**Investigation of critical binding pattern in SARS-CoV-2 spike glycoprotein with angiotensin-converting enzyme 2: an *in silico* analysis**

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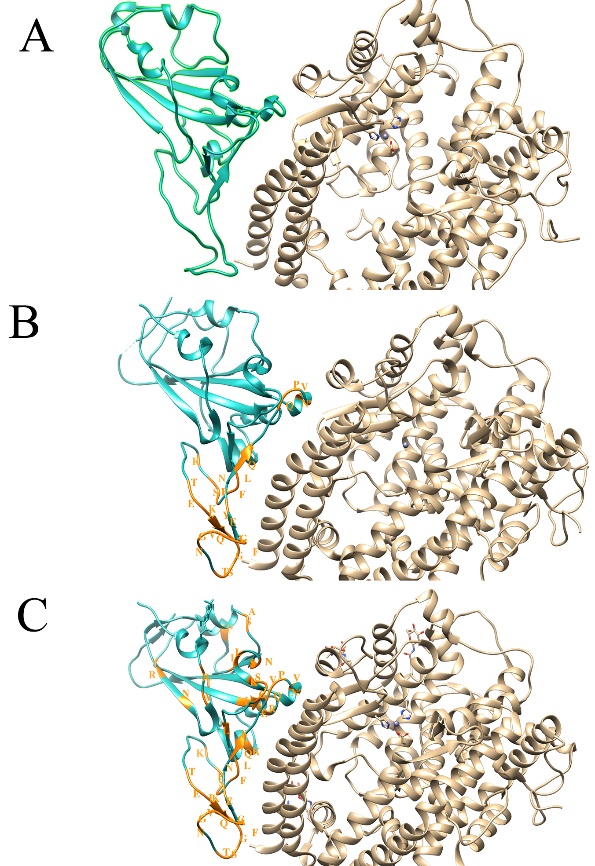
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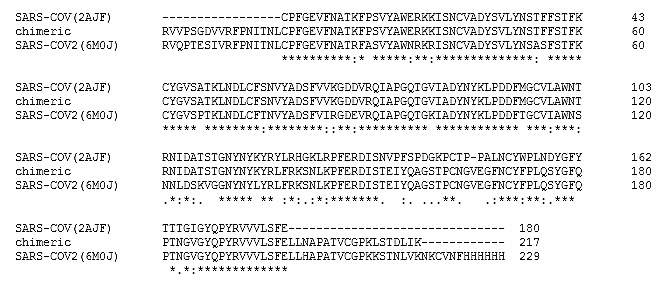
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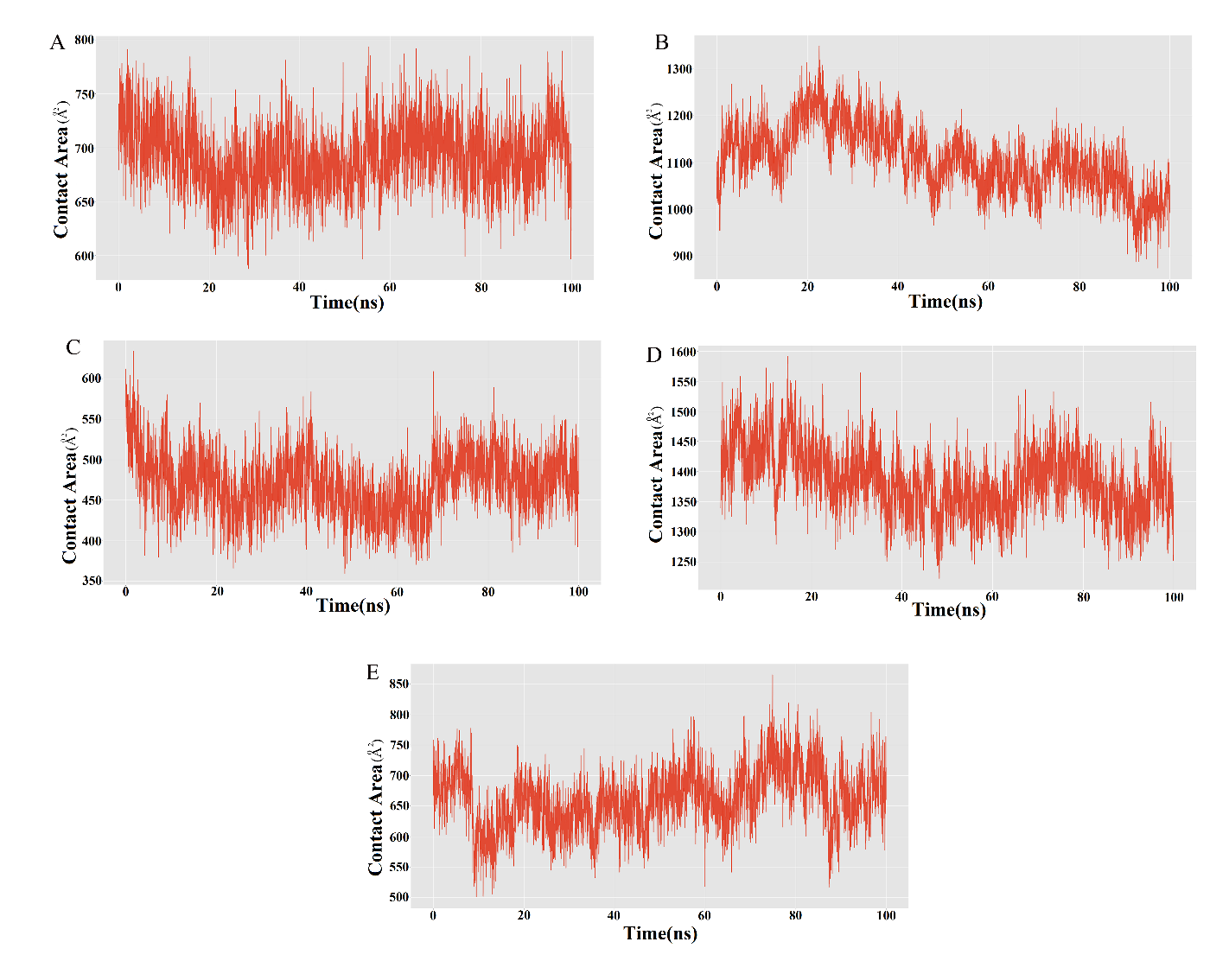
**Supplementary Figures**



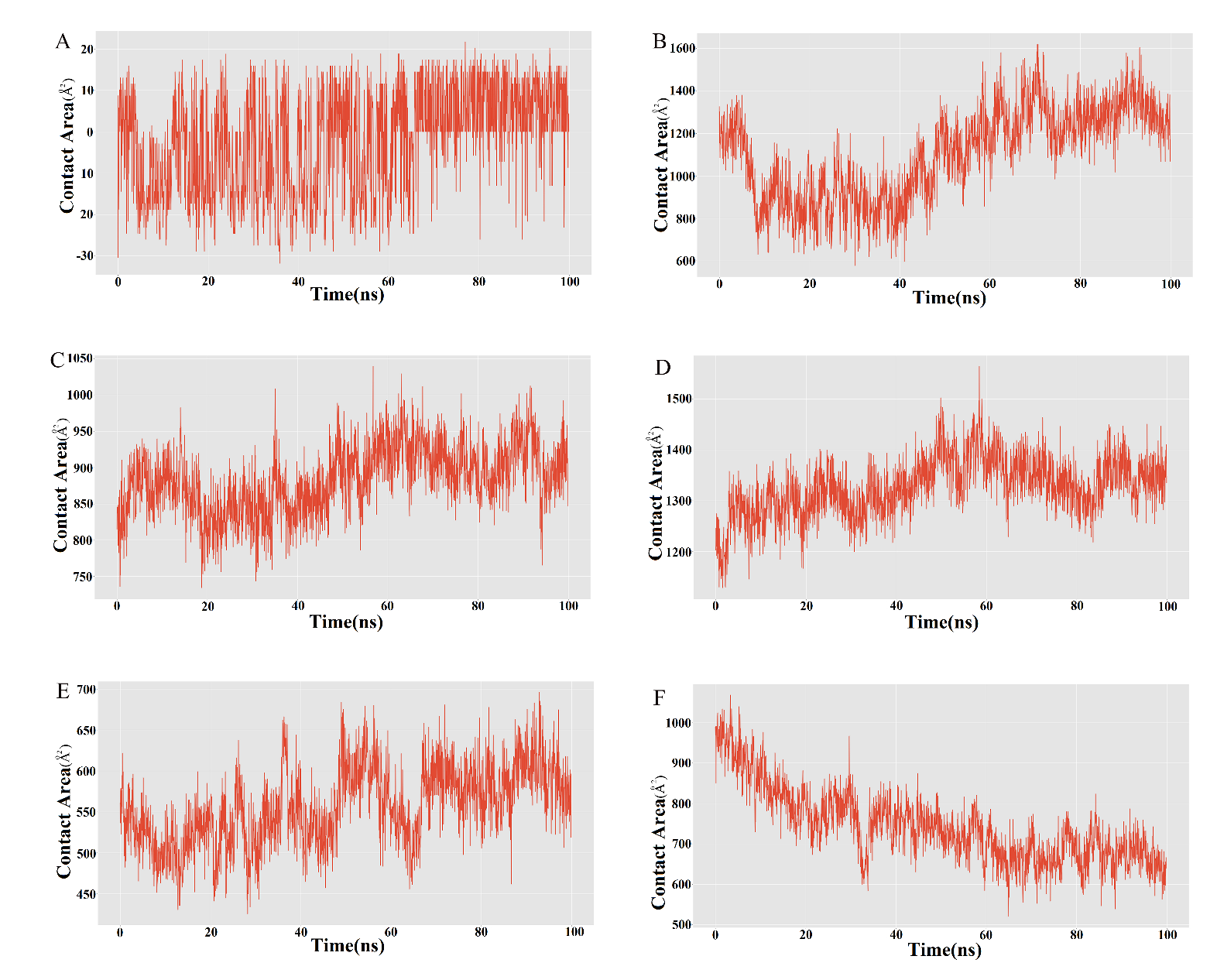
**Figure S1: structural representations for three structures of spike-ACE2 complexes**: (A) SARS-CoV, (B) chimeric structure and (C) SARS-CoV-2. Mutations are highlighted for chimeric and SARS-CoV-2 structures.



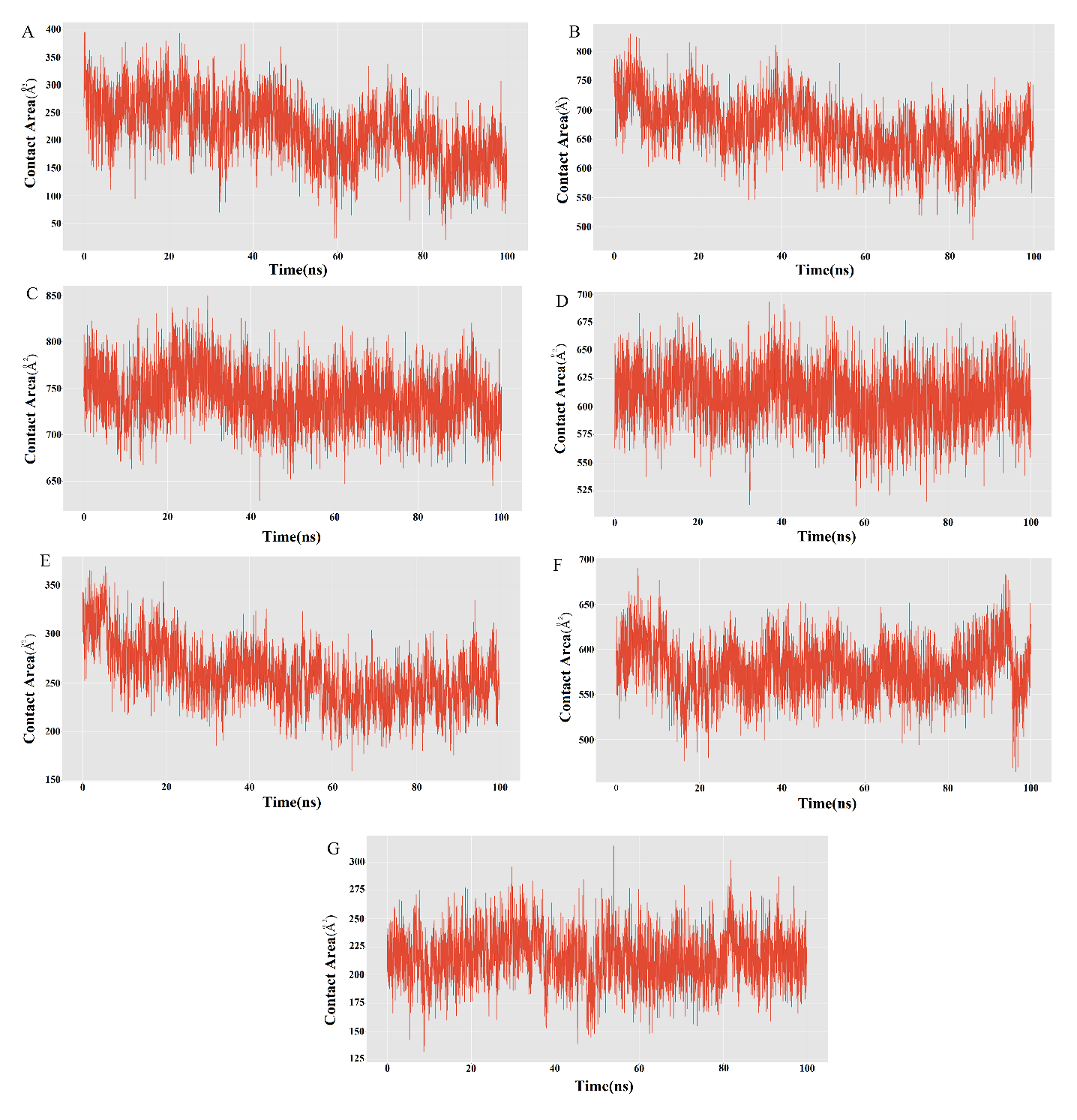
**Figure S2: Sequence alignment of spike proteins:** SARS-CoV (2ajf), chimeric structure (6vw1) and SARS-CoV-2 (6m0j).



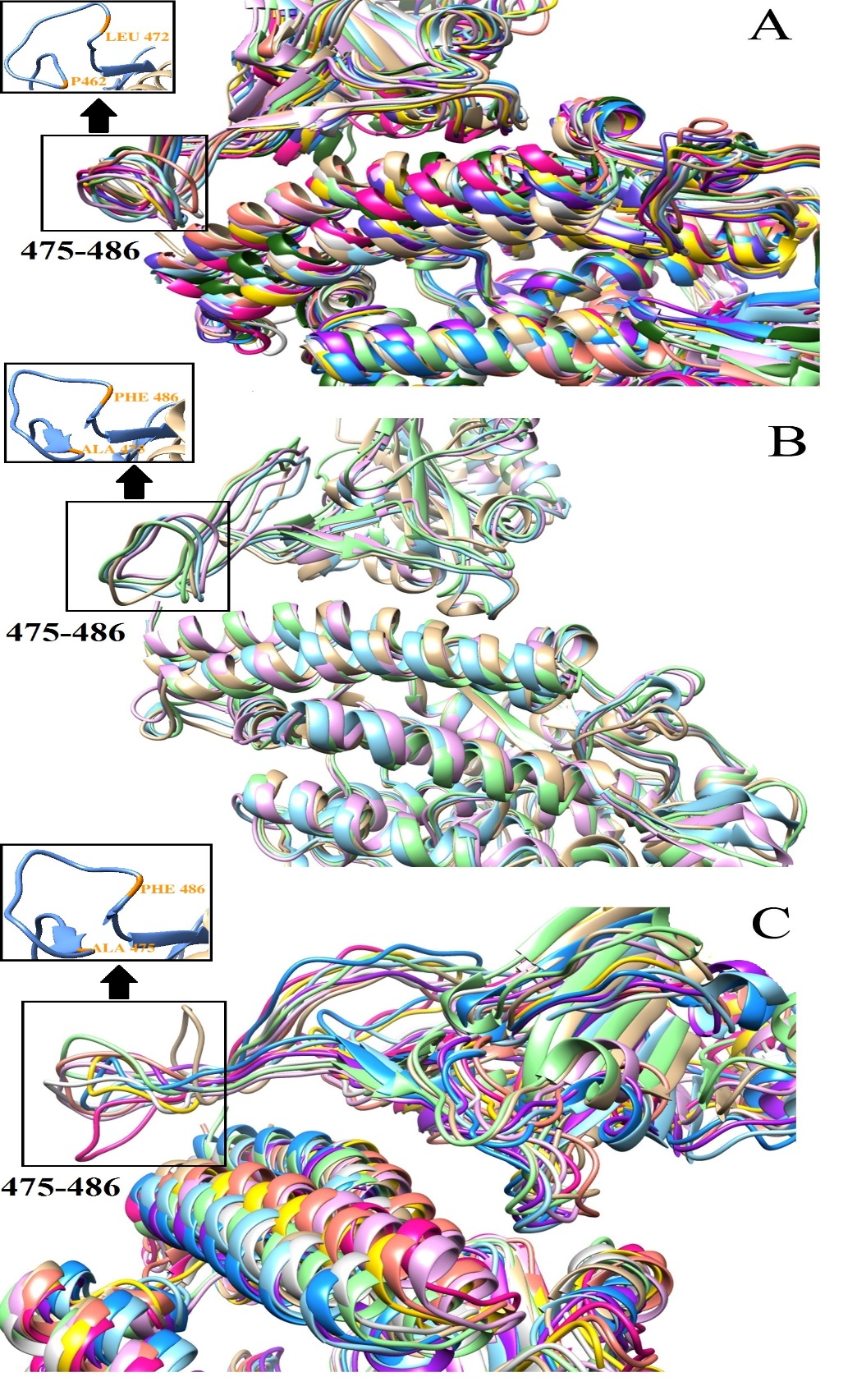
**Figure S3. Contact area graphs for SARS-CoV-ACE2 complex (2ajf) during simulation.** The contact areas between receptor-binding motif of SARS-CoV spike protein and different regions of ACE2 including residues 19-33 (A), 35-194 (B), 196-227 (C), 266-344 (D) and 541-616 (E).



**Figure S4. Contact area graphs for SARS-CoV-2-ACE2 complex (6m0j) during simulation.** The contact areas between receptor-binding motif of SARS-CoV-2 spike protein and different regions of ACE2 including residues 19-33 (A), 35-194 (B), 242-264 (C), 266-344 (D), 346-372 (E) and 541-616 (F).



**Figure S5. Contact area graphs for chimeric structure-ACE2 complex (6vw1) during simulation.** The contact areas between receptor-binding motif of chimeric structure and different regions of ACE2 including residues 1-4 (A), 16-175 (B), 224-246 (C), 248-326 (D), 328-364 (E), 471-468 (F) and 523-596 (G).



**Figure S6.** Comparison of proteins structures during simulation (with more focus on the binding area) showed that, the most fluctuated regions are located at residues 475-486 for SARS-COV2 and 462-472 for SARS-CoV.

**Supplementary Tables**

**Table S1. List of amino acid mutations in receptor-binding motif of SARS-CoV-2 compared to SARS-CoV**

|  |  |
| --- | --- |
| SARS-COV | SARS-COV2 |
| Tyr442 | **Leu455** |
| Leu443 | **Phe456** |
| His445 | **Lys458** |
| Gly446 | **Ser459** |
| Lys447 | **Asn460** |
| Arg449 | **Lys462** |
| Asn457 | **Thr470** |
| Val458 | **Glu471** |
| Pro459 | **Ile472** |
| Phe460 | **Tyr473** |
| Ser461 | **Gln474** |
| Pro462 | **Ala475** |
| Asp 463 | **Gly476** |
| Gly464 | **Ser477** |
| Lys465 | **Thr478** |
| Thr468 | **Asn481** |
| Pro469 | **Gly482** |
| Pro470 | **Glu484** |
| Ala471 | **Gly485** |
| Leu472 | **Phe486** |
| Trp476 | **Phe490** |
| Asn479 | **Gln493** |
| Asp480 | **Ser494** |
| Tyr484 | **Gln498** |
| Thr485 | **Pro499** |
| Thr487 | **Asn501** |
| Ile 489 | **Val503** |

**Table S2. Free energy decomposition of the SARS-CoV residues in spike-ACE2 complex**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Residue | Van der Waals | Electrostatic | Polar Solvation | Non-Polar Solv | TOTAL |
| Tyr440 | **-0.5155** | **0.0577** | **-0.0475** | **-0.0853** | **-0.0861±0.1095** |
| Arg441 | **-0.06075** | **-94.0605** | **94.1639** | **0.0** | **0.04274±0.0100** |
| Tyr442 | **-1.2877** | **-4.4947** | **4.5442** | **-0.4156** | **-1.6539±0.4785** |
| Leu443 | **-0.55025** | **-0.2584** | **0.4032** | **-0.1094** | **-0.5149±0.0363** |
| Arg444 | **0.0387** | **-87.2232** | **87.2925** | **0.0** | **0.0304±0.0070** |
| His445 | **-0.0270** | **0.9190** | **-0.8239** | **0.0** | **0.0680±0.0041** |
| Gly446 | **-0.0075** | **0.33444** | **-0.3127** | **0.0** | **0.01424±0.0020** |
| Lys447 | **-0.0155** | **-93.0455** | **93.0662** | **0.0** | **0.00524±0.0126** |
| Leu448 | **-0.0072** | **0.00024** | **0.00599** | **0.0** | **-0.00100±0.00079** |
| Arg449 | **-0.0037** | **-74.6127** | **74.5985** | **0.0** | **-0.0180±0.0006** |
| Pro450 | **-0.00200** | **-0.439249** | **0.4385** | **0.0** | **-0.002749±0.000892** |
| Phe451 | **-0.00200** | **-0.695499** | **0.694750** | **0.0** | **-0.002749±0.00021** |
| Glu452 | **-0.002999** | **78.6145** | **-78.583** | **0.0** | **0.028500±0.0055396** |
| Arg453 | **-0.00400** | **-81.79400** | **81.773,2** | **0.0** | **-0.025000±0.003409** |
| Asp454 | **-0.00625** | **88.57325** | **-88.497** | **0.0** | **0.069999±0.0046233** |
| Ile455 | **-0.00500** | **0.62100** | **-0.610250** | **0.0** | **0.005750±0.0011** |
| Ser456 | **-0.00675** | **1.797499** | **-1.7675** | **0.0** | **0.02325000±0.001138** |
| Asn457 | **-0.0127** | **0.53275** | **-0.45125** | **0.0** | **0.0687499±0.01212628** |
| VAL458 | **-0.01624** | **0.667250** | **0.2663** | **0.0** | **0.027500±0.00192** |
| Pro459 | **-0.0384** | **-1.5322** | **1.5877** | **0.0** | **0.017000±0.0054** |
| Phe460 | **-0.264750** | **0.847249** | **-0.598749** | **-0.0133379** | **-0.0295880±0.04822** |
| Ser461 | **-0.17799** | **-2.074** | **2.33775** | **0.0** | **0857499±0.00397** |
| Pro462 | **-2.07824** | **-3.249250** | **3.6332499** | **-0.3785706** | **-2.0728206±0.6382928** |
| Asp463 | **-0.99375** | **94.6152** | **-93.225000** | **-0.1227563** | **0.27374359±0.153622** |
| Gly464 | **-0.052499** | **-1.076499** | **1.1467500** | **0.0** | **0.01774999±0.015282** |
| Lys465 | **-0.219500** | **-98.7687500** | **99.376** | **-0.0205073** | **0.36724259±0.1875600** |
| Pro466 | **-0.021750** | **0.116749** | **-0.0952500** | **0.0** | **-0.0002500±0.0040369** |
| Cyc467 | **-0.04899** | **-0.554749** | **0.6692499** | **0.0** | **0.065500±0.0008291** |
| Thr468 | **-0.042500** | **0.066500** | **0.108499** | **0.0** | **0.132499±0.03570** |
| Pro469 | **-0.15774** | **-2.17500** | **2.35325** | **0.0** | **0.020499±0.007652** |
| Pro470 | **-0.748250** | **-0.29700** | **0.7505000** | **-0.177561** | **-0.47231100±0.45591** |
| Ala471 | **-0.50225** | **1.0507499** | **-0.559499** | **-0.0628848** | **-0.07388480±0.181488** |
| Leu472 | **-2.24925** | **-2.41200** | **3.10499** | **-0.5715666** | **-2.127816±0.30896** |
| Asn473 | **-2.507749** | **-2.682500** | **4.24,0** | **-0.3706758** | **-1.320925±0.383652** |
| CYS 474 | **-0.306250** | **1.0912500** | **-0.6817500** | **-0.000239400** | **0.10301060±0.0312150** |
| TYR 475 | **-3.7625** | **-1.071500** | **3.24625** | **-0.6433883** | **-2.2311383±0.339181** |
| TRP 476 | **-0.2217500** | **-2.9794999** | **1.98324999** | **-0.0560771** | **-1.274077200±0.807167** |
| PRO 477 | **-0.18049** | **1.034999** | **-0.9515** | **0.0** | **-0.096999±0.0351158** |
| LEU 478 | **-0.273749** | **-1.33075** | **1.09525** | **-0.0177839** | **-0.5270339±0.32764** |
| ASN 479 | **-1.83100** | **-7.953499** | **8.044** | **-0.3598308** | **-2.10033079±0.7966147** |
| ASP480 | **-1.02300** | **128.9555** | **-125.962** | **-0.190553** | **1.77994659±0.1701833** |
| TYR 481 | **-0.714249** | **-0.01500** | **0.75899999** | **-0.0959778** | **-0.0662277±0.0739066** |
| GLY 482 | **-0.96150** | **-0.4837499** | **0.9595** | **-0.1228518** | **-0.608601799±0.071494** |
| PHE 483 | **,-0.381500** | **-0.55724** | **0.685750** | **0.0** | **-0.2530±0.06141** |
| TYR 484 | **-3.42924** | **-6.82199** | **5.89849** | **-0.64378** | **-4.996536±0.813662** |
| THR 485 | **-0.39400** | **0.3257500** | **0.469249** | **-0.00118** | **0.399810±0.02827** |
| THR 486 | **-2.4535** | **-5.73374** | **5.51825** | **-0.788527** | **-3.4575277±0.423244** |
| THR 487 | **-2.80725** | **-3.038999** | **3.073499** | **-0.218493** | **-2.991242±0.178196** |
| GLY 488 | **-1.21624** | **-3.105499** | **2.88975** | **0.015074** | **-1.671684±0.08438** |
| ILE 489 | **-0.9** | **-1.33374** | **1.374** | **-0.236890** | **-1.0966408±0.26180** |
| GLY 490 | **-0.1640** | **-1.80075** | **1.991750** | **0.0** | **0.02699±0.02699** |
| TYR 491 | **-3.67949** | **-2.69575** | **4.369499** | **-0.57746** | **-2.58321±0.1613** |
| GLN 492 | **-0.37750** | **-3.75749** | **4.43325** | **-0.03858** | **0.259665±0.03482** |
| PRO 493 | **-0.062499** | **0.830249** | **-0.8345** | **0.0** | **-0.066750±0.0085** |

**Table S3. Free energy decomposition of the SARS-CoV-2 residues in spike-ACE2 complex**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Residue | Van der Waals | Electrostatic | Polar Solvation | Non-Polar Solv | TOTAL |
| Leu452 | **-0.0620** | **-0.9402** | **0.984** | **0.0** | **0.0174±0.0037** |
| Tyr453 | **-0.9207** | **-0.5615** | **1.7677** | **-0.1430** | **0.1424±0.0960** |
| Arg454 | **-0.0935** | **-94.970** | **95.1544** | **0.0** | **0.0900±0.0066** |
| Leu455 | **-2.69** | **1.7307** | **-1.5465** | **-0.2712** | **-2.7769±0.17222** |
| Phe456 | **-2.147** | **-0.29300** | **1.1042** | **-0.3387** | **-1.6744±0.2814** |
| Arg457 | **-0.0742** | **-87.5895** | **87.7722** | **0.0** | **0.1084±0.0092** |
| Lys458 | **-0.06699** | **-83.2865** | **83.4644** | **0.0** | **-0.11099±0.03423** |
| Ser459 | **-0.0197** | **0.8854** | **-0.8342** | **0.0** | **0.0314±0.0028** |
| Asn460 | **-0.0179** | **-1.4041** | **1.47375** | **0.0** | **-0.04425±0.0170** |
| Lus461 | **-0.011** | **0.38074** | **-0.3777** | **0.0** | **-0.0079±0.0023** |
| Lys462 | **-0.0044** | **-78.2182** | **78.2075** | **0.0** | **-0.0152±0.00143** |
| Pro463 | **-0.0033** | **-0.6742** | **0.6729** | **0.0** | **-0.0042±0.0002** |
| Phe464 | **-0.003** | **-0.9977** | **0.997** | **0.0** | **-0.0037±0.00021** |
| Glu465 | **-0.00425** | **82.993** | **-82.93875** | **0.0** | **0.04999±0.00372** |
| Arg466 | **-0.00574** | **-83.85675** | **83.8375** | **0.0** | **-0.02499±0.001499** |
| Asp467 | **-0.00999** | **89.5095** | **-89.42725** | **0.0** | **0.07224999±0.006493** |
| Ile468 | **-0.008249** | **0.5979999** | **-0.57775** | **0.0** | **0.012000±0.001274** |
| Ser469 | **-0.009249** | **1.7684999** | **-1.73775** | **0.0** | **0.0214999±0.0046165** |
| Thr470 | **-0.01900** | **0.261249** | **-0.1747499** | **0.0** | **0.0674999±0.007462** |
| Glu471 | **-0.02474** | **85.77875** | **-85.59025** | **0.0** | **0.163750±0.009429** |
| Ile472 | **-0.0814999** | **-1.06925000** | **1.1137499** | **0.0** | **-0.0369999±0.0070799** |
| Tyr473 | **-0.751500** | **-0.7197500** | **1.3787500** | **-0.103559** | **-0.1960594±0.0736** |
| Gln474 | **-0.374500** | **-2.604999** | **3.4457500** | **0.0** | **0.4662500±0.014006** |
| Ala475 | **-2.4** | **-5.4** | **0.93** | **0.0** | **-6.86±0.346735** |
| Gly476 | **-1.33** | **-0.819** | **0.210** | **0.0** | **-1.941±-0.5075** |
| SER477 | **-0.2575** | **-1.019** | **0.236** | **0.0** | **-1.0412±0.285** |
| THR478 | **-0.235** | **-0.319** | **0.2847** | **0.0** | **-0.27024±0.099** |
| PRO479 | **-0.0482** | **2.0280** | **-0.471** | **0.0** | **1.5087±0.080** |
| CYS480 | **-0.0392** | **-0.10** | **0.02849** | **0.0** | **-0.1107±0.485405** |
| ASN481 | **-0.02050** | **-1.5867** | **0.36874** | **0.0** | **-1.2385±0.2653** |
| GLY482 insertion | **-0.0130** | **-0.2397** | **0.06024** | **0.0** | **-0.1924±0.0615** |
| VAL483 | **-0.0657** | **-0.7180** | **0.15175** | **0.0** | **-0.6320±0.13198** |
| GLU484 | **-0.2472** | **94.5339** | **-20.826** | **0.0** | **73.46074±2.36857** |
| GLY485 | **-0.8525,** | **-0.9514** | **0.3054** | **0.0** | **-1.49850±0.1309** |
| PHE486 | **-4.986** | **-2.78225** | **0.95475** | **0.0** | **-6.81350±0.3228268** |
| ASN487 | **-2.16875** | **-4.966999** | **4.66175** | **-0.1221767** | **-2.596176±0.462978** |
| CYS488 | **-0.40399** | **0.468250** | **-0.00749** | **0.0** | **0.0567500±0.045828** |
| TYR489 | **-4.4825** | **-2.253250** | **3.984** | **-0.6036408** | **-3.355390±0.159110** |
| PHE490 | **-0.70149** | **0.88350** | **-0.406500** | **-0.05899** | **-0.2834949±0.28366** |
| PRO491 | **-0.1750** | **1.77700** | **-1.7545** | **0.0** | **-0.152499±0.027978** |
| LEU492 | **-0.2450** | **-1.16200** | **1.186499** | **-0.0053496** | **-0.225849±0.304708** |
| GLN493 | **-2.54999** | **-13.47625** | **12.46575** | **-0.5655132** | **-4.126013±0.46159** |
| SER494 | **-0.35450** | **2.19399** | **-1.487500** | **-0.0140579** | **0.33794±0.02797** |
| TYR495 | **-0.411249** | **-0.23124** | **0.905999** | **-0.000637** | **0.262862±0.010493** |
| GLY496 | **-1.247** | **-4.69324** | **2.67925** | **-0.213111** | **-3.47411±0.334680** |
| PHE497 | **-0.41799** | **0.014250** | **0.34525** | **0.0** | **-0.05849±0.07135** |
| GLN498 | **-1.38974** | **-8.909749** | **6.52150** | **-0.4337334** | **-4.211733±0.50664** |
| PRO499 | **-0.43949** | **1.74700** | **-1.365** | **-0.035546** | **-0.093046±0.0411** |
| THR500 | **-3.15799** | **-1.998249** | **3.2177499** | **-0.711802** | **-2.650302±0.68225** |
| ASN501 | **-3.316,0** | **-3.60650** | **4.4354999** | **-0.189327** | **-2.6763275±0.52674** |
| GLY502 | **-1.3195** | **-3.22649** | **2.99075** | **-0.30320** | **-1.8584581±0.05986** |
| VAL503 | **-0.74** | **-1.45700** | **1.67075** | **-0.1462068** | **-0.6724567±0.19391** |
| GLY504 | **-0.23475** | **-1.84649** | **2.08875** | **-0.002628** | **0.0048720±0.02468** |
| TYR505 | **-4.3575** | **-2.80150** | **4.218** | **-0.744759** | **-3.6857590±0.3645386** |
| GLN506 | **-0.525249** | **-3.357499** | **3.85425** | **-0.09606** | **-0.1245623±0.3135** |
| PRO507 | **-0.07099** | **0.815250** | **-0.857500** | **0.0** | **-0.11324±0.00537** |
| TYR508 | **-0.055750** | **-0.243749** | **0.30950** | **0.0** | **0.0100±0.00791** |