTAATGAGACCGGTCAGGTGCAGTTAGTGG
BZ-VHtoUQ-R	GGCCCTTGGTGGAAGCGCTCGAGACGGTGACCAGG
BZ-nCoV396-VLtoUQ-F	CTCTAATGAGACTAGTCAGCTGGTGCTGACCCAG
BZ-nCoV396-VLtolaCL-R	CGGGAACAGAGTGACGCTGGGTGCGGCCTTCGG
BZ-laCL-F	GTCACTCTGTTCCCGCCCTC
BZ-LCtoUQ-R	CGTCCTTGTAGTCGGATCCGCCCCCTTCTGTAG
Cov2_N-Nde1(+)	AAATTCATATGTCCGACAACGGTCCCCAG
Cov2_N-Hind3(-)	ATTTAAGCTTAAGCCTGGGTGGAGTCAGCAG
Optimized VH and VL sequence of nCoV396 anti-SARS-CoV-2 N protein antibody	5'- ACCGGTCAGGTGCAGTTAGTGGAAAGCGGCGGTGGCGTGGTGCAGCCGG GTCGCAGCTTACGTTTATCGTGCGCCGCGTCGGGCTTTACCTTTAGCAGCT ACATTATGCACTGGGTTCGTCAAGCCCCTGGTAAAGGCCTGGAATGGGTG GCGGTGATCAGCTATGATGGTAGCAATGAAGCCTATGCCGATAGCGTGAA AGGTCGCTTCACCATTAGCCGCGATAATAGCAAAAACACCCTGTATCTGC AGATGTCGAGCCTGCGTGCCGAAGATACGGGTGTGTACTATTGCGCCCGC GAAACGGGTGATTACAGCTCGTCATGGTATGATAGCTGGGGCCGTGGAAC CCTGGTCACCGTCTCGAGCGGTGGAGGCGGTTCAGGCGGAGGTGGCTCTG GCGGTGGCGGATCTCAGCTGGTGCTGACCCAGTCGCCGAGCGCCAGCGCG TCGCTGGGTGCGAGCGTGAAATTAACCTGCACGCTGTCGAGCGGCCATAG CAATTATGCCATTGCCTGGCATCAGCAACAGCCTGAGAAAGGCCCTCGTT ATCTGATGAAAGTTAATTCGGACGGCAGCCACACGAAAGGCGATGGTATT CCTGATCGCTTTAGCGGTAGCTCAAGCGGCGCGGAACGTTATTTAACGAT TAGCTCGCTGCAGTCGGAAGATGAGGCGGATTATTATTGCCAGACGTGGG GCACGGGCATTCAGGTGTTTGGCGGTGGCACGAAGCTGACCGTGTTAGGC CAGCCGAAGGCCGCACCCAGC -3′
Synthesized Lambda CL sequence	5'- GTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTCCAAGCCAACAAGGCCAC TCTAGTGTGTCTGATCAGTGACTTCTACCCGGGAGCTGTGACAGTGGCCT GGAAGGCAGATGGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCAAACC CTCCAAACAGAGCAACAACAAGTACGCGGCCAGCAGCTACCTGAGCCTG ACGCCCGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGC ATGAAGGGAGCACCGTGGAGAAGACAGTGGCCCCTACAGAAGGGGGCGG ATCC -3′

Supplementary Figures

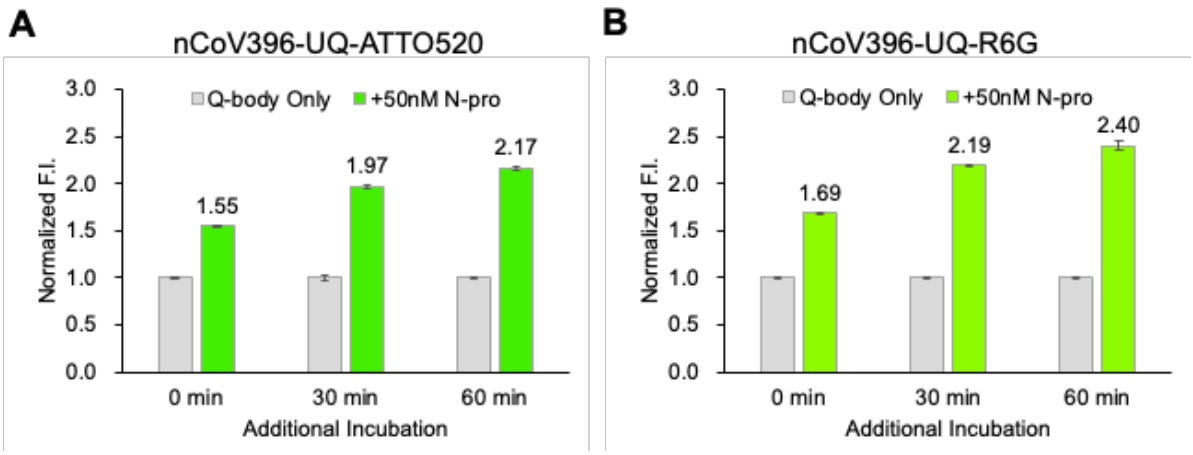


Figure S1. The responses of the nCoV396-UQ-ATTO520 (A) and nCoV396-UQ-R6G (B) Ultra-Q-bodies to 50 nM N protein after 30 min and 60 min incubation. Ultra-Q-body concentration: 1 nM; n = 3; Data were expressed as mean ± standard deviation.

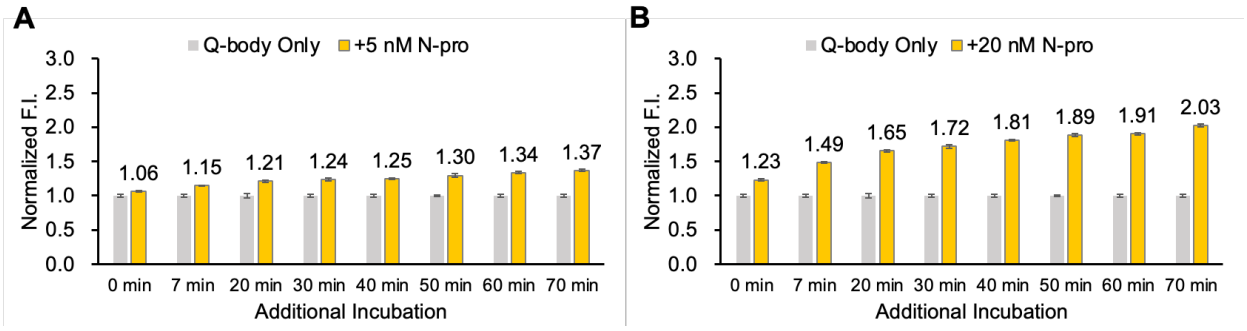


Figure S2. The responses of the nCoV396-UQ-TAMRA immunosensor to 5 nM (A) and 20 nM (B) N protein. Ultra-Q-body concentration: 1 nM; n = 3; Data were expressed as mean ± standard deviation.

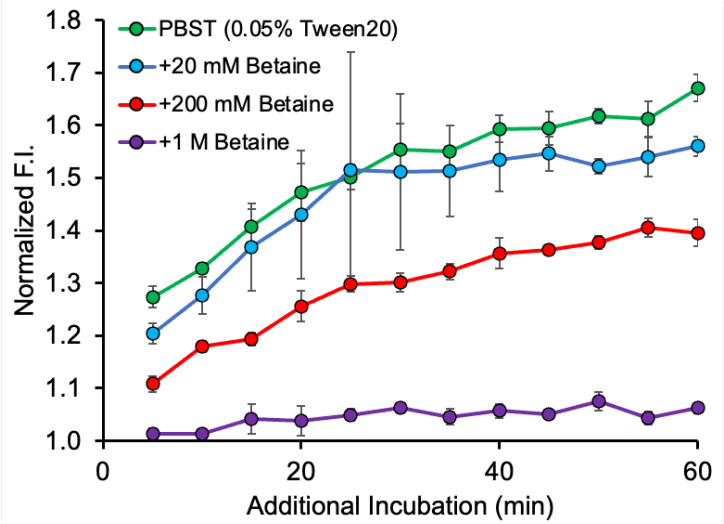


Figure S3. Effect of the betaine concentration on the response speed of the nCoV396-UQ-TAMRA. Ultra-Q-body concentration: 1 nM; N protein concentration: 10 nM; n = 3; Data were expressed as mean ± standard deviation.

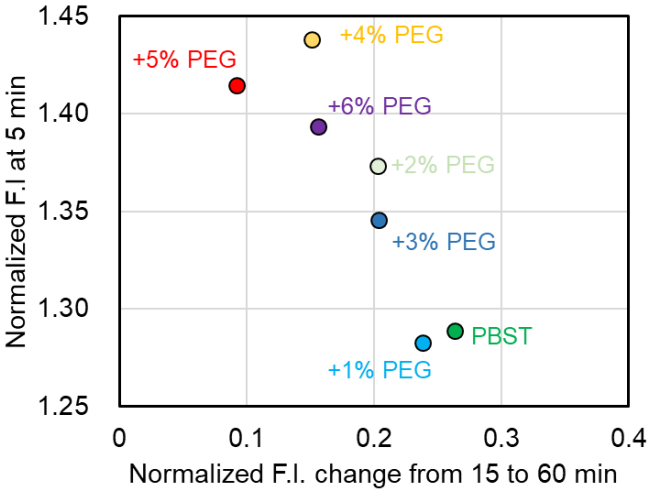
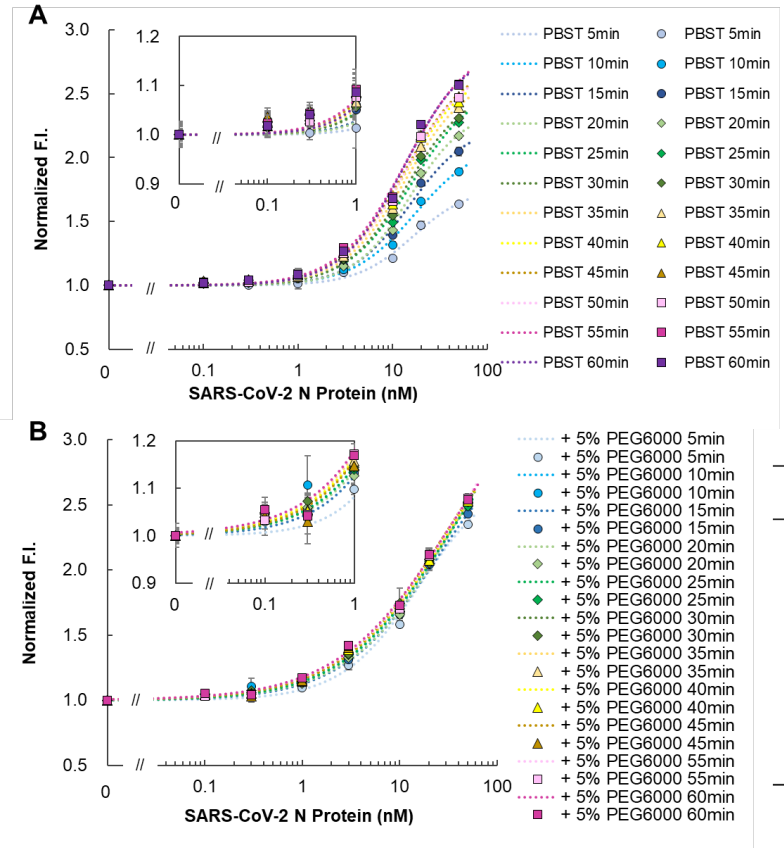


Figure S4. Fluorescence intensity change after 15 min incubation and 5 min response plot of the nCoV396-UQ-TAMRA Ultra-Q-body in the presence of different concentrations of PEG6000. Ultra-Q-body concentration: 1 nM; N protein concentration: 10 nM; n = 3.



Incubation Time (min)	LOD (nM)
	PBST
5	**2.69**
10	**1.75**
15	**1.68**
20	**0.900**
25	**1.20**
30	**1.26**
35	**0.666**
40	**1.14**
45	**0.623**
50	**1.27**
55	**1.05**
60	**1.05**

Incubation Time (min)	LOD (nM)
	PBST + 5% PEG6000
5	**0.290**
10	**0.191**
15	**0.538**
20	**0.139**
25	**0.158**
30	**0.183**
35	**0.0827**
40	**0.217**
45	**0.0936**
50	**0.110**
55	**0.0682**
60	**0.120**

Figure S5. Dose dependency of nCoV396-UQ-TAMRA after additional incubation from 5 to 60 min without (A) and with (B) the addition of crowding agent PEG6000. The inset shows an enlarged view in the low concertation region. LOD: limit of detection. Ultra-Q-body concentration: 1 nM; n = 4; Data were expressed as mean ± standard deviation.

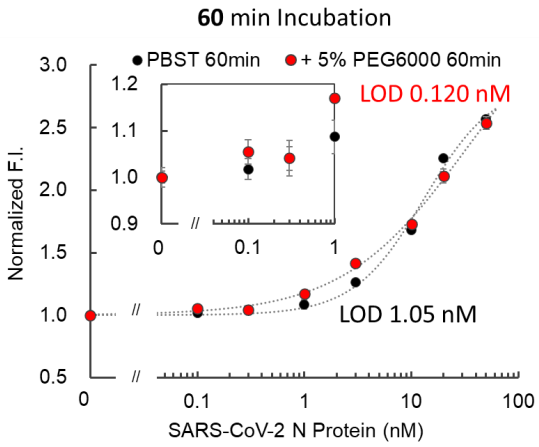


Figure S6. Dose dependency of nCoV396-UQ-TAMRA after 60 min incubation with and without the addition of crowding agent PEG6000. The inset shows an enlarged view in the low concertation region. LOD: limit of detection. Ultra-Q-body concentration: 1 nM; n = 4; Data were expressed as mean ± standard deviation.

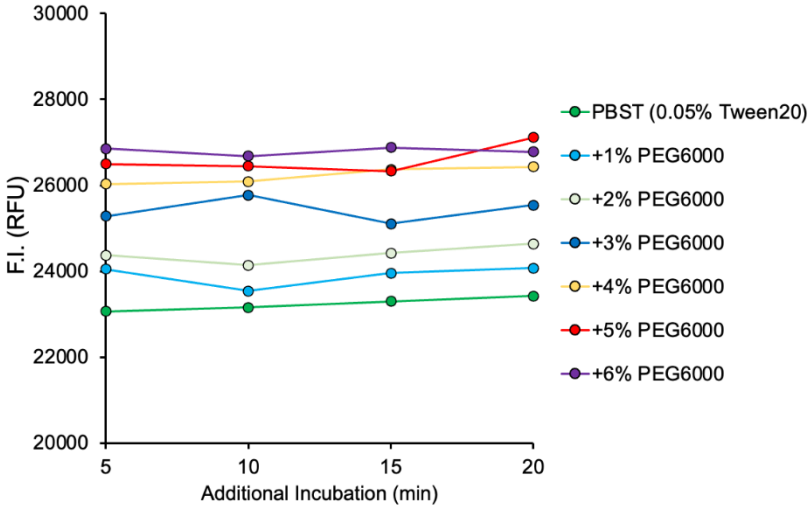


Figure S7. Background fluorescence intensity of the nCoV396-UQ-TAMRA Ultra-Q-body in buffers containing different concentrations of PEG6000. Ultra-Q-body concentration: 1 nM; n = 3.

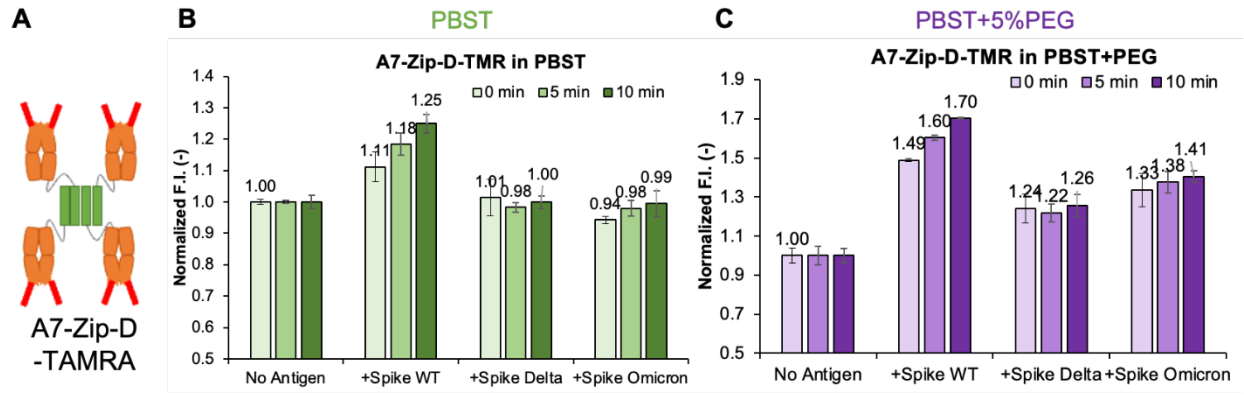


Figure S8. The performance of the SARS-CoV-2 spike protein-recognizing Ultra-Q-body in PEG6000-containing buffer. (A) Illustration of the design of the SARS-CoV-2 spike protein-recognizing Ultra-Q-body. (B) Response of the immunosensor to 10 nM spike protein trimers in PBST. (C) Response of the immunosensor to 10 nM spike protein trimers in PBST containing 5% PEG6000. Ultra-Q-body concentration: 0.5 nM (Fab unit); n = 3; Data were expressed as mean ± standard deviation. WT: spike protein of wild-type SARS-CoV-2, 10549-CV-100, R&D Systems. Delta: spike protein of SARS-CoV-2 variant of concern delta, 40589-V08B16, Sino Biological. Omicron: spike protein of SARS-CoV-2 variant of concern omicron, 40589-V08H26, Sino Biological.

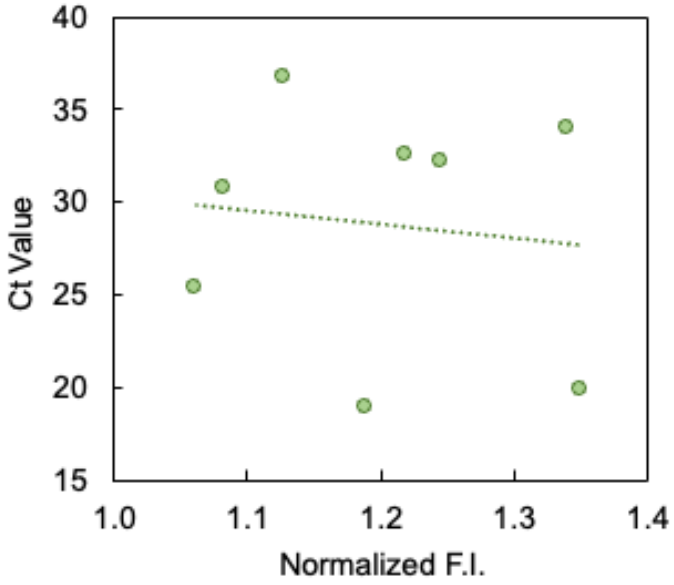


Figure S9. Correlation between RT-PCR threshold cycle (Ct) value and fluorescence intensity (F.I.) change in N protein Ultra-Q-body immunoassay of the positive clinical specimens.

Reference

1. F. W. Studier, *Protein Expr. Purif.*, 2005, **41**, 207-234.