Molecular Dynamics Studies on the Interactions between SARS-CoV-2 Spike Protein and hACE2 or mAbs

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The first step of SARS-CoV-2 infection is the binding of its spike protein to human ACE2.

The spike protein on the surface of the virus particle is in a state of a trimer.

The domain to interact with ACE2 is called RBD, which has up- and down-Conformations.
Experiments showed contradictory binding affinities

- **ACE2-RBD Binding Affinity**

<table>
<thead>
<tr>
<th>Protein coated</th>
<th>$K_d$ (M)</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>SARS-CoV-RBD-His tag</td>
<td>$1.85 \times 10^{-7}$</td>
<td>SPR</td>
</tr>
<tr>
<td>SARS-CoV-2-RBD-His tag</td>
<td>$4.42 \times 10^{-8}$</td>
<td>SPR</td>
</tr>
</tbody>
</table>

- **ACE2-Spike Binding Affinity**

- The binding of the RBD of SARS-2 spike to ACE2 is **stronger** than that of SARS

- **The ACE2 binding affinities of RBD and full length spike are contradictory**;

- **Why**?


PNAS, 117, 11729–11734 (2020)
### ΔG calculated by MM/GBSA with 100 ns MD simulation

<table>
<thead>
<tr>
<th>Energy term</th>
<th>CoV-2-S RBD</th>
<th>CoV-S RBD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{vdw}$</td>
<td>-86.91±0.06</td>
<td>-80.73±0.07</td>
</tr>
<tr>
<td>$E_{ele}$</td>
<td>-697.07±0.56</td>
<td>-742.78±0.71</td>
</tr>
<tr>
<td>$E_{gb}$</td>
<td>760.94±0.51</td>
<td>812.86±0.67</td>
</tr>
<tr>
<td>$E_{np}$</td>
<td>-12.05±0.06</td>
<td>-10.34±0.10</td>
</tr>
<tr>
<td>$\Delta H$</td>
<td>-35.10±0.62</td>
<td>-20.98±0.64</td>
</tr>
<tr>
<td>$-T\Delta S$</td>
<td>-10.24±0.56</td>
<td>-10.94±0.69</td>
</tr>
<tr>
<td>$\Delta G$</td>
<td>-24.86±0.59</td>
<td>-10.04±0.66</td>
</tr>
</tbody>
</table>

Methods: Amber16, Amber ff03, 100 ns MD simulation, 50-100 ns trajectory for MM/GBSA calculation Temperate: 2AJF and 6M0J (SARS RBD-ACE2)

- The binding of ACE2 to RBD of SARS-2 is calculated to be stronger than SARS, which is in well agreement with the experimental results.
Spike-ACE2 binding affinity simulated by MD

Δ\(G\) calculated by MM/GBSA with 100 ns MD simulation

<table>
<thead>
<tr>
<th></th>
<th>6ACG</th>
<th>6ACJ</th>
<th>6CS2</th>
<th>6ACK</th>
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</thead>
<tbody>
<tr>
<td>SARS-CoV-2</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(E_{vdw})</td>
<td>-81.34±0.47</td>
<td>-95.90±0.53</td>
<td>-100.86±0.72</td>
<td>-106.25±0.56</td>
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<tr>
<td>(E_{ele})</td>
<td>-816.09±0.59</td>
<td>-796.20±2.59</td>
<td>-763.73±3.59</td>
<td>-763.10±2.86</td>
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<tr>
<td>(E_{gb})</td>
<td>875.10±0.52</td>
<td>866.54±2.59</td>
<td>830.25±3.43</td>
<td>828.67±2.66</td>
</tr>
<tr>
<td>(E_{ap})</td>
<td>-11.14±0.07</td>
<td>-13.98±0.06</td>
<td>-14.61±0.08</td>
<td>-15.21±0.05</td>
</tr>
<tr>
<td>(\Delta H)</td>
<td>-33.47±0.71</td>
<td>-39.55±0.56</td>
<td>-48.95±0.95</td>
<td>-55.89±0.56</td>
</tr>
<tr>
<td>(-T\Delta S)</td>
<td>-15.47±0.98</td>
<td>-14.45±0.81</td>
<td>-16.55±0.78</td>
<td>-16.37±0.66</td>
</tr>
<tr>
<td>(\Delta G)</td>
<td>-18.00±0.84</td>
<td>-25.10±0.68</td>
<td>-32.40±0.86</td>
<td>-39.52±0.61</td>
</tr>
<tr>
<td>SARS-CoV</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E_{vdw})</td>
<td>-74.67±0.60</td>
<td>-84.99±0.54</td>
<td>-81.48±0.69</td>
<td>-86.15±0.47</td>
</tr>
<tr>
<td>(E_{ele})</td>
<td>18.69±0.66</td>
<td>53.59±0.56</td>
<td>109.67±3.05</td>
<td>120.10±3.29</td>
</tr>
<tr>
<td>(E_{gb})</td>
<td>34.91±0.82</td>
<td>122.73±0.68</td>
<td>173.18±3.09</td>
<td>182.77±3.26</td>
</tr>
<tr>
<td>(E_{ap})</td>
<td>-9.72±0.08</td>
<td>-11.10±0.07</td>
<td>-10.42±0.10</td>
<td>-12.04±0.06</td>
</tr>
<tr>
<td>(\Delta H)</td>
<td>-24.79±0.61</td>
<td>-26.96±0.56</td>
<td>-28.39±0.70</td>
<td>-35.52±0.54</td>
</tr>
<tr>
<td>(-T\Delta S)</td>
<td>-14.20±0.62</td>
<td>-14.57±0.80</td>
<td>-16.85±0.74</td>
<td>-14.89±0.67</td>
</tr>
<tr>
<td>(\Delta G)</td>
<td>-10.59±0.62</td>
<td>-12.39±0.68</td>
<td>-11.54±0.72</td>
<td>-20.63±0.60</td>
</tr>
</tbody>
</table>

Methods: Amber16, Amber ff03, 100 ns MD simulation, 50-100 ns trajectory for MM/GBSA calculation

- The calculated \(\Delta G\) of ACE2 to spike of CoV-2 with different models are always stronger than SARS, which are contradictory to experimental results.
- **Different models have different conformation, implying that conformation matters?**
Do the RBD-up and -down conformations matter?

**Accessible and inaccessible conformations for spike binding ACE2**

- **RBD-angle** was defined to be $\angle$D405-V633-V991.
- **ACE2** was docked to the conformations with different RBD-angles.
- **RBD-angle of $\geq 52.2^\circ$** is required for binding **ACE2**, the larger the stronger.
- The experimentally observed weaker SARS2 spike-ACE2 binding can not be interpreted.
- Any other reason affecting the binding of the spike to ACE2? Accessible conformation distribution?
Method for conformation sampling

Method:
• Trimers of the SARS and SARS-2 spikes
• 48 replica for each systems
• 100 ns vsREMD simulation with Gromacs5.1.4

Significantly different conformation distributions

- The accessible conformations of SARS-2 is 5.5% while that of SARS is 22.7%.

- Transition from inaccessible to accessible conformation of SARS-2 has higher barrier (2.6-4.4 kcal/mol) than that of SARS (1.7 kcal/mol).

- Remarkably, the SARS spike has evenly distributed conformation space, while the SARS-2 are mainly located at inaccessible ones.

- Although the SARS-2 spike RBD has stronger binding affinity to ACE2, the SARS-2 spike has much less accessible conformation and higher transition barrier, making the SARS-2 spike difficult to bind ACE2.

- In terms of infectiousness of SARS-2, human being is quite lucky this time.

- Why does the SARS-2 RBD bind to ACE2 stronger?
The origin of the stronger binding affinity of SARS2 RBD-ACE2

In comparison with SARS, some mutation enhanced the spike-ACE2 binding of SARS-2.

Could mutations significantly affect the spike binding mAbs?

• A variant harbored a E484K mutation (B.1.351) was first sequenced on 15/12/2020.
• The variant may have stronger binding to ACE2, indicating potential severe infectiousness.
• The neutralization by some mAbs against the variant was weakened, indicating potential immune evasion risk.

What is the reason of the high infectiousness and diminished neutralization?

Calculated binding affinity of ACE2-RBD harbored E484K mutation

- The E484K mutation of the spike resulted in a strong binding to ACE2 than WT, indicating high infectiousness of the new variant.

- Any changes in binding mAbs?

Methods: Amber18, Amber ff14SB, 4-20 ns MD simulation trajectory for MM/GBSA calculation
Predicted binding affinity of the variant RBD to 25 mAbs

- 22/25 mAbs (88%) showed decreased binding affinity to the E484K mutated RBD;
- Only 7K9Z (4%) showed increased binding affinity;
- Indeed, 3 of them were reported having decreased affinity.
Contribution of the residue E484/K484 to $\Delta G$

- E484 is favorable to binding ACE2 in 21/25 (86%) systems, while K484 is only in 3/25 (12%) (7CWO);
- The negative E484 is attractive to the mAb 7CWO, while the K484 is repulsive to;
- 7 systems have $\Delta G$ weakened by $\geq 5$ kcal/mol, indicating high immune evasion risk.
Effect of E484K mutation on the spike-ACE2 binding

L Wu, W. Zhu, et al., Briefings in Bioinformatics 2022, 23 (1), bbab383,
Other potential mutation with immune evasion risk predicted

- Besides E484, there are additional 10 residues that are important to binding mAbs (>30% mAbs);

- Among them, Y489, Y449, F486, Q493, F456 and N487 are important to binding ACE2, their mutations may result in weaker infectiousness;

- But, the mutations of F490, V483, G485 and S494 might be highly risk to infectiousness and immune evasion.

mAb-Spike interaction:
Infectiousness of Omicron vs Delta

- Omicron variant (B.1.1.529) was first reported to WHO on 24/11/2021;
- No experimental results reported on its transmissibility and immune risk;

Omicron has 15 mutations on RBD, while Delta has 4.
Calculated and Experimentally Determined Binding Affinity

### Binding affinity of RBD-ACE2

**Calculated**
- **MM/GBSA**
  - WT: -33.13 kcal/mol
  - Delta: -42.76 kcal/mol
  - Omicron: -29.43 kcal/mol

**Experimentally**
- **ELISA**
  - WT: $6.01 \times 10^{-5}$
  - Delta: $26.91 \times 10^{-5}$
  - Omicron: $0.37 \times 10^{-5}$

### Binding affinity of RBD-mAbs

- **Etesevimab**: 76.95 kcal/mol
- **Bamlanivimab**: 84.45 kcal/mol
- **Regdanivimab**: 67.78 kcal/mol
- **BD-368-2**: 68.38 kcal/mol
- **Bebtelovimab**: 56.38 kcal/mol

**Remarks**
- **RBD_{Omicron}** possesses much *weaker binding affinity* to ACE2 than **RBD_{Delta}**
- **Omicron variant** has high risk of *immune evasion*

### Energy contributions of RBD residues

- **WT**
- **Delta**
- **Omicron**

Methods: Gromacs2020.2, Amber ff14SB, 50-200 ns MD simulation trajectory for MM/GBSA calculation

L Wu, W Zhu, et al., *Signal Transduction and Targeted Therapy* 2022, 7 (1), 8
1. The RBD of SARS-2 spike has stronger binding affinity to ACE2 than SARS, but the accessible conformation of the SARS-2 spike is significantly less than SARS spike, which should be one of the reasons why SARS-2 spike has weaker binding affinity.

2. E484K mutant has weaker binding affinity to most mAbs due to the weakened electrostatic interaction or the increased electrostatic repulsion, possibly leading to high risk of immune evasion.

3. Omicron RBD has lower binding affinity than Delta RBD to ACE2, but has great potential risk of immune evasion to most mAbs.


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