FRET-based hACE2 receptor mimic peptide conjugated nanoprobe for simple detection of SARS-CoV-2

Byunghoon Kang¹, Youngjin Lee², Jaewoo Lim¹,³, Dongeun Yong⁴, Young Ki Choi⁵,⁶, Sun Woo Yoon¹, Seungbeom Seo¹,⁷, Soojin Jang¹,³, Seong Uk Son¹,³, Taejoon Kang¹, Juyeon Jung¹,³, Kyu-Sun Lee¹, Myung Hee Kim², *, and Eun-Kyung Lim¹,³, *

¹BioNanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology (KIRIBB), 125 Gwahak-ro, Yuseong-gu, Daejeon 34141, Republic of Korea

²Metabolic Regulation Research Center, Korea Research Institute of Bioscience and Biotechnology (KIRIBB), 125 Gwahak-ro, Yuseong-gu, Daejeon 34141, Republic of Korea

³Department of Nanobiotechnology, KIRIBB School of Biotechnology, University of Science and Technology (UST), 217 Gajeong-ro, Yuseong-gu, Daejeon 34113, Republic of Korea

⁴Department of Laboratory Medicine and Research Institute of Bacterial Resistance, College of Medicine, Yonsei University, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea

⁵Department of Microbiology, College of Medicine and Medical Research Institute, Chungbuk National University, 776 Isunhwan-ro, Seowon-gu, Cheongju 28644, Republic of Korea

⁶Center for Study of Emerging and Re-emerging Viruses, Korea Virus Research Institute, Institute for Basic Science (IBS), Daejeon 34126, Republic of Korea
Department of Cogno-Mechatronics Engineering, Pusan National University, 2 Busandaehak-ro, Gumjeong-gu, Busan 46241, Republic of Korea

**Corresponding Author**

Eun-Kyung Lim*
Tel: 82-42-879-8456; Fax: 82-42-879-8492; E-mail: eklim1112@kribb.re.kr

Myung Hee Kim*
Tel: 82-42-879-8219; E-mail: mhk8n@kribb.re.kr
Fig. S1. Synthetic scheme for SARS-CoV-2 detection using hACE2 mimic peptide-beacon (COVID19-PEB) [Cy3-TTTTGGGG- I EEQA KTFL DKFN HEAE DLFY QSSL ASWK-CCCCCAAAA-BHQ2].
**Fig. S2.** Interacting residues between human angiotensin-converting enzyme 2 (hACE2) and spike (S) protein. Residues (colored circles), salt bridge (red line), hydrogen bonds (blue lines), and hydrophobic interactions (orange dashed-lines) are illustrated in this figure. This figure was generated using the PDBsum server.[1]
**Fig. S3.** Western blot image of full membrane showing detection of SARS-CoV-2 S protein RBD and Bovine Serum Albumin by ACE2 mimic peptide-biotin.
**Fig. S4.** Fluorescence intensity according to time change after COVID19-PEB treatment to i) SARS-CoV-2 (blue), ii) S protein RBD (red), and iii) BSA (yellow) ($n = 3$).
**Fig. S5.** Fluorescence detection of different concentrations of variant spike (S) protein receptor-binding domain (RBD) (a: alpha variant, b: beta variant, c: gamma variant and d: delta variant) using COVID19-PEB: i) Fluorescence intensity *<0.05, **<0.005 ***<0.0005 (n = 3) and ii) relative fluorescence intensity (F_{NT} : fluorescence intensity in the absence of target protein
(nontreatment, NT)).

**Anti-biotin HRP**

**a)**
SARS-CoV-2 alpha variant S protein RBD + hACE2 mimic peptide-biotin

**b)**
SARS-CoV-2 beta variant S protein RBD + hACE2 mimic peptide-biotin

**c)**
SARS-CoV-2 gamma variant S protein RBD + hACE2 mimic peptide-biotin

**d)**
SARS-CoV-2 delta variant S protein RBD + hACE2 mimic peptide-biotin

**e)**
Bovine Serum Albumin + hACE2 mimic peptide-biotin

**Fig. S6.** Western blot images depicting detection of a) SARS-CoV-2 alpha variant S protein RBD, b) SARS-CoV-2 beta variant S protein RBD, c) SARS-CoV-2 gamma variant S protein RBD, d) SARS-CoV-2 delta variant S protein RBD, and e) Bovine Serum Albumin by hACE2 mimic peptide-biotin.
| Number | E Ct value | RdRp Ct value | N Ct value |
|--------|-----------|---------------|-----------|
| 1      | 26.87     | 26.76         | 26.42     |
| 2      | 32.13     | 32.44         | 32.58     |
| 3      | 35.49     | 37.5          | 36.05     |
| 4      | 28.19     | 28.21         | 27.95     |
| 5      | 34.68     | 33.74         | 34.45     |
| 6      | 35.35     | 35.56         | 34.64     |
| 7      | 35.72     | 34.37         | 36.43     |
| 8      | 32.7      | 32.91         | 32.12     |
| 9      | 34.64     | 36.26         | 33.57     |
| 10     | 21.98     | 21.31         | 21.88     |
| 11     | 29.13     | 29            | 29.11     |
| 12     | 27.58     | 27.4          | 28.13     |
| 13     | 32.83     | 32.41         | 31.86     |
| 14     | 36.13     | 36.26         | 37.26     |
| 15     | 21.27     | 20.73         | 21.73     |
|   | 16 | 35.01 | 34.03 | 32.86 |
|---|----|-------|-------|-------|
| 17| 23.31 | 22.75 | 23.4  |
| 18| 22.63 | 22.11 | 23.48 |
| 19| 25.8  | 26.25 | 26.44 |
| 20| 35.22 | 35.75 | 34.73 |
| 21| 36.25 | 36.01 | 36.35 |
| 22| 32.52 | 33.32 | 31.2  |
| 23| 32.97 | 33.6  | 32.83 |
| 24| 33.76 | 33.61 | 33.54 |
| 25| 30.65 | 31.15 | 31.04 |
| 26| 38.14 | 38.09 | 37.2  |
| 27| 18.16 | 17.82 | 17.75 |
| 28| 10.79 | 12.27 | 12.9  |
| 29| 11.87 | 13.25 | 13.17 |
| 30| 31.53 | 32.32 | 31.25 |

**Table S1.** Diagnostic result of COVID-19 patients using PCR at Yonsei University Severance Hospital. (Sample type: Positive nasopharyngeal swab specimens, N=30)
References

[1] R.A. Laskowski, J. Jablonska, L. Pravda, R.S. Varekova, J.M. Thornton, PDBsum: Structural summaries of PDB entries, Protein Sci 27 (2018) 129-134.