

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

*Byunghoon Kang<sup>1</sup>, Youngjin Lee<sup>2</sup>, Jaewoo Lim<sup>1,3</sup>, Dongeun Yong<sup>4</sup>, Young Ki Choi<sup>5,6</sup>, Sun Woo Yoon<sup>1</sup>, Seungbeom Seo<sup>1,7</sup>, Soojin Jang<sup>1,3</sup>, Seong Uk Son<sup>1,3</sup>, Taejoon Kang<sup>1</sup>, Juyeon Jung<sup>1,3</sup>, Kyu-Sun Lee<sup>1</sup>, Myung Hee Kim<sup>2</sup>, \* and Eun-Kyung Lim<sup>1,3</sup>, \**

<sup>1</sup>BioNanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), 125 Gwahak-ro, Yuseong-gu, Daejeon 34141, Republic of Korea

<sup>2</sup>Metabolic Regulation Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), 125 Gwahak-ro, Yuseong-gu, Daejeon 34141, Republic of Korea

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

<sup>4</sup>Department 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

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

<sup>6</sup>Center for Study of Emerging and Re-emerging Viruses, Korea Virus Research Institute, Institute for Basic Science (IBS), Daejeon 34126, Republic of Korea

<sup>7</sup>Department of Cogno-Mechatronics Engineering, Pusan National University, 2 Busandaehak-ro, Gumjeong-gu, Busan 46241, Republic of Korea

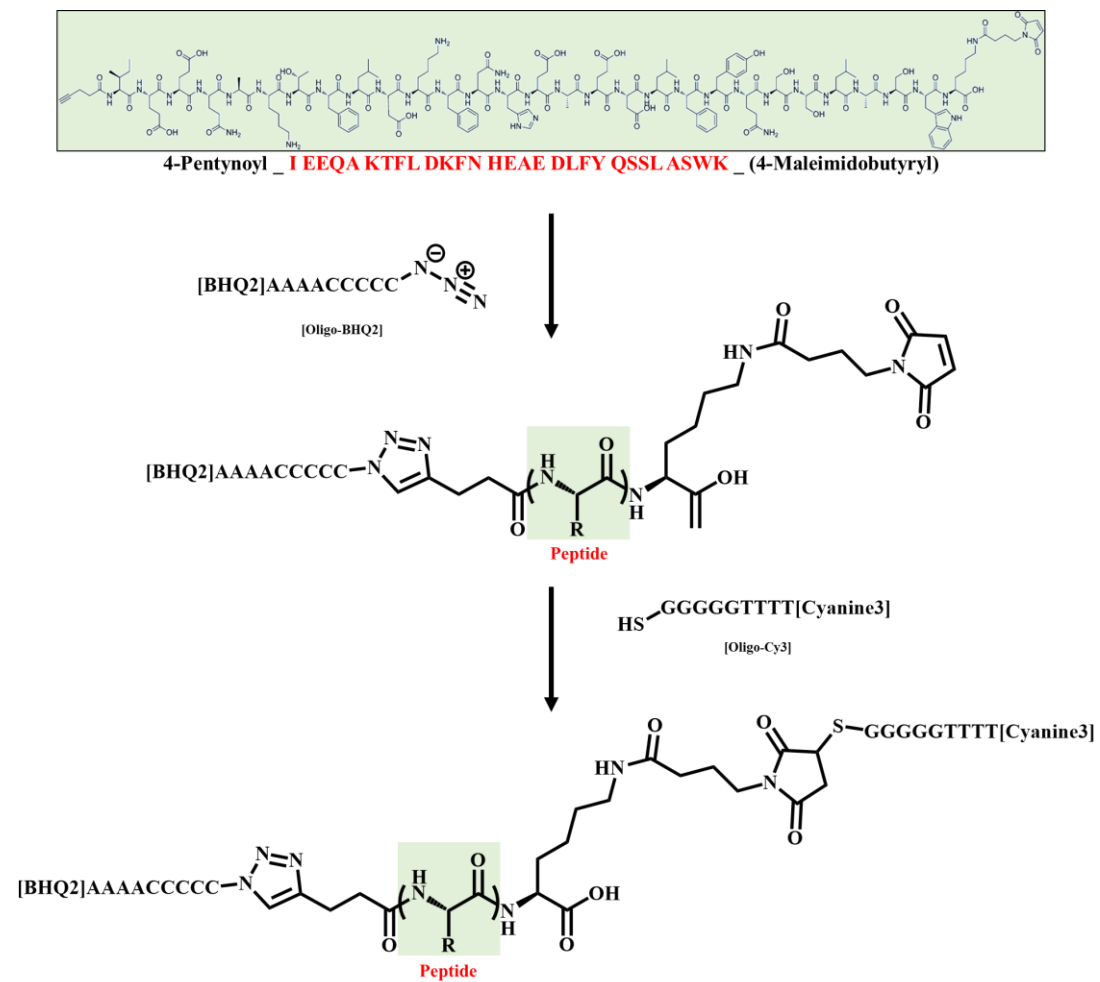
### **Corresponding Author**

Eun-Kyung Lim \*

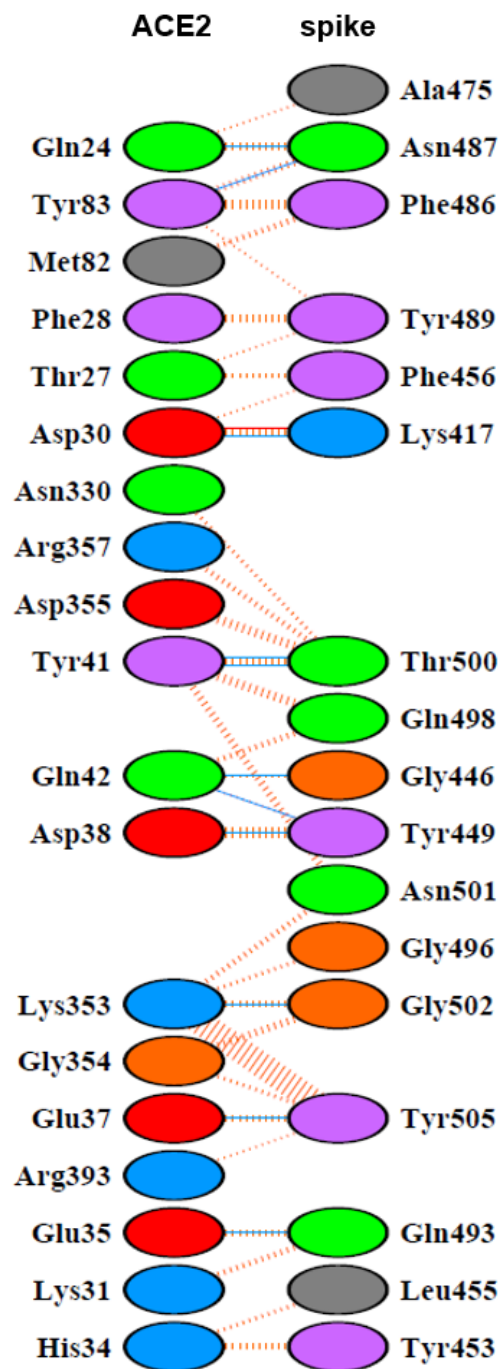
Tel: 82-42-879-8456; Fax: 82-42-879-8492; E-mail: [eklim1112@kribb.re.kr](mailto:eklim1112@kribb.re.kr)

Myung Hee Kim \*

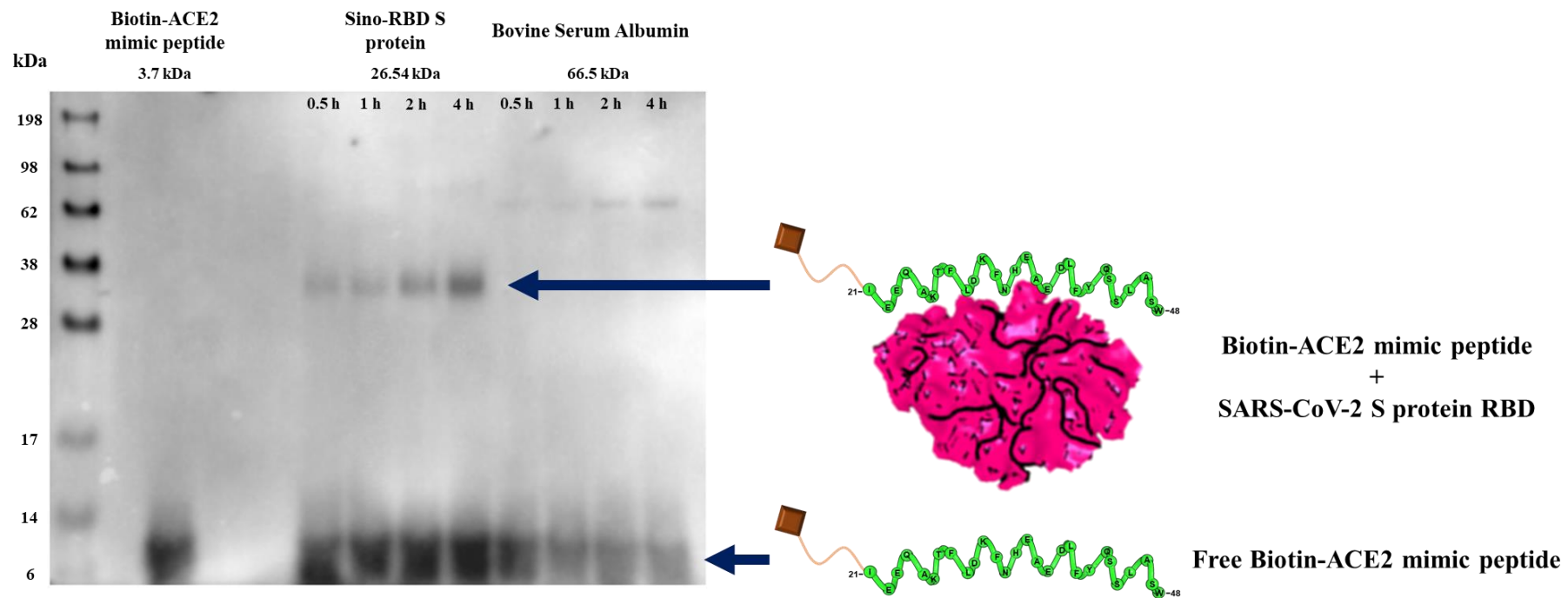
Tel: 82-42-879-8219; E-mail: [mhk8n@kribb.re.kr](mailto:mhk8n@kribb.re.kr)



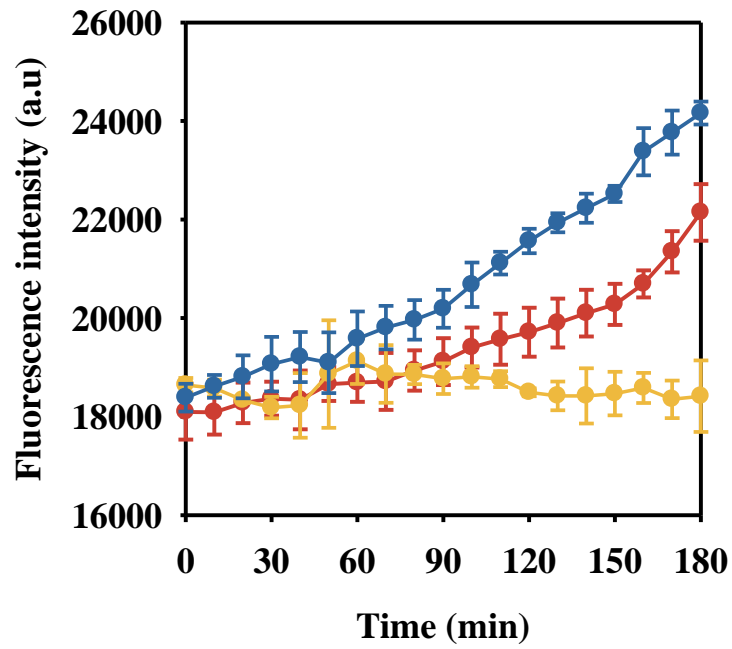
**Fig. S1.** Synthetic scheme for SARS-CoV-2 detection using hACE2 mimic peptide-beacon (COVID19-PEB) [Cy3-TTTTGGGGG- I EEQA KTFL DKFN HEAE DLFY QSSL ASWK-CCCCCAAAA-BHQ2].



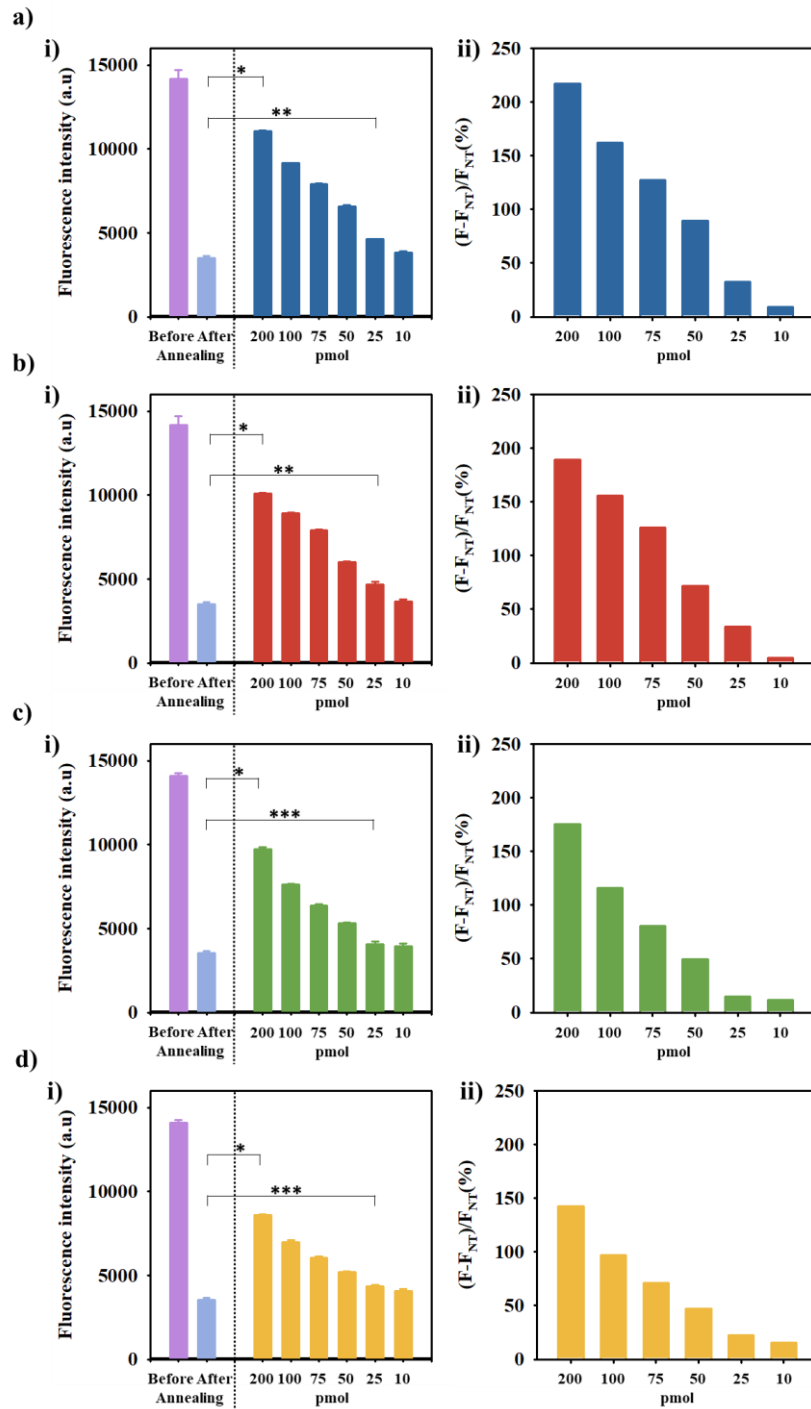
**Fig. S2.** Interacting residues between human angiotensin-converting enzyme 2 (hACE2) and spike (S) protein. Residues (colored circles), salt bridge (red line), hydrogen bonds (blue lines), and hydrophobic interactions (orange dashed-lines) are illustrated in this figure. This figure was generated 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.

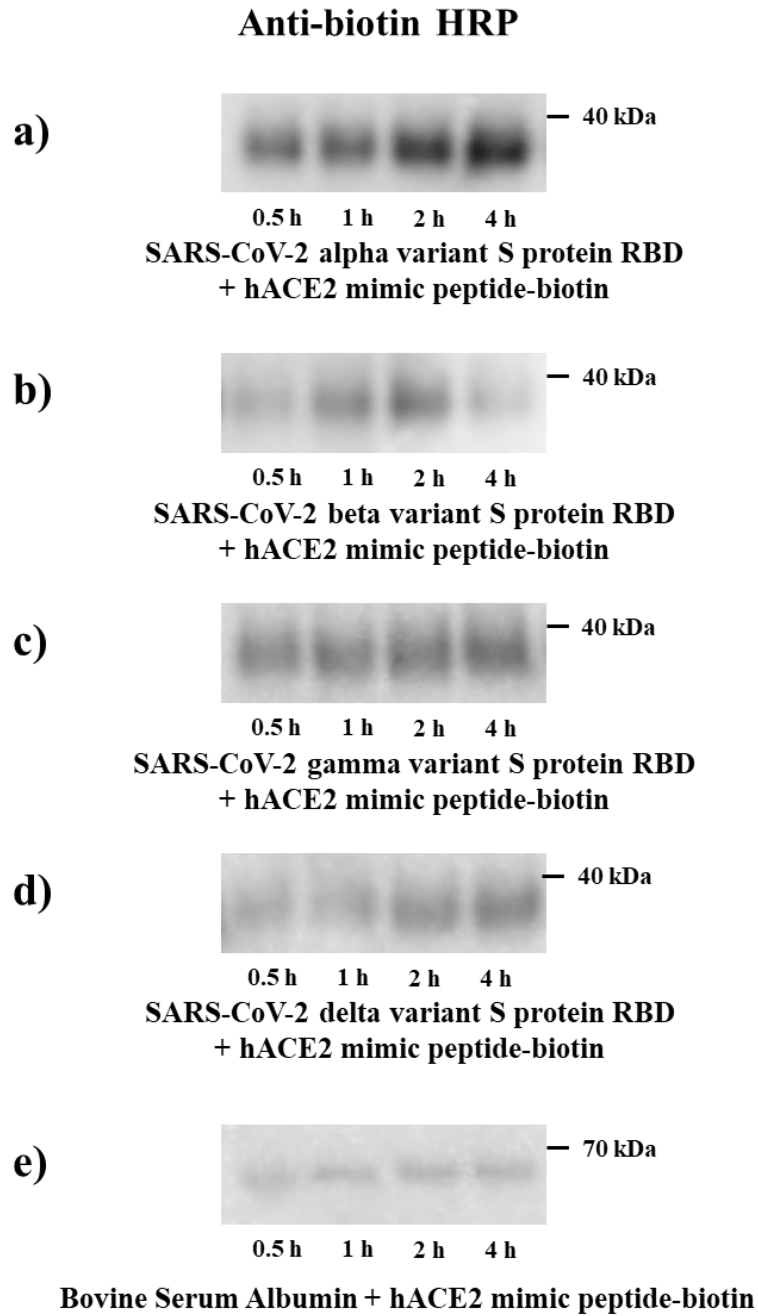


**Fig. S4.** Fluorescence intensity according to time change after COVID19-PEB treatment to i) SARS-CoV-2 (blue), ii) S protein RBD (red), and iii) BSA (yellow) ( $n = 3$ ).



**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)).



**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.



<b>Number</b>	<b>E Ct value</b>	<b>RdRp Ct value</b>	<b>N Ct value</b>
<b>1</b>	26.87	26.76	26.42
<b>2</b>	32.13	32.44	32.58
<b>3</b>	35.49	37.5	36.05
<b>4</b>	28.19	28.21	27.95
<b>5</b>	34.68	33.74	34.45
<b>6</b>	35.35	35.56	34.64
<b>7</b>	35.72	34.37	36.43
<b>8</b>	32.7	32.91	32.12
<b>9</b>	34.64	36.26	33.57
<b>10</b>	21.98	21.31	21.88
<b>11</b>	29.13	29	29.11
<b>12</b>	27.58	27.4	28.13
<b>13</b>	32.83	32.41	31.86
<b>14</b>	36.13	36.26	37.26
<b>15</b>	21.27	20.73	21.73

<b>16</b>	35.01	34.03	32.86
<b>17</b>	23.31	22.75	23.4
<b>18</b>	22.63	22.11	23.48
<b>19</b>	25.8	26.25	26.44
<b>20</b>	35.22	35.75	34.73
<b>21</b>	36.25	36.01	36.35
<b>22</b>	32.52	33.32	31.2
<b>23</b>	32.97	33.6	32.83
<b>24</b>	33.76	33.61	33.54
<b>25</b>	30.65	31.15	31.04
<b>26</b>	38.14	38.09	37.2
<b>27</b>	18.16	17.82	17.75
<b>28</b>	10.79	12.27	12.9
<b>29</b>	11.87	13.25	13.17
<b>30</b>	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.