SARS-CoV-2 Variants Increase Kinetic Stability of Open Spike Conformations as an Evolutionary Strategy

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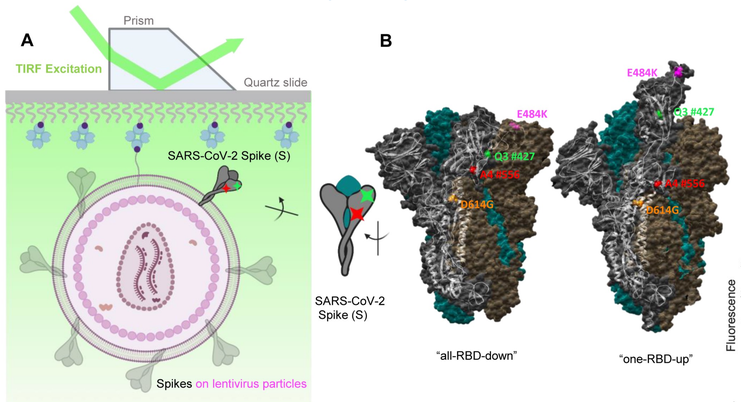
#These authors contributed equally to this work

BACKGROUND

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants of concern (VOCs) harbor mutations in the spike (S) glycoprotein that confer more efficient transmission and dampen the efficacy of COVID-19 vaccines and antibody therapies. S mediates virus entry and is the primary target for antibody responses, with structural studies of soluble S variants revealing an increased propensity toward conformations accessible to the human angiotensin-converting enzyme 2 (hACE2) receptor. However, real-time observations of conformational dynamics that govern the structural equilibriums of the S variants have been lacking. Here, we report single molecule Förster resonance energy transfer (smFRET) studies of critical mutations observed in VOCs, including D614G and E484K, in the context of virus particles.

METHODS

Employment of smFRET to monitor the dynamic and conformational landscape of Spike variants on the virus



Previous Highlights

Lu et al., Cell Host & Microbe (2020)

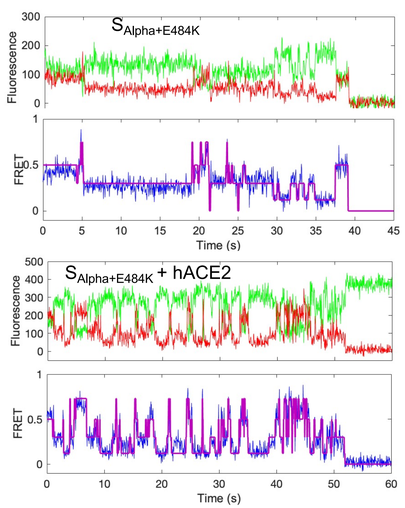
- SARS-CoV-2 S protein dynamically samples at least 4 distinct conformational states
- hACE2 activates S from the ground state to the activated state via an intermediate
- Antibodies can antagonize S by two different mechanisms of neutralization

Early Spike variants show a propensity towards receptor-accessible conformational states with *decelerated transition dynamics* than that of receptor-inaccessible states

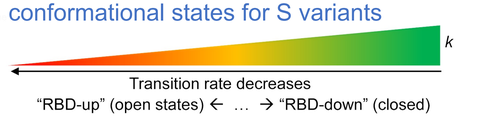
RESULTS

1. We provided a time dimension, using smFRET, to delineate the temporal prevalence of distinct structures of S in the context of virus particles
2. D614G substitution induces a conformational shift of virus-associated Spikes towards receptor-accessible states.
3. E484K carrying S_{Alpha} resides predominately in partially open "one/two-RBD-up" receptor-accessible conformations.
4. Spike variants show a propensity towards "RBD-up" conformations with decelerated transition dynamics than that of "RBD-down."

1 - Real-time conformational dynamics of Spikes on the virus



4 - Rates of transitions between all observed conformational states for S variants



Spike variants on virus particles	$k_{0.11->0.30}$ (s^{-1})	$k_{0.30->0.11}$ (s^{-1})	$k_{0.30->0.50}$ (s^{-1})	$k_{0.50->0.30}$ (s^{-1})	$k_{0.50->0.75}$ (s^{-1})	$k_{0.75->0.50}$ (s^{-1})
S_{D614} Ligand-free	1.98 +/- 0.03	2.99 +/- 0.03	2.84 +/- 0.03	3.18 +/- 0.04	3.09 +/- 0.02	3.68 +/- 0.04
S_{D614} +hACE2	1.28 +/- 0.01	1.96 +/- 0.01	2.02 +/- 0.02	3.41 +/- 0.03	3.26 +/- 0.04	3.12 +/- 0.03
S_{G614} Ligand-free	1.55 +/- 0.02	2.13 +/- 0.02	1.97 +/- 0.02	2.73 +/- 0.02	3.29 +/- 0.03	3.74 +/- 0.03
S_{G614} +hACE2	1.17 +/- 0.01	1.92 +/- 0.03	1.96 +/- 0.02	2.64 +/- 0.03	2.97 +/- 0.02	3.22 +/- 0.03
S_{Alpha} Ligand-free	1.63 +/- 0.02	2.29 +/- 0.04	2.24 +/- 0.03	2.43 +/- 0.02	2.56 +/- 0.02	3.57 +/- 0.03
S_{Alpha} +hACE2	1.24 +/- 0.01	2.25 +/- 0.02	1.96 +/- 0.03	3.15 +/- 0.03	3.41 +/- 0.03	4.05 +/- 0.02
$S_{Alpha+E484K}$ Ligand-free	1.6 +/- 0.01	1.92 +/- 0.02	1.84 +/- 0.02	2.81 +/- 0.02	3.1 +/- 0.03	3.59 +/- 0.04
$S_{Alpha+E484K}$ +hACE2	1.37 +/- 0.01	1.99 +/- 0.02	1.81 +/- 0.02	2.33 +/- 0.02	2.64 +/- 0.02	3.54 +/- 0.03

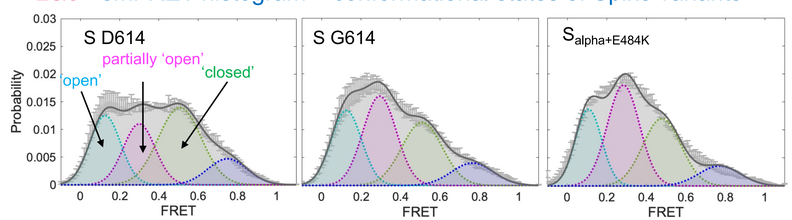
CONCLUSIONS

- D614G- and E484K-carrying S variants predominately occupied more open hACE2-accessible conformations.
- The transition between different conformations is noticeably decelerated when Spikes are more open.
- Higher receptor binding competence and transmission rate may confer a higher fitness advantage for the spread of SARS-CoV-2 than a higher degree of protection against host immunity.
- Our finding of increased S kinetic stability in the open conformation provides a new perspective on SARS-CoV-2 adaptation to the human population.

ADDITIONAL KEY INFORMATION

- This work is currently in press (mBio, 2022)
- Address correspondence to: Maolin Lu (maolin.lu@uthct.edu)
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2&3 - smFRET histogram – conformational states of Spike variants



2&3 - Quantification of state-occupancy of Spike variants

