**Supplementary Material**

# Molecular insights and optimization strategies for the competitive binding of engineered ACE2 proteins: a multiple replica molecular dynamics study

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**Table S1** Crystal structure and size of the system on which the initial conformation of each system is based

| Complex | Initial conformation | Numbers of residues | Numbers of atoms |
|---|---|---|---|
| **WT-WT** | 6m0j | 793 | 12543 |
| **3N39-WT** | 7dmu | 793 | 12550 |
| **3N94-WT** | 7dmu | 793 | 12556 |
| **WT-Delta** | 6m0j | 793 | 12556 |
| **3N39-Delta** | 7dmu | 793 | 12563 |
| **3N94-Delta** | 7dmu | 793 | 12569 |
| **WT-BA.1** | 7dmu | 793 | 12601 |
| **3N39-BA.1** | 7dmu | 793 | 12608 |
| **3N94-BA.1** | 7u0n | 793 | 12614 |
| **WT-BA.2** | 7xb0 | 793 | 12577 |
| **3N39-BA.2** | 7xb0 | 793 | 12584 |
| **3N94-BA.2** | 7xb0 | 793 | 12590 |
| **WT-BA.4/5** | 7xo9 | 793 | 12577 |
| **3N39-BA.4/5** | 7xo9 | 793 | 12584 |
| **3N94-BA.4/5** | 7xo9 | 793 | 12590 |

**Table S2** Solvent accessible surface area calculated using PyMOL

| Components[a] / Systems | ACE2 | RBD | ACE2-RBD | ΔSASA |
|---|---|---|---|---|
| **WT-WT** | 26850.02 | 10755.91 | 35899.39 | 1706.54 |
| **3N39-WT** | 24992.08 | 10780.06 | 33492.88 | 2279.26 |
| **3N94-WT** | 26284.18 | 10881.08 | 35004.41 | 2160.84 |
| **WT-Delta** | 25678.57 | 11149.80 | 34583.13 | 2245.24 |
| **3N39-Delta** | 26158.58 | 10811.13 | 34994.74 | 1974.97 |
| **3N94-Delta** | 25643.67 | 10929.85 | 34260.11 | 2313.42 |
| **WT-BA1** | 25865.58 | 10777.34 | 34494.83 | 2148.09 |
| **3N39-BA1** | 25967.62 | 11092.49 | 34912.73 | 2147.37 |
| **3N94-BA1** | 25965.97 | 11530.90 | 35509.97 | 1986.91 |
| **WT-BA2** | 25652.72 | 10941.45 | 34321.64 | 2272.53 |
| **3N39-BA2** | 26041.53 | 11213.13 | 35457.50 | 1797.16 |
| **3N94-BA2** | 26283.89 | 10670.19 | 35007.18 | 1946.90 |
| **WT-BA4/5** | 26835.46 | 11133.72 | 36060.79 | 1908.39 |
| **3N39-BA4/5** | 26057.80 | 10658.51 | 34797.30 | 1919.00 |
| **3N94-BA4/5** | 26689.90 | 10941.27 | 35515.18 | 2115.99 |

[a] All components of solvent accessible surface areas are in $Å^2$.

**Table S3** Hydrogen bonding interactions of WT ACE2 with RBDs

| Complexes | Hydrogen bonds | Distance[a] (Å) | Angle[a] (deg) | Occupancy[b] (%) |
|---|---|---|---|---|
| WT-WT | K353-O···G502-H-N | 2.91 | 161.69 | 99.64 |
| | D355-OD2···T500-HG1-OG1 | 2.77 | 158.60 | 83.19 |
| | N487-OD1···Y83-HH-OH | 2.79 | 157.94 | 95.20 |
| WT-Delta | K353-O···G502-H-N | 2.93 | 163.30 | 99.75 |
| | Y83-OH···Y489-HH-OH | 2.83 | 162.40 | 98.40 |
| | D355-OD2···T500-HG1-OG1 | 2.70 | 163.44 | 94.02 |
| | A386-O···Y505-HH-OH | 2.76 | 155.56 | 76.43 |
| | N487-OD1···Y83-HH-OH | 2.75 | 158.91 | 88.05 |
| WT-BA.1 | Y41-OH···G502-H-N | 3.02 | 153.83 | 88.42 |
| | E35-OE1···R493-HE-NE | 2.98 | 152.46 | 61.45 |
| | E35-OE2···R493-HE-NE | 3.01 | 149.62 | 59.84 |
| | N487-OD1···Y83-HH-OH | 2.78 | 158.79 | 86.92 |
| WT-BA.2 | K353-O···G502-H-N | 2.98 | 162.89 | 98.76 |
| | D355-OD2···T500-HG1-OG1 | 2.70 | 162.80 | 95.49 |
| | D38-OD1···Y449-HH-OH | 2.88 | 150.27 | 65.90 |
| | D38-OD2···Y449-HH-OH | 2.89 | 148.87 | 64.97 |
| | Q24-OE1···N487-HD21-ND2 | 2.99 | 147.18 | 61.98 |
| | N487-OD1···Y83-HH-OH | 2.76 | 159.29 | 96.62 |
| | A475-O···S19-HG-OG | 2.73 | 161.33 | 64.81 |
| WT-BA.4/5 | K353-O···G502-H-N | 2.94 | 163.63 | 99.78 |
| | D355-OD2···T500-HG1-OG1 | 2.77 | 159.69 | 75.49` |
| | D38-OD1···Y449-HH-OH | 2.88 | 150.71 | 72.64 |
| | D38-OD2···Y449-HH-OH | 2.87 | 150.42 | 71.91 |
| | N487-OD1···Y83-HH-OH | 2.81 | 156.47 | 77.71 |

[a] Hydrogen bonding is determined by an acceptor···donor distance of <3.5 Å and an acceptor···H-donor angle of >120°.

[b] Occupancy is defined as the percentage of simulation time that a particular hydrogen bond is present.

**Table S4** Hydrogen bonding interactions of 3N39 with RBDs

| Complexes | Hydrogen bonds | Distance[a] (Å) | Angle[a] (deg) | Occupancy[b] (%) |
|---|---|---|---|---|
| 3N39-WT | K353-O···G502-H-N | 2.96 | 162.07 | 99.47 |
| | D355-OD2···T500-HG1-OG1 | 2.73 | 162.10 | 90.15 |
| | N487-OD1···Y83-HH-OH | 2.76 | 159.84 | 98.09 |
| 3N39-Delta | K353-O···G502-H-N | 2.93 | 162.38 | 99.55 |
| | D355-OD2···T500-HG1-OG1 | 2.72 | 162.82 | 91.45 |
| | N487-OD1···Y83-HH-OH | 2.76 | 159.71 | 97.87 |
| 3N39-BA.1 | K353-O···G502-H-N | 2.94 | 162.97 | 99.53 |
| | D355-OD2···T500-HG1-OG1 | 2.72 | 162.41 | 93.95 |
| | Q24-OE1···N487-HD21-ND2 | 2.97 | 148.09 | 61.19 |
| | D38-OD1···Y449-HH-OH | 2.88 | 151.67 | 57.17 |
| | D38-OD2···Y449-HH-OH | 2.88 | 148.74 | 56.02 |
| | N487-OD1···Y83-HH-OH | 2.77 | 160.14 | 95.70 |
| 3N39-BA.2 | K353-O···G502-H-N | 2.94 | 162.64 | 99.52 |
| | D355-OD2···T500-HG1-OG1 | 2.74 | 161.62 | 94.78 |
| | D38-OD2···R493-HH21-NH2 | 2.90 | 153.36 | 78.30 |
| | D38-OD1···R493-HH21-NH2 | 3.07 | 145.35 | 66.34 |
| | D38-OD1···R493-HE-NE | 2.97 | 159.25 | 61.45 |
| | Q24-OE1···N487-HD21-ND2 | 2.98 | 146.28 | 60.89 |
| | N487-OD1···Y83-HH-OH | 2.77 | 158.53 | 97.44 |
| 3N39-BA.4/5 | K353-O···G502-H-N | 2.96 | 163.39 | 99.65 |
| | D355-OD2···T500-HG1-OG1 | 2.76 | 160.00 | 81.16` |
| | H34-ND1···Q493-HE22-NE2 | 3.01 | 160.59 | 76.45 |
| | N487-OD1···Y83-HH-OH | 2.79 | 158.39 | 93.06 |

[a] Hydrogen bonding is determined by an acceptor···donor distance of <3.5 Å and an acceptor···H-donor angle of >120°.

[b] Occupancy is defined as the percentage of simulation time that a particular hydrogen bond is present.

**Table S5** Hydrogen bonding interactions of 3N94 with RBDs

| Complexes | Hydrogen bonds | Distance[a] (Å) | Angle[a] (deg) | Occupancy[b] (%) |
|---|---|---|---|---|
| 3N94-WT | K353-O···G502-H-N | 2.90 | 162.65 | 99.93 |
| | D355-OD2···T500-HG1-OG1 | 2.77 | 159.70 | 80.99 |
| | Q24-OE1···N487-HD21-ND2 | 2.97 | 145.93 | 61.24 |
| | N487-OD1···Y83-HH-OH | 2.76 | 160.83 | 98.74 |
| 3N94-Delta | K353-O···G502-H-N | 2.91 | 162.70 | 99.85 |
| | D355-OD2···T500-HG1-OG1 | 2.72 | 160.73 | 85.37 |
| | Y83-OH···Y489-HH-OH | 2.90 | 157.17 | 77.00 |
| | Q24-OE1···N487-HD21-ND2 | 2.94 | 155.40 | 59.45 |
| | N487-OD1···Y83-HH-OH | 2.78 | 157.30 | 77.06 |
| 3N94-BA.1 | K353-O···G502-H-N | 2.98 | 163.37 | 98.83 |
| | D355-OD2···T500-HG1-OG1 | 2.75 | 160.26 | 83.43 |
| | D38-OD2···R493-HH22-NH2 | 2.94 | 149.27 | 79.59 |
| | D38-OD1···R493-HH22-NH2 | 3.00 | 146.79 | 79.34 |
| | N487-OD1···Y83-HH-OH | 2.76 | 159.79 | 96.38 |
| 3N94-BA.2 | K353-O···G502-H-N | 2.94 | 163.40 | 99.73 |
| | D355-OD2···T500-HG1-OG1 | 2.74 | 160.06 | 84.70 |
| | D38-OD2···Y449-HH-OH | 2.86 | 149.61 | 71.47 |
| | D38-OD1···Y449-HH-OH | 2.87 | 149.33 | 70.69 |
| | N487-OD1···Y83-HH-OH | 2.77 | 159.35 | 97.35 |
| 3N94-BA.4/5 | K353-O···G502-H-N | 2.97 | 163.84 | 99.07 |
| | D355-OD2···T500-HG1-OG1 | 2.76 | 160.12 | 74.99` |
| | N487-OD1···Y83-HH-OH | 2.79 | 158.25 | 93.14 |
| | A475-O···S19-HG-OG | 2.76 | 161.10 | 58.25 |

[a] Hydrogen bonding is determined by an acceptor···donor distance of <3.5 Å and an acceptor···H-donor angle of >120°.

[b] Occupancy is defined as the percentage of simulation time that a particular hydrogen bond is present.

**Table S6** Energetic contributions of separate residues to bindings of ACE2s to WT RBD

| Energy (kcal/mol) | | $T_{vdW}$ | | | $T_{ele}$ | | | $T_{gbsolv}$ | | | $T_{gbtot}$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **WT** | **3N39** | **3N94** | **WT** | **3N39** | **3N94** | **WT** | **3N39** | **3N94** | **WT** | **3N39** | **3N94** |
| **ACE2** | **S19** | -0.77 | -0.73 | -0.40 | 14.83 | 14.91 | 14.19 | -11.86 | -11.93 | -12.70 | 2.19 | 2.25 | 1.09 |
| | **Q24** | -2.83 | -2.71 | -2.69 | -3.11 | -3.29 | -2.87 | 3.77 | 3.92 | 3.76 | -2.18 | -2.09 | -1.79 |
| | **T27** | -3.07 | -3.03 | -4.55(Y) | -1.49 | -1.58 | -1.56(Y) | 2.52 | 2.42 | 3.04(Y) | -2.04 | -2.19 | -3.07(Y) |
| | **H34** | -3.09 | -3.14 | -3.08(I) | -2.40 | -0.64 | -0.38(I) | 4.49 | 2.94 | 0.41(I) | -1.01 | -0.84 | -3.05(I) |
| | **Y41** | -2.18 | -2.98 | -2.82 | 0.84 | 0.93 | 0.01 | 0.15 | 0.28 | 0.73 | -1.18 | -1.77 | -2.09 |
| | **L79** | -1.17 | -2.43(F) | -2.20(F) | -0.36 | -0.72(F) | -0.41(F) | 0.53 | 1.19(F) | 0.93(F) | -1.00 | -1.96(F) | -1.68(F) |
| | **Y83** | -1.40 | -1.65 | -1.59 | -3.64 | -4.25 | -3.58 | 2.59 | 2.92 | 2.56 | -2.45 | -2.98 | -2.61 |
| | **K353** | -4.84 | -5.35 | -4.65 | 10.23 | 2.89 | 8.10 | -8.09 | -1.57 | -6.98 | -2.70 | -4.04 | -3.53 |
| | **D355** | -1.76 | -1.80 | -1.75 | -26.89 | -30.17 | -29.90 | 24.29 | 26.87 | 27.29 | -4.35 | -5.11 | -4.36 |
| **RBD** | **D405** | -0.27 | -0.23 | -0.16 | 131.93 | 138.85 | 161.47 | -129.12 | -135.93 | -158.49 | 2.54 | 2.69 | 2.82 |
| | **E406** | -0.13 | -0.14 | -0.10 | 123.81 | 135.44 | 157.45 | -121.51 | -132.98 | -154.89 | 2.17 | 2.32 | 2.46 |
| | **K417** | -0.23 | -0.32 | 0.22 | -143.23 | -174.14 | -200.32 | 140.85 | 170.87 | 195.19 | -2.60 | -3.59 | -4.91 |
| | **Y449** | -0.02 | -0.45 | -1.02 | -1.43 | -7.37 | -2.67 | 1.50 | 4.88 | 2.19 | 0.05 | -2.93 | -1.50 |
| | **L455** | -2.30 | -2.39 | -2.09 | 1.60 | 3.25 | 3.80 | -1.50 | -3.11 | -3.75 | -2.19 | -2.25 | -2.04 |
| | **F456** | -2.57 | -2.68 | -2.76 | -1.09 | -2.41 | -2.60 | 1.64 | 2.91 | 3.10 | -2.02 | -2.17 | -2.26 |
| | **E484** | -0.23 | -0.44 | -0.31 | 103.59 | 111.02 | 124.99 | -101.37 | -108.33 | -122.32 | 2.00 | 2.26 | 2.36 |
| | **F486** | -3.93 | -5.03 | -4.92 | -3.36 | -3.51 | -3.78 | 4.29 | 4.51 | 4.77 | -3.00 | -4.02 | -3.94 |
| | **Y489** | -0.45 | -4.39 | -3.82 | 0.62 | -1.79 | -0.64 | -0.23 | 3.42 | 2.74 | -0.06 | -2.76 | -1.73 |
| | **Q493** | -1.95 | -2.28 | -1.65 | -14.39 | -9.42 | -10.64 | 12.94 | 10.03 | 10.07 | -3.40 | -1.68 | -2.21 |
| | **Y495** | -0.42 | -0.52 | -0.51 | -2.34 | -4.17 | -4.15 | 2.79 | 2.76 | 4.44 | 0.02 | -1.92 | -0.22 |
| | **G496** | -0.78 | -0.58 | -0.31 | -4.59 | -0.89 | -5.11 | 2.86 | 0.44 | 3.53 | -2.51 | -1.02 | -1.90 |
| | **Q498** | -2.02 | -2.47 | -2.21 | -5.92 | -7.08 | -6.66 | 4.88 | 4.79 | 3.47 | -3.06 | -4.76 | -5.41 |
| | **T500** | -3.07 | -3.03 | -3.60 | -3.30 | -4.09 | -3.63 | 3.77 | 4.32 | 3.99 | -2.60 | -2.81 | -3.25 |
| | **N501** | -3.48 | -4.06 | -3.95 | -2.81 | -11.12 | -12.89 | 4.33 | 11.75 | 12.99 | -1.96 | -3.43 | -3.84 |
| | **Y505** | -3.92 | -4.50 | -4.51 | -6.06 | -6.80 | -3.83 | 5.57 | 6.39 | 4.72 | -4.40 | -4.92 | -3.62 |

**Table S7** Energetic contributions of separate residues to bindings of ACE2s to Delta

| Energy (kcal/mol) | | $T_{vdW}$ | | | $T_{ele}$ | | | $T_{gbsolv}$ | | | $T_{gbtot}$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **WT** | **3N39** | **3N94** | **WT** | **3N39** | **3N94** | **WT** | **3N39** | **3N94** | **WT** | **3N39** | **3N94** |
| **ACE2** | **S19** | -0.61 | -0.56 | -1.02 | 32.42 | 29.19 | 37.20 | -29.57 | -26.56 | -33.09 | 2.23 | 2.07 | 3.08 |
| | **Q24** | -2.99 | -2.63 | -2.70 | -2.75 | -3.61 | -4.37 | 3.85 | 4.19 | 5.13 | -1.90 | -2.04 | -1.95 |
| | **T27** | -2.74 | -2.81 | -7.11(Y) | -1.06 | -1.43 | -3.27(Y) | 1.90 | 2.27 | 4.88(Y) | -1.91 | -1.97 | -5.50(Y) |
| | **K31** | -4.25 | -3.05(N) | -3.37(N) | 17.77 | 0.22(N) | -1.24(N) | -15.66 | 1.21(N) | 3.26(N) | -2.14 | -1.62(N) | -1.35(N) |
| | **H34** | -3.15 | -3.07 | -3.46(I) | -0.44 | -1.14 | 0.68(I) | 2.94 | 3.47 | -0.58(I) | -0.66 | -0.73 | -3.36(I) |
| | **Y41** | -3.46 | -3.01 | -1.90 | 0.76 | 1.31 | 1.63 | 0.25 | -0.13 | -0.56 | -2.45 | -1.84 | -0.83 |
| | **L79** | -1.73 | -2.42(F) | -3.06(F) | -0.38 | -0.69(F) | -0.76(F) | 0.54 | 1.18 (F) | 1.35(F) | -1.57 | -1.93(F) | -2.46(F) |
| | **Y83** | -1.16 | -1.64 | -1.63 | -5.66 | -3.80 | -4.61 | 3.14 | 2.52 | 2.70 | -3.68 | -2.93 | -3.54 |
| | **K353** | -5.52 | -4.56 | -5.33 | 36.22 | 25.07 | 33.80 | -32.63 | -23.53 | -30.03 | -1.92 | -3.02 | -1.56 |
| | **D355** | -1.71 | -1.74 | -1.49 | -43.41 | -42.97 | -37.70 | 39.65 | 39.34 | 34.80 | -5.46 | -5.36 | -4.40 |
| **RBD** | **R403** | -0.89 | -0.77 | -0.58 | -145.13 | -152.17 | -179.18 | 144.37 | 149.97 | 178.06 | -1.66 | -2.96 | -1.70 |
| | **D405** | -0.44 | -0.22 | -0.21 | 130.87 | 139.41 | 164.02 | -127.73 | -136.71 | -160.79 | 2.71 | 2.48 | 3.02 |
| | **E406** | -0.16 | -0.12 | -0.10 | 126.38 | 134.18 | 159.85 | -123.94 | -131.93 | -157.24 | 2.28 | 2.13 | 2.51 |
| | **K417** | -0.49 | -0.23 | -0.19 | -144.49 | -174.05 | -206.49 | 142.49 | 170.68 | 203.04 | -2.49 | -3.60 | -3.64 |
| | **Y449** | -0.78 | -0.42 | -0.34 | -1.18 | -7.95 | -2.54 | 1.49 | 5.10 | 2.03 | -0.46 | -3.27 | -0.85 |
| | **L455** | -2.86 | -2.25 | -2.84 | 1.94 | 3.06 | 2.31 | -1.75 | -2.93 | -2.05 | -2.68 | -2.12 | -2.57 |
| | **F456** | -2.69 | -2.61 | -2.29 | -0.90 | -2.37 | -1.31 | 1.37 | 2.88 | 1.69 | -2.22 | -2.11 | -1.91 |
| | **A475** | -1.17 | -1.42 | -2.60 | -3.79 | -3.99 | -6.10 | 3.60 | 3.89 | 6.11 | -1.36 | -1.52 | -2.59 |
| | **E484** | -0.27 | -0.37 | -0.22 | 106.95 | 112.64 | 121.90 | -104.63 | -109.96 | -119.62 | 2.06 | 2.31 | 2.06 |
| | **F486** | -4.50 | -5.00 | -4.99 | -3.33 | -3.53 | -4.01 | 4.28 | 4.56 | 4.70 | -3.56 | -3.96 | -4.30 |
| | **N487** | -1.83 | -1.73 | -2.29 | -3.81 | -4.23 | -4.89 | 3.99 | 4.32 | 5.09 | -1.65 | -1.65 | -2.10 |
| | **Y489** | -4.80 | -4.38 | -5.56 | -2.86 | -1.92 | -5.75 | 3.72 | 3.52 | 6.51 | -3.94 | -2.78 | -4.80 |
| | **Q493** | -2.40 | -2.19 | -4.80 | -18.04 | -8.71 | -6.31 | 15.84 | 9.49 | 7.01 | -4.60 | -1.41 | -1.50 |
| | **Y495** | -0.67 | -0.52 | -0.64 | -2.63 | -4.17 | -3.52 | 2.88 | 2.76 | 4.04 | -0.41 | -1.92 | -0.13 |
| | **G496** | -0.94 | -0.26 | -0.95 | -2.40 | -4.20 | -2.95 | 3.13 | 2.74 | 1.97 | -0.22 | -1.72 | -1.94 |
| | **Q498** | -3.06 | -2.39 | -2.33 | -7.10 | -6.70 | -2.54 | 4.92 | 4.93 | 3.09 | -5.24 | -4.16 | -1.78 |
| | **T500** | -2.83 | -3.00 | -3.40 | -4.73 | -4.63 | -3.08 | 4.82 | 4.57 | 2.84 | -2.75 | -3.05 | -3.64 |
| | **N501** | -4.19 | -4.04 | -3.13 | -10.55 | -10.74 | -1.84 | 11.14 | 11.54 | 2.99 | -3.60 | -3.24 | -1.98 |
| | **Y505** | -5.35 | -4.39 | -4.24 | -6.09 | -7.39 | -6.94 | 6.25 | 6.71 | 6.14 | -5.19 | -5.08 | -5.04 |

**Table S8** Energetic contributions of separate residues to bindings of ACE2s to BA.1

| Energy (kcal/mol) | | $T_{vdW}$ | | | $T_{ele}$ | | | $T_{gbsolv}$ | | | $T_{gbtot}$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | WT | 3N39 | 3N94 | WT | 3N39 | 3N94 | WT | 3N39 | 3N94 | WT | 3N39 | 3N94 |
| ACE2 | S19 | -0.88 | -0.90 | -0.41 | 33.88 | 34.14 | 32.75 | -30.71 | -30.74 | -30.85 | 2.29 | 2.50 | 1.49 |
| | Q24 | -3.08 | -2.90 | -2.80 | -3.29 | -4.02 | -3.38 | 4.28 | 4.97 | 4.47 | -2.08 | -1.95 | -1.71 |
| | T27 | -3.12 | -2.74 | -4.43(Y) | -1.45 | -1.16 | -1.18(Y) | 2.56 | 2.08 | 2.87(Y) | -2.00 | -1.83 | -2.75(Y) |
| | K31 | -3.35 | -2.79(N) | -2.71(N) | 58.05 | -7.43(N) | -4.33(N) | -54.78 | 8.51(N) | 6.06(N) | -0.09 | -1.71(N) | -0.98(N) |
| | H34 | -4.22 | -3.02 | -3.06(I) | -9.54 | -6.16 | -3.04(I) | 12.07 | 8.13 | 3.37(I) | -1.68 | -1.06 | -2.74(I) |
| | E35 | -0.97 | -1.43(K) | -1.38(K) | -93.28 | 64.17(K) | 62.45(K) | 92.35 | -61.77(K) | -59.58(K) | -1.91 | 0.97(K) | 1.49(K) |
| | D38 | -0.69 | -0.43 | 0.27 | -106.80 | -99.33 | -101.59 | 105.24 | 96.12 | 98.27 | -2.25 | -3.65 | -3.05 |
| | Y41 | -3.84 | -3.10 | -2.59 | -0.04 | 1.63 | 1.08 | 1.11 | -0.29 | 0.20 | -2.76 | -1.76 | -1.30 |
| | L79 | -0.89 | -2.02(F) | -2.09(F) | 0.37 | 0.19(F) | 0.23(F) | -0.22 | 0.29(F) | 0.23(F) | -0.75 | -1.55(F) | -1.63(F) |
| | Y83 | -1.23 | -1.68 | -1.62 | -2.81 | -2.88 | -3.06 | 1.95 | 2.07 | 2.15 | -2.09 | -2.50 | -2.54 |
| | K353 | -4.62 | -5.57 | -5.37 | 49.35 | 67.16 | 81.78 | -45.45 | -63.22 | -77.37 | -0.72 | -1.63 | -0.96 |
| | D355 | -0.85 | -1.81 | -1.97 | -51.58 | -65.82 | -67.46 | 51.76 | 62.68 | 64.63 | -0.67 | -4.95 | -4.80 |
| RBD | R403 | -1.75 | -0.60 | -0.52 | -153.37 | -137.89 | -172.67 | 152.00 | 137.33 | 171.23 | -3.12 | -1.17 | -1.97 |
| | D405 | -1.31 | -0.18 | -0.18 | 126.95 | 133.30 | 161.26 | -123.25 | -130.73 | -158.24 | 2.39 | 2.39 | 2.84 |
| | E406 | -0.32 | -0.11 | -0.10 | 124.79 | 128.90 | 156.80 | -122.57 | -126.69 | -154.24 | 1.90 | 2.10 | 2.46 |
| | K417N | -0.73 | -0.28 | -0.18 | -1.92 | -3.78 | -5.09 | 2.69 | 4.15 | 5.36 | 0.04 | 0.10 | 0.09 |
| | N440K | -0.02 | -0.09 | -0.29 | -113.14 | -131.12 | -154.76 | 111.86 | 129.86 | 153.15 | -1.31 | -1.36 | -1.89 |
| | Y449 | -0.24 | -0.36 | -0.27 | -0.22 | -5.49 | -0.41 | 0.54 | 3.59 | 0.70 | 0.08 | -2.26 | 0.02 |
| | L455 | -2.52 | -1.96 | -1.44 | 0.99 | 1.74 | 1.67 | -0.77 | -1.56 | -1.54 | -2.30 | -1.78 | -1.32 |
| | F456 | -2.67 | -2.38 | -2.50 | -0.95 | -2.51 | -2.49 | 1.55 | 3.08 | 3.08 | -2.07 | -1.81 | -1.91 |
| | A475 | -1.86 | -1.52 | -1.76 | -5.62 | -4.67 | -3.32 | 5.40 | 4.49 | 3.45 | -2.08 | -1.70 | -1.63 |
| | E484A | -0.07 | -0.13 | -0.15 | -0.38 | -0.92 | -1.01 | 0.50 | 1.08 | 1.18 | 0.05 | 0.03 | 0.02 |
| | F486 | -3.26 | -4.63 | -4.76 | -2.98 | -3.36 | -3.65 | 3.88 | 4.29 | 4.60 | -2.35 | -3.70 | -3.81 |
| | N487 | -1.98 | -1.79 | -1.81 | -3.58 | -3.45 | -2.91 | 4.34 | 3.54 | 3.15 | -1.23 | -1.70 | -1.57 |
| | Y489 | -3.60 | -3.76 | -3.91 | -0.23 | -1.32 | -1.14 | 2.17 | 3.35 | 3.27 | -1.66 | -1.73 | -1.78 |
| | Q493R | -1.85 | -2.55 | -2.02 | -191.21 | -142.83 | -183.20 | 180.33 | 141.69 | 177.65 | -12.73 | -3.68 | -7.57 |
| | Y495 | -0.34 | -0.33 | -0.23 | -4.55 | -1.72 | -2.61 | 4.66 | 2.13 | 3.02 | -0.23 | 0.08 | 0.18 |
| | G496S | -0.42 | -0.95 | -0.66 | -5.33 | -2.85 | -0.08 | 4.42 | 2.21 | 0.31 | -1.33 | -1.59 | -0.43 |
| | Q498R | -1.17 | -2.67 | -2.31 | -133.76 | -156.42 | -161.40 | 133.64 | 153.11 | 161.26 | -1.29 | -5.97 | -2.46 |
| | T500 | -1.19 | -3.05 | -3.76 | 0.96 | -4.20 | -3.96 | 0.38 | 4.07 | 3.55 | 0.14 | -3.18 | -4.16 |
| | N501Y | -3.23 | -5.41 | -5.17 | -4.42 | -2.68 | -3.36 | 4.72 | 3.13 | 3.91 | -2.93 | -4.96 | -4.62 |
| | Y505H | -4.57 | -3.40 | -3.27 | -7.97 | -7.37 | -7.75 | 9.67 | 8.28 | 8.71 | -2.88 | -2.49 | -2.31 |

**Table S9** Energetic contributions of separate residues to bindings of ACE2s to BA.2

| Energy (kcal/mol) | | $T_{vdW}$ | | | $T_{ele}$ | | | $T_{gbsolv}$ | | | $T_{gbtot}$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **WT** | **3N39** | **3N94** | **WT** | **3N39** | **3N94** | **WT** | **3N39** | **3N94** | **WT** | **3N39** | **3N94** |
| **ACE2** | **S19** | -0.82 | -0.87 | -0.13 | 32.80 | 30.29 | 31.83 | -29.75 | -27.33 | -30.78 | 2.24 | 2.10 | 0.91 |
| | **Q24** | -2.96 | -2.87 | -2.62 | -4.65 | -4.30 | -4.25 | 5.44 | 5.19 | 5.07 | -2.17 | -1.98 | -1.80 |
| | **T27** | -3.10 | -2.54 | -4.73(Y) | -1.47 | -0.81 | -2.65(Y) | 2.56 | 1.69 | 4.27(Y) | -2.02 | -1.65 | -3.11(Y) |
| | **K31** | -4.03 | -2.72(N) | -3.37(N) | 59.97 | -3.55(N) | -11.34(N) | -56.28 | 5.20(N) | 11.82(N) | -0.33 | -1.07(N) | -2.89(N) |
| | **H34** | -3.49 | -3.79 | -3.03 (I) | -7.48 | -9.77 | 0.84 (I) | 9.13 | 11.86 | -0.61 (I) | -1.85 | -1.70 | -2.81(I) |
| | **E35** | -1.57 | -0.93(K) | -0.93(K) | -82.40 | 56.09(K) | 66.78(K) | 82.17 | -53.62(K) | -64.74(K) | -1.80 | 1.53(K) | 1.12(K) |
| | **D38** | -0.45 | -0.30 | -0.20 | -102.11 | -95.40 | -91.07 | 99.30 | 92.14 | 88.52 | -3.26 | -3.56 | -2.75 |
| | **Y41** | -3.08 | -2.66 | -2.90 | 2.36 | 0.95 | 1.50 | -0.96 | 0.39 | -0.15 | -1.69 | -1.31 | -1.55 |
| | **L79** | -1.44 | -2.32(F) | -1.89(F) | 0.28 | -0.03(F) | 0.17(F) | -0.11 | 0.50(F) | 0.25(F) | -1.27 | -1.86(F) | -1.48(F) |
| | **Y83** | -1.41 | -1.67 | -1.65 | -4.46 | -2.95 | -3.35 | 2.73 | 2.21 | 2.46 | -3.14 | -2.41 | -2.54 |
| | **K353** | -5.75 | -5.15 | -5.22 | 64.38 | 74.58 | 64.10 | -60.37 | -69.95 | -60.35 | -1.74 | -0.52 | -1.47 |
| | **D355** | -1.90 | -1.91 | -1.74 | -62.93 | -65.62 | -59.89 | 59.59 | 62.91 | 57.24 | -5.23 | -4.63 | -4.38 |
| **RBD** | **R403** | -1.25 | -0.47 | -0.57 | -136.24 | -151.98 | -166.17 | 135.82 | 149.94 | 165.43 | -1.67 | -2.51 | -1.31 |
| | **E406** | -0.20 | -0.11 | -0.08 | 123.19 | 136.36 | 153.52 | -121.04 | -134.10 | -151.22 | 1.95 | 2.15 | 2.22 |
| | **K417N** | -0.27 | -0.29 | -0.16 | -2.59 | -3.41 | -5.15 | 2.98 | 3.90 | 5.43 | 0.12 | 0.19 | 0.13 |
| | **N440K** | -0.05 | -0.05 | -0.06 | -113.54 | -116.03 | -127.89 | 112.29 | 114.74 | 126.48 | -1.29 | -1.34 | -1.47 |
| | **Y449** | -0.27 | -0.31 | -0.34 | -7.00 | -0.04 | -6.90 | 4.65 | 0.41 | 4.33 | -2.63 | 0.06 | -2.91 |
| | **L455** | -2.54 | -1.72 | -1.71 | 1.28 | 1.71 | 1.07 | -1.00 | -1.49 | -0.94 | -2.26 | -1.50 | -1.59 |
| | **F456** | -2.78 | -2.45 | -2.79 | -0.74 | -2.32 | -2.62 | 1.34 | 2.90 | 3.24 | -2.18 | -1.87 | -2.17 |
| | **A475** | -1.44 | -1.40 | -1.68 | -6.11 | -4.66 | -0.86 | 5.70 | 4.48 | 1.54 | -1.85 | -1.58 | -1.00 |
| | **E484A** | -0.10 | -0.16 | -0.15 | -0.33 | -0.56 | -1.68 | 0.48 | 0.77 | 1.85 | 0.05 | 0.04 | 0.02 |
| | **F486** | -4.32 | -4.98 | -4.70 | -3.30 | -3.57 | -3.73 | 4.27 | 4.60 | 4.73 | -3.35 | -3.95 | -3.70 |
| | **N487** | -1.60 | -1.81 | -1.72 | -5.42 | -3.96 | -3.62 | 5.09 | 4.17 | 3.70 | -1.92 | -1.60 | -1.65 |
| | **Y489** | -4.10 | -4.03 | -4.38 | -1.55 | -1.13 | -3.58 | 3.03 | 3.18 | 5.38 | -2.62 | -1.98 | -2.58 |
| | **Q493R** | -3.23 | -2.11 | -2.44 | -165.01 | -186.87 | -165.57 | 161.83 | 176.97 | 164.78 | -6.41 | -12.00 | -3.23 |
| | **Y495** | -0.63 | -0.37 | -0.53 | -0.07 | 0.54 | 0.12 | 0.28 | -0.17 | 0.24 | -0.41 | 0.01 | -0.18 |
| | **G496** | -0.44 | -0.25 | -0.37 | 2.44 | 1.91 | 2.96 | -2.11 | -1.54 | -2.65 | -0.11 | 0.11 | -0.05 |
| | **Q498R** | -2.47 | -2.50 | -2.58 | -161.37 | -140.86 | -177.12 | 157.22 | 141.29 | 172.82 | -6.62 | -2.07 | -6.88 |
| | **T500** | -3.00 | -3.15 | -3.60 | -4.28 | -4.34 | -3.81 | 4.13 | 4.04 | 3.65 | -3.16 | -3.46 | -3.76 |
| | **N501Y** | -5.78 | -5.06 | -5.33 | -2.36 | -3.37 | -3.10 | 2.90 | 3.86 | 3.66 | -5.24 | -4.58 | -4.77 |
| | **Y505H** | -4.39 | -3.08 | -3.35 | -9.28 | -3.92 | -7.21 | 9.35 | 5.17 | 8.37 | -4.32 | -1.83 | -2.19 |

**Table S10** Energetic contributions of separate residues to bindings of ACE2s to BA.4/5

| Energy (kcal/mol) | | $T_{vdW}$ | | | $T_{ele}$ | | | $T_{gbsolv}$ | | | $T_{gbtot}$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | WT | 3N39 | 3N94 | WT | 3N39 | 3N94 | WT | 3N39 | 3N94 | WT | 3N39 | 3N94 |
| ACE2 | S19 | -0.10 | -0.59 | -0.79 | 32.99 | 31.14 | 29.94 | -31.96 | -28.78 | -27.60 | 0.93 | 1.77 | 1.55 |
| | Q24 | -2.81 | -2.71 | -2.80 | -3.53 | -3.91 | -3.90 | 4.43 | 4.84 | 5.10 | -1.91 | -1.78 | -1.60 |
| | T27 | -2.87 | -2.73 | -4.55(Y) | -0.87 | -0.57 | -1.22(Y) | 1.95 | 1.68 | 2.98(Y) | -1.79 | -1.63 | -2.79(Y) |
| | K31 | -3.31 | -3.20(N) | -3.02(N) | 51.99 | -1.61(N) | -2.18(N) | -49.42 | 3.42(N) | 3.87(N) | -0.74 | -1.39(N) | -1.33(N) |
| | H34 | -3.33 | -3.88 | -2.76(I) | -4.59 | -2.70 | -0.80(I) | 6.20 | 3.89 | 0.87(I) | -1.72 | -2.69 | -2.69(I) |
| | E35 | -0.85 | -1.84(K) | -0.94(K) | -67.56 | 50.17(K) | 52.05(K) | 68.18 | -47.63(K) | -49.85(K) | -0.23 | 0.70(K) | 1.26(K) |
| | D38 | -0.28 | -0.94 | -0.76 | -95.45 | -79.73 | -75.38 | 93.06 | 81.09 | 76.70 | -2.67 | 0.41 | 0.56 |
| | Y41 | -3.01 | -2.86 | -2.83 | 0.76 | 0.61 | 0.45 | 0.31 | 0.72 | 0.64 | -1.94 | -1.53 | -1.74 |
| | L79 | -0.76 | -1.68(F) | -1.67(F) | 0.19 | -0.03(F) | -0.05(F) | -0.13 | 0.36(F) | 0.38(F) | -0.69 | -1.35(F) | -1.34(F) |
| | Y83 | -0.66 | -0.87 | -0.85 | -1.97 | -2.57 | -2.52 | 1.39 | 1.83 | 1.77 | -1.25 | -1.61 | -1.60 |
| | K353 | -5.33 | -5.08 | -5.45 | 65.41 | 61.96 | 61.90 | -61.70 | -58.76 | -57.98 | -1.62 | -1.87 | -1.52 |
| | D355 | -1.86 | -1.86 | -1.75 | -63.80 | -64.38 | -67.92 | 61.27 | 61.60 | 65.58 | -4.39 | -4.63 | -4.09 |
| RBD | R403 | -0.78 | -0.88 | -0.57 | -129.43 | -133.37 | -165.45 | 129.11 | 133.51 | 164.69 | -1.09 | -0.74 | -1.32 |
| | D405N | -0.19 | -0.22 | -0.20 | -0.38 | -0.14 | -1.21 | 0.88 | 0.68 | 1.69 | 0.31 | 0.32 | 0.29 |
| | E406 | -0.10 | -0.11 | -0.08 | 118.50 | 129.82 | 152.01 | -116.61 | -127.77 | -149.74 | 1.79 | 1.94 | 2.18 |
| | K417N | -0.20 | -0.21 | -0.15 | -2.43 | -4.43 | -4.66 | 2.83 | 4.75 | 4.95 | 0.20 | 0.11 | 0.14 |
| | N440K | -0.05 | -0.05 | -0.06 | -113.53 | -115.85 | -127.51 | 112.28 | 114.56 | 126.11 | -1.30 | -1.34 | -1.47 |
| | Y449 | -0.36 | -0.56 | -0.51 | -7.62 | -6.20 | -4.04 | 4.97 | 4.16 | 2.97 | -3.00 | -2.60 | -1.58 |
| | L455 | -1.96 | -1.89 | -1.68 | 0.93 | 2.94 | 1.81 | -0.82 | -2.77 | -1.68 | -1.85 | -1.71 | -1.55 |
| | F456 | -2.43 | -2.47 | -2.66 | -1.20 | -2.53 | -2.41 | 1.76 | 3.01 | 3.01 | -1.87 | -1.98 | -2.05 |
| | A475 | -1.50 | -1.29 | -1.69 | -1.73 | -4.07 | -3.89 | 2.35 | 3.86 | 3.87 | -0.88 | -1.50 | -1.70 |
| | E484A | -0.08 | -0.13 | -0.13 | -0.36 | -0.57 | -0.87 | 0.50 | 0.75 | 1.06 | 0.06 | 0.05 | 0.06 |
| | F486V | -1.64 | -2.53 | -2.76 | -2.39 | -2.93 | -3.41 | 2.53 | 3.05 | 3.48 | -1.50 | -2.41 | -2.69 |
| | N487 | -2.04 | -1.93 | -1.97 | -3.15 | -3.24 | -3.39 | 4.32 | 3.91 | 4.07 | -0.87 | -1.27 | -1.29 |
| | Y489 | -3.54 | -4.20 | -4.11 | -0.13 | -1.51 | -2.03 | 2.16 | 3.34 | 3.82 | -1.51 | -2.37 | -2.32 |
| | Q493 | -2.03 | -2.35 | -1.81 | -12.48 | -9.48 | -5.59 | 11.33 | 9.08 | 6.70 | -3.18 | -2.75 | -0.69 |
| | Y495 | -0.69 | -0.88 | -0.48 | 0.03 | 0.63 | -0.49 | 0.32 | 0.07 | 0.55 | -0.34 | -0.18 | -0.42 |
| | G496 | -0.40 | -0.37 | -0.31 | 2.35 | 2.34 | 2.76 | -2.10 | -1.99 | -2.49 | -0.14 | -0.03 | -0.05 |
| | Q498R | -2.56 | -2.90 | -3.16 | -161.32 | -146.59 | -179.70 | 156.63 | 147.30 | 179.57 | -7.24 | -2.19 | -3.29 |
| | T500 | -3.00 | -3.04 | -3.49 | -3.78 | -3.67 | -3.55 | 4.17 | 3.92 | 3.37 | -2.60 | -2.79 | -3.68 |
| | N501Y | -5.36 | -5.28 | -4.64 | -2.06 | -2.54 | -3.39 | 2.62 | 3.28 | 3.74 | -4.80 | -4.54 | -4.29 |
| | Y505H | -3.56 | -3.72 | -3.30 | -6.75 | -8.02 | -8.26 | 8.11 | 9.53 | 9.58 | -2.20 | -2.20 | -1.99 |

**Table S11** Statistical analysis of hotspot residues and differentiating residues

| Residue types<br>RBD types | Hot spot residue | Potentially engineerable beneficial residues | Potentially engineerable detrimental residues |
|---|---|---|---|
| **WT** | S19, Q24, T27, Y83, K353, D355, | S19, T27Y, H34I, Y41, L79F, K353 | |
| | D405, E406, K417, L455, F456, E484, F486, Q493, G496, Q498, T500, N501, Y505 | K417, Y449, F486, Y489, Y495, Q498, N501 | Q493, G496 |
| **Delta** | S19, Q24, T27, K31, Y41, Y83, K353, D355 | T27Y, H34I, K353, | Y41, D355 |
| | D405, E406, K417, L455, F456, E484, F486, Y489, Q493, Q498, T500, N501, Y505 | R403, K417, Y449, A475, Y495, G496 | Y489, Q493, Q498, N501 |
| **BA.1** | S19, Q24, T27, E35, D38, Y41, Y83 | K31N, H34I, D38, K353, D355 | E35K, Y41 |
| | R403, D405, E406, L455, F456, A475, F486, Q493R, N501Y, Y505H | Y449, F486, Q498R, T500, N501Y | R403, L455, Q493R, G496S |
| **BA.2** | S19, Q24, T27, D38, Y83, D355 | S19, T27Y, K31N, H34I | E35K, K353 |
| | E406, Y449, L455, F456, F486, N487, Y489, Q493R, Q498R, T500, N501Y, Y505H | Q493R | Y449, Q493R, Q498R, Y505H |
| **BA.4/5** | Q24, D38, Y41, D355 | T27Y, H34I | E35K, D38 |
| | Y449, Q493, Q498R, T500, N501Y, Y505H | F486V, T500 | Y449, Q493, Q498R |

Hotspot residues: Residues with absolute value contributions to binding free energy of 2 kcal/mol or higher. Potential engineered hotspots: Residues in engineered ACE2s that show a decrease in binding free energy contribution of approximately 1 kcal/mol or higher compared to WT ACE2.Potential engineered unfavorable residues: Residues in engineered ACE2s that show an increase in binding free energy contribution of approximately 1 kcal/mol or higher compared to WT ACE2.Red font represents unfavorable contributions to binding free energy. Red bold font indicates mutations in 3N39. Orange bold font represents mutations present in both 3N39 and 3N94.Blue background represents effects observed in 3N39. Green background represents effects observed in 3N94. Pink background represents effects observed in both 3N39 and 3N94.

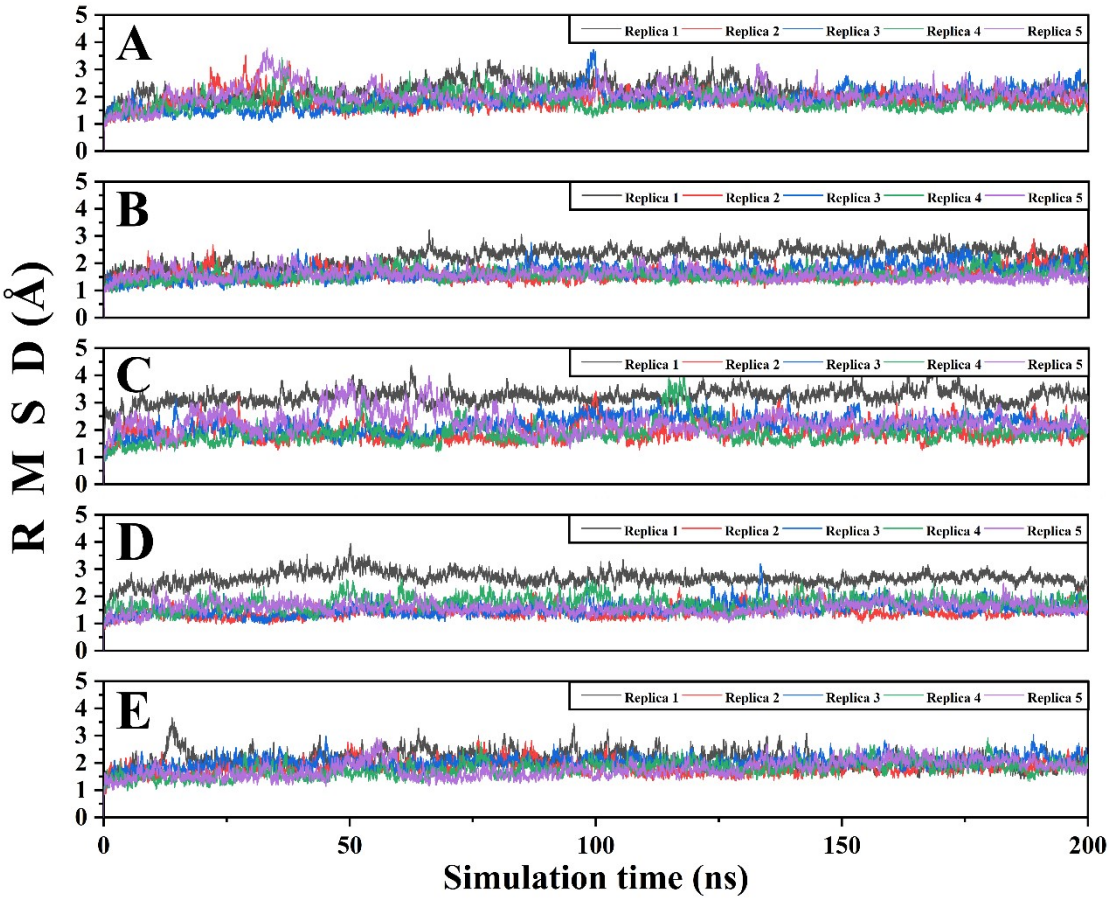


**Fig. S1** Root-mean-square deviations (RMSDs) of backbone atoms in the WT ACE2-RBDs: (A) the WT ACE2-WT, (B) the WT ACE2-Delta, (C) the WT ACE2-BA.1, (D) the WT ACE2-BA.2, (E) the WT ACE2-BA.4/5.

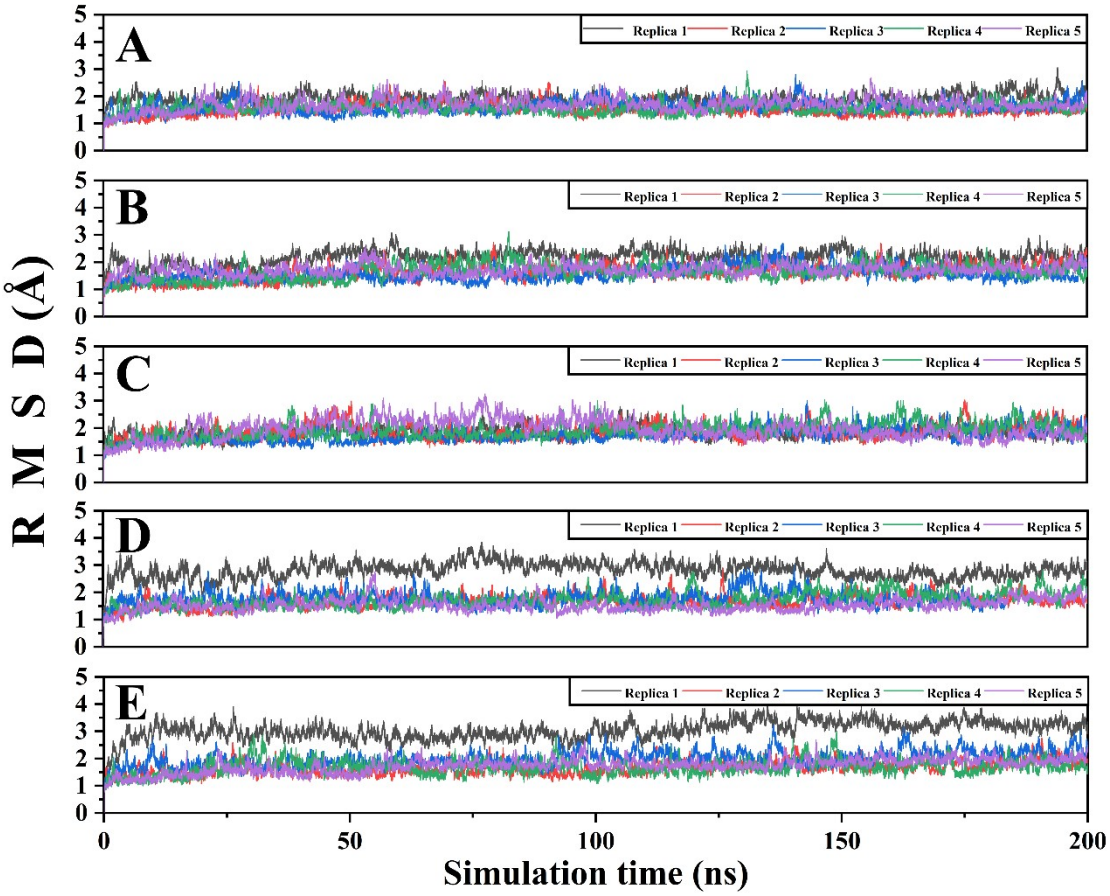


**Fig. S2** Root-mean-square deviations (RMSDs) of backbone atoms in the 3N39-RBDs: (A) the 3N39-WT, (B) the 3N39-Delta, (C) the 3N39-BA.1, (D) the 3N39-BA.2, (E) the 3N39-BA.4/5.

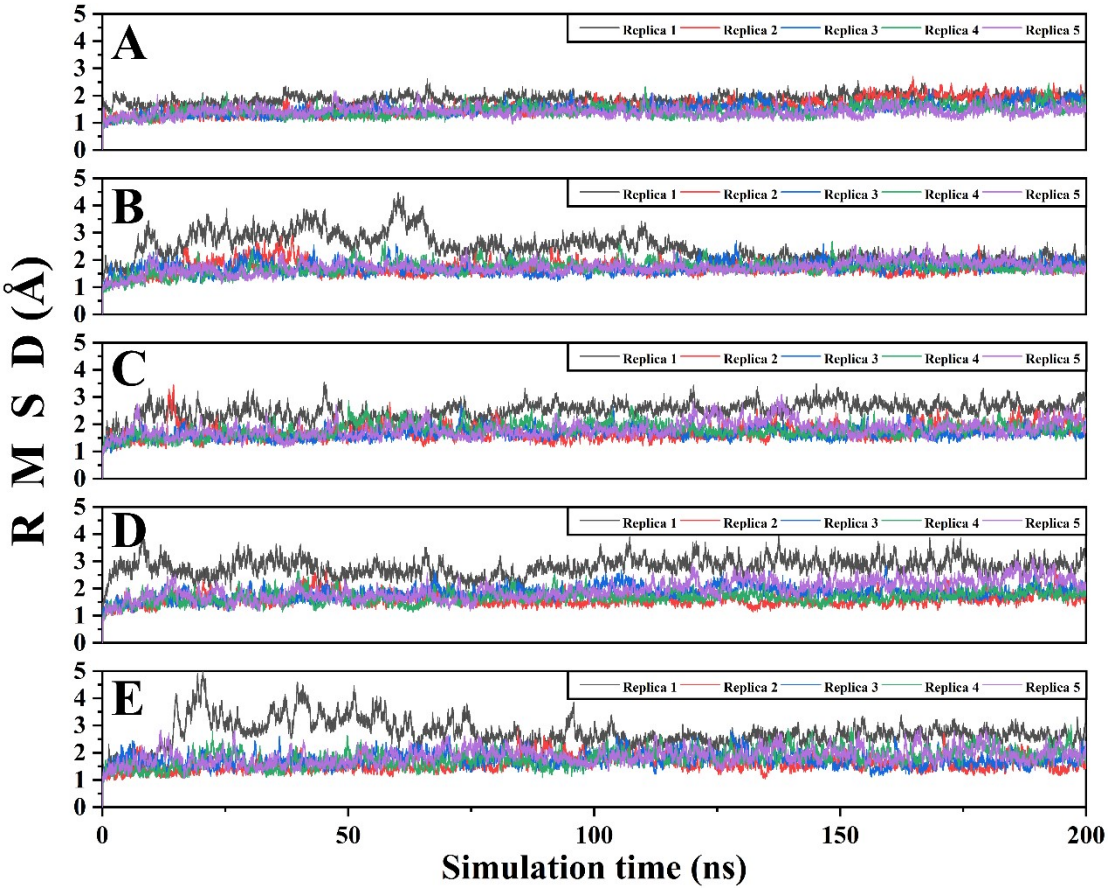


**Fig. S3** Root-mean-square deviations (RMSDs) of backbone atoms in the 3N94-RBDs: (A) the 3N94-WT, (B) the 3N94-Delta, (C) the 3N94-BA.1, (D) the 3N94-BA.2, (E) the 3N94-BA.4/5.

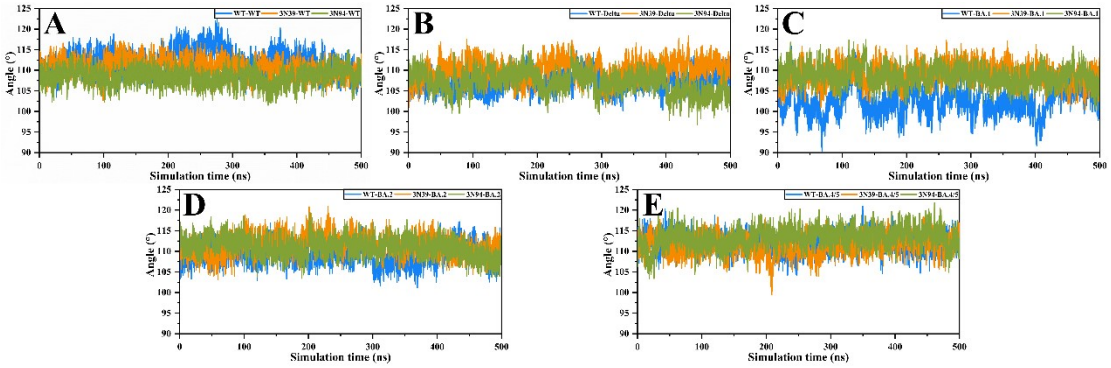


**Fig. S4** The change of the angle of the geometric centers of ACE2, RBD and its RBM region during simulation using the single joined trajectory. (A) the ACE2s-WT, (B) the ACE2s-Delta, (C) the ACE2s-BA.1, (D) the ACE2s-BA.2, (E) the ACE2s-BA.4/5.

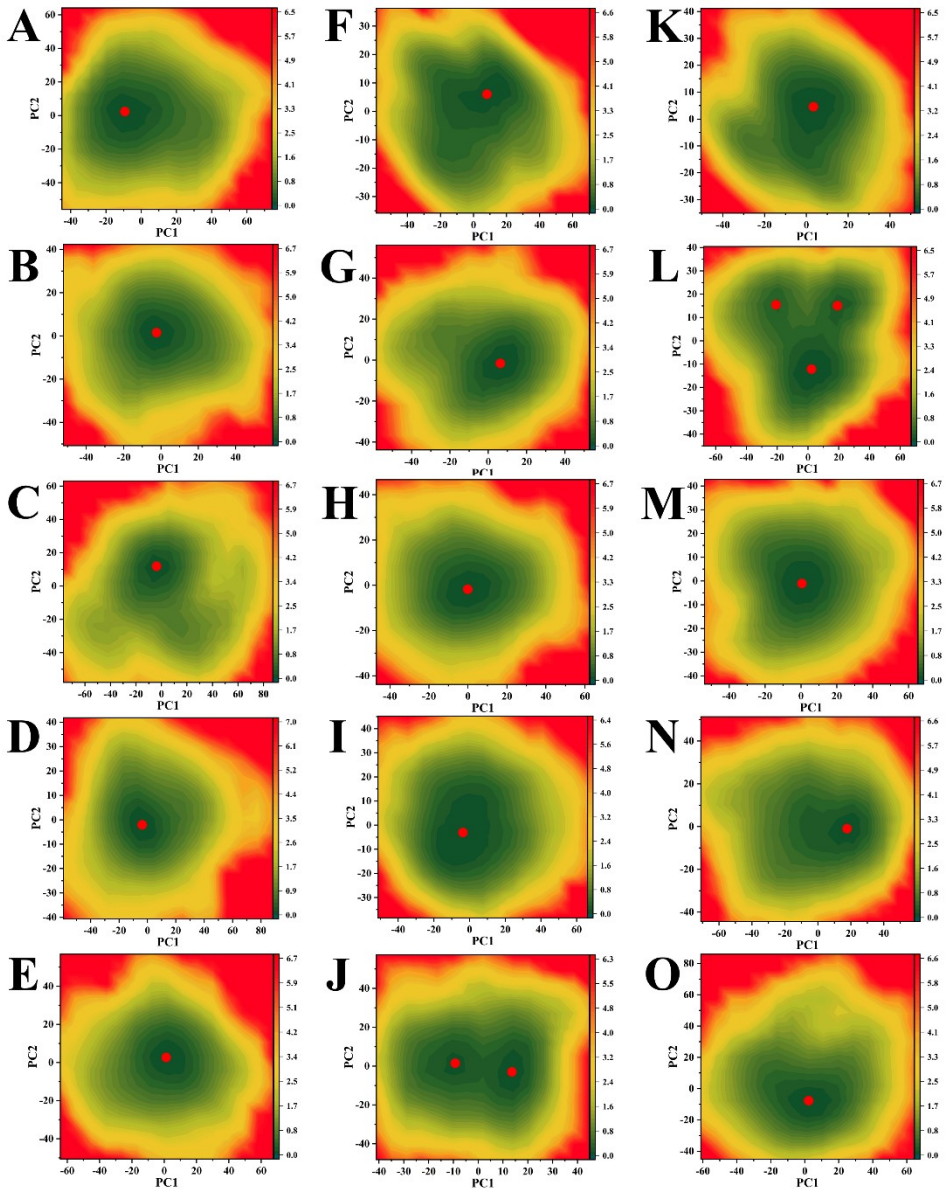


**Fig. S5** Free energy landscapes of ACE2s-RBDs. (A), (B), (C), (D) and (E): WT ACE2-WT RBD/Delta/BA.1/BA.2/BA.4/5. (F), (G), (H), (I) and (J): 3N39-WT RBD/Delta/BA.1/BA.2/BA.4/5. (K), (L), (M), (N) and (O): 3N94-WT RBD/Delta/BA.1/BA.2/BA.4/5.

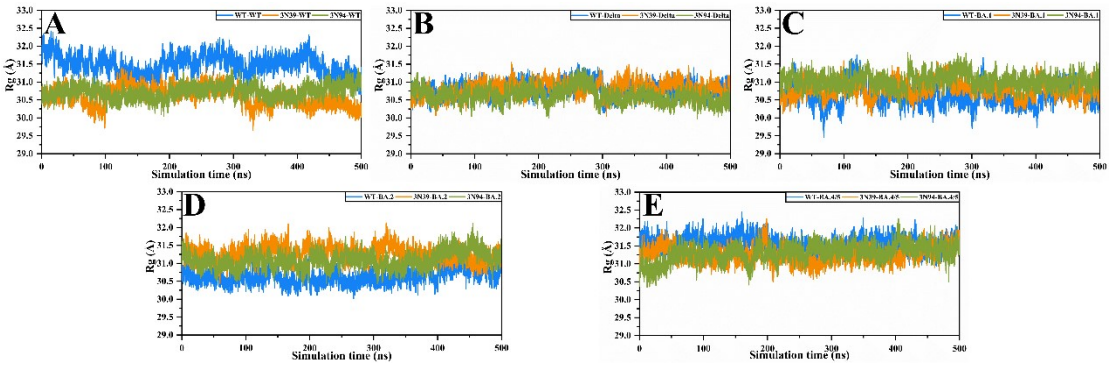


**Fig. S6** The radius of gyration of the whole systems. (A) the ACE2s-WT, (B) the ACE2s-Delta, (C) the ACE2s-BA.1, (D) the ACE2s-BA.2, (E) the ACE2s-BA.4/5.

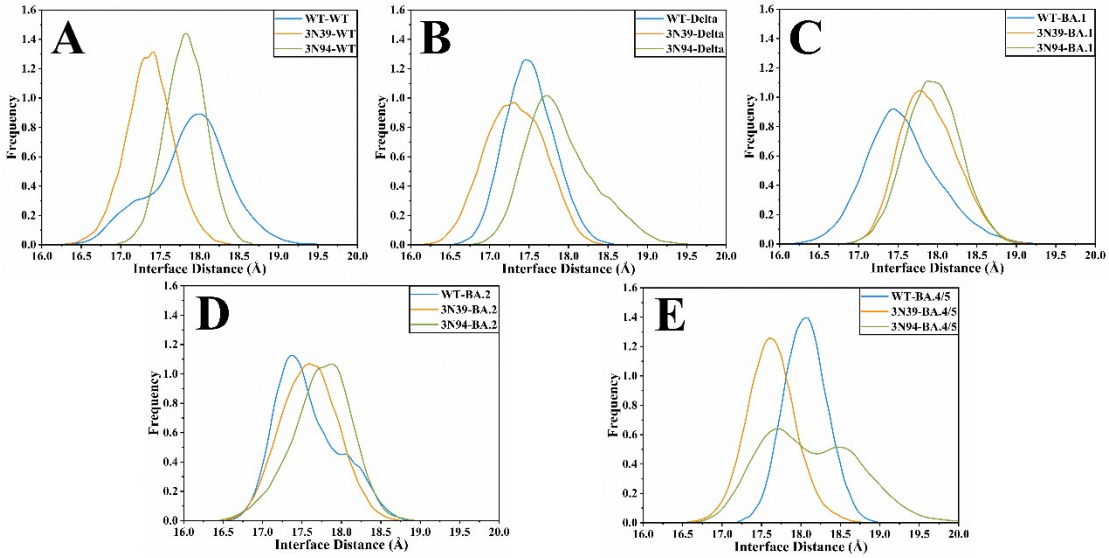


**Fig. S7** The distance between the geometric centers of the binding region of ACE2 and the RBM region of RBD during the simulation process. (A) the ACE2s-WT RBD, (B) the ACE2s-Delta, (C) the ACE2s-BA.1, (D) the ACE2s-BA.2, (E) the ACE2s-BA.4/5.

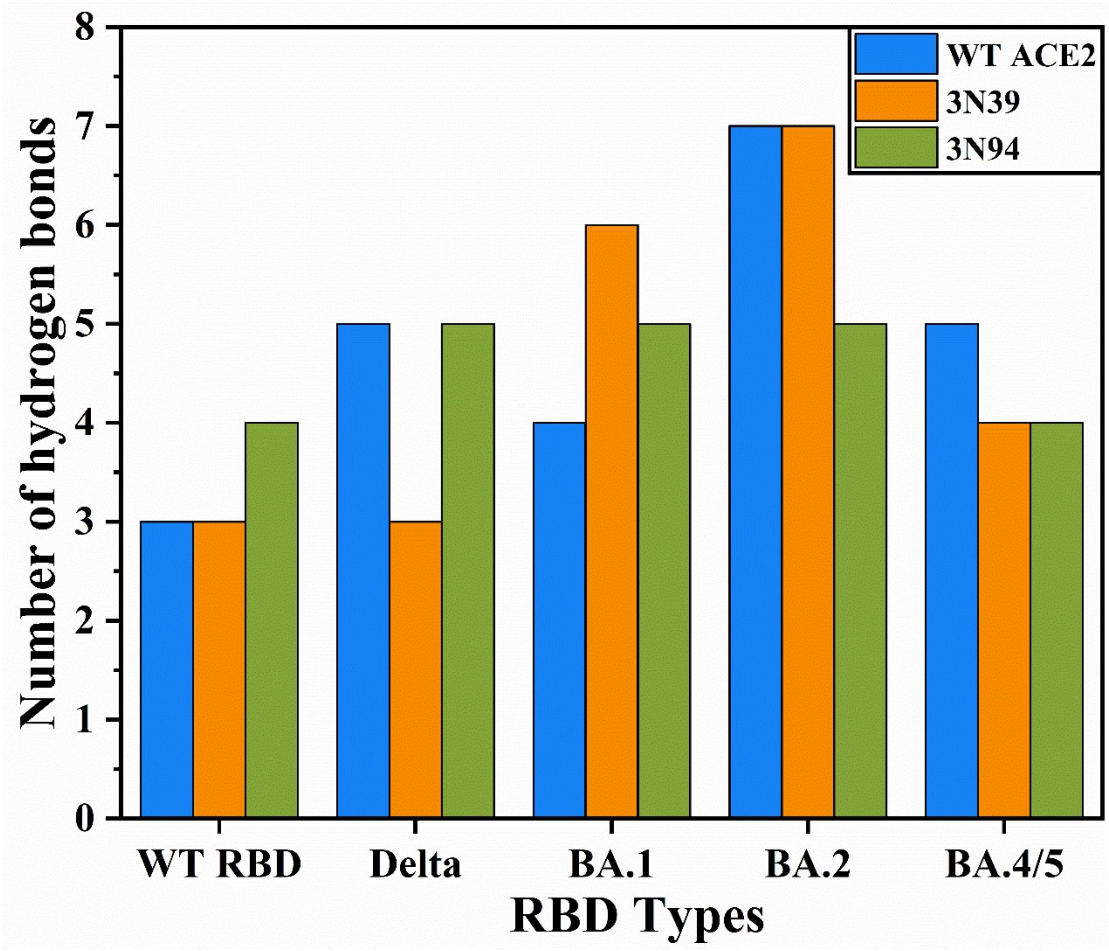


**Fig. S8** Comparison of the number of hydrogen bonds in different systems.

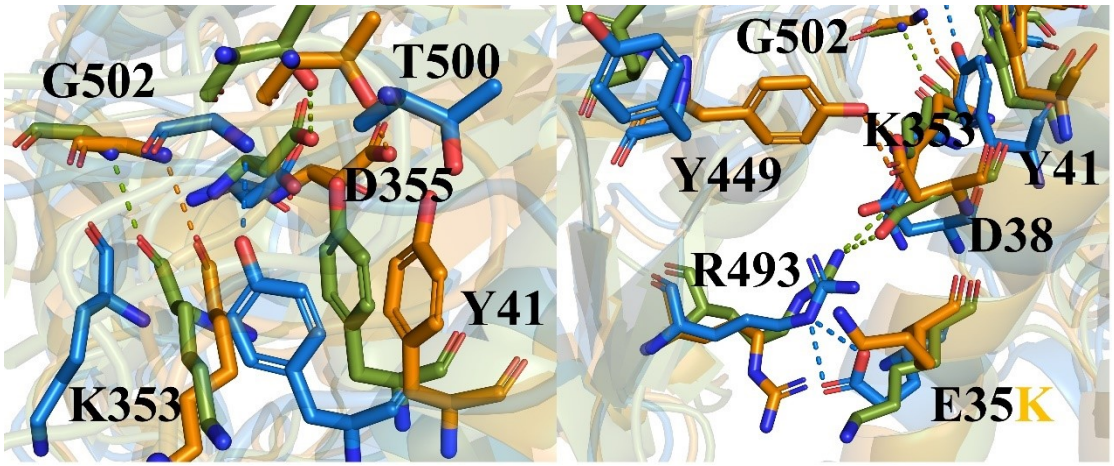


**Fig. S9** Superposition of representative structures of ACE2s-BA.1 RBD, residues involved in hydrogen bond formation are shown as sticks, blue orange and green represent WT ACE2-BA.1,3N39-BA.1,3N94-BA.1 respectively. The orange font represents mutations shared by 3N39 and 3N94.

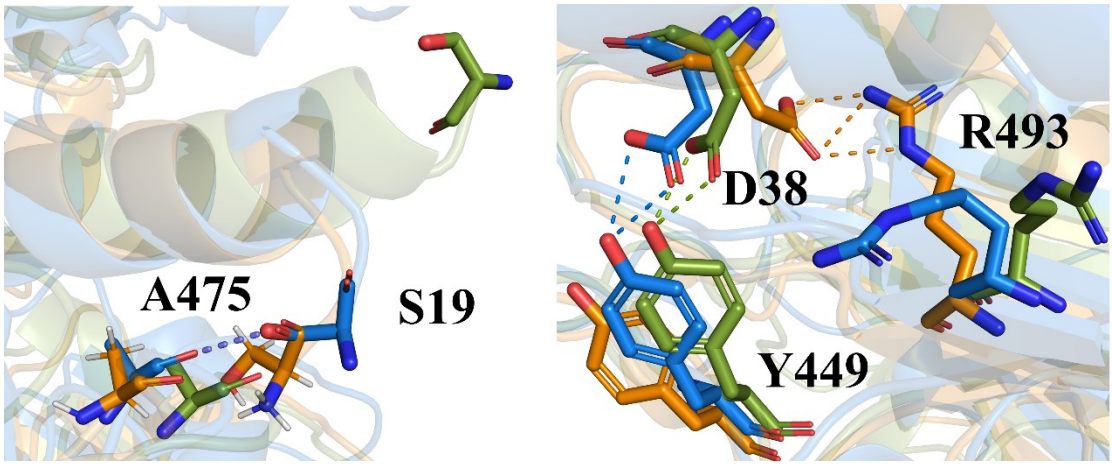


**Fig. S10** Superposition of representative structures of ACE2s-BA.2 RBD, residues involved in hydrogen bond formation are shown as sticks, blue orange and green represent WT ACE2-BA.2,3N39-BA.2,3N94-BA.2 respectively.

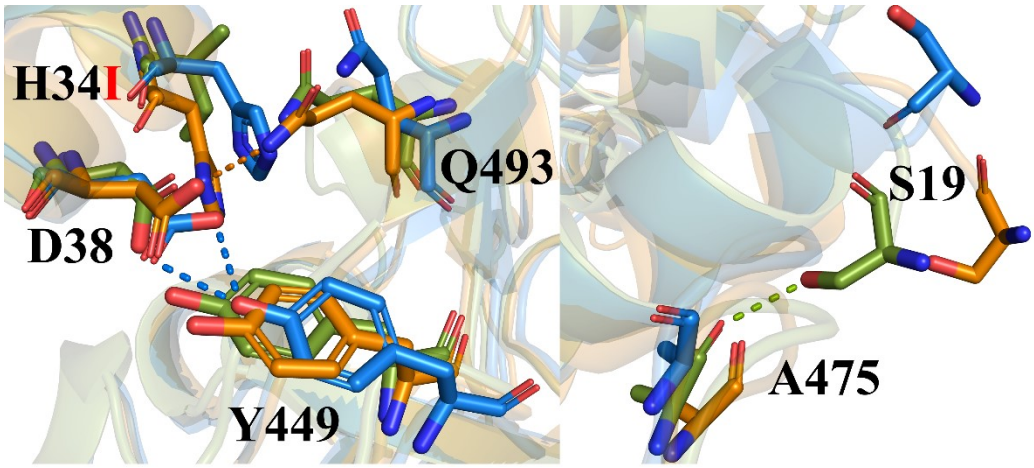


**Fig. S11** Superposition of representative structures of ACE2s-BA.4/5 RBD, residues involved in hydrogen bond formation are shown as sticks, blue orange and green represent WT ACE2-BA.2,3N39-BA.2,3N94-BA.2 respectively. The red font represents mutations unique to 3N94.