ANALYSIS OF THE SARS-COV-2 SPIKE PROTEIN TAGGED WITH DYE, CY3 AND CY5, ATTACHMENT UNDER SMFRET ENVIRONMENT

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INTRODUCTION

- Since the rise of the COVID-19 pandemic, researchers have pushed for development of efficient therapeutics and vaccines to assist in preventing the severity of its viral effects. A major target has been the trimeric SARS-CoV-2 spike protein (S).
- There have been various experimental and computational efforts to characterise the structural dynamics of this protein at different stages of its mechanisms. Among these methods are single molecule fluorescence resonance energy transfer (smFRET) experiments as well as molecular dynamics (MD) simulations and, more effectively, in combination.

- The purpose of this work is to combine the existing experimental data involving smFRET experiments and MD simulations to provide a more complete picture of prefusion SARS-CoV-2 S conformational dynamics. We have particularly examined the conformational dynamics of several variants of concern (VOCs) such as the wild-type, the alpha, beta, delta, gamma and epsilon.

- Specifically, we have examined multiple computational methods based on "implicit screening" approach within different approximations to predict dye-dye distance and FRET efficiency distributions to provide reliable interpretations for both smFRET experimental data and MD simulation data.
- Initial distance analysis was inducted onto the alpha carbon at residue position 427 and 556 for each protomer (A, B and C) for each trimeric VOC and wild-type S.

- To compare analysis and incorporate smFRET, photostable dyes CY3B(S) (donor) and CY5(S) (acceptor) were implicitly attached at the same residue position, 427 and 556, respectively, and in replacement of the α-C.
- This work sheds light on the structural heterogeneity of the spike protein in different variants of SARS-CoV-2.

Figure 1

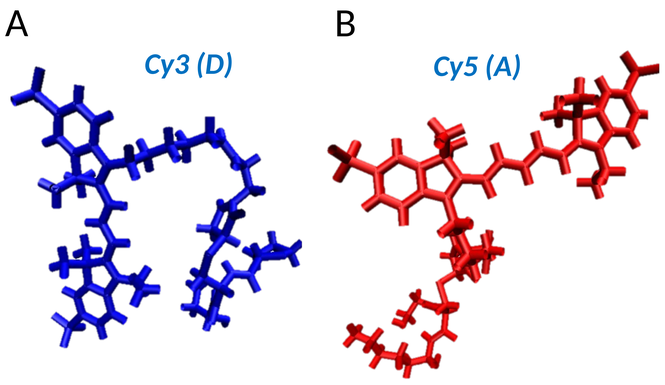


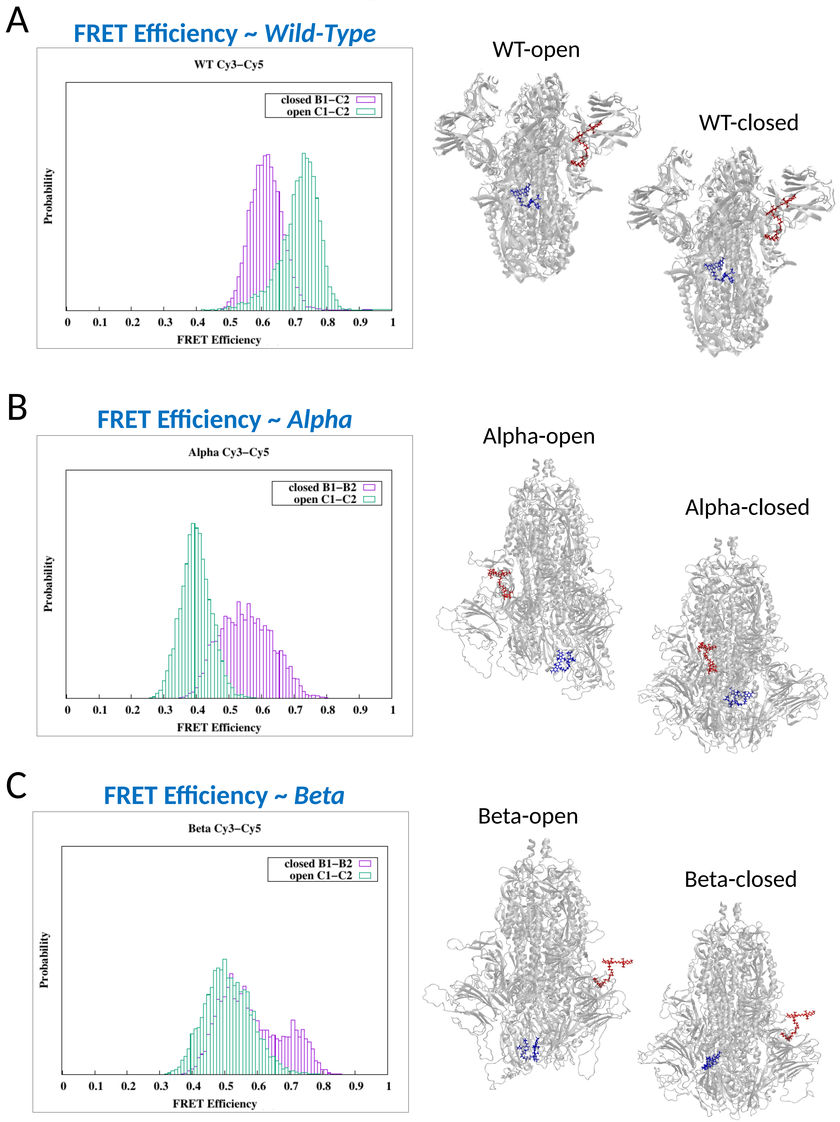
Fig 1. The dyes that were used in this study. A) Cy3 (shown in blue) was placed on each system at residue position 427. B) Cy5 (shown in red) was placed on each system at residue position 556.

METHODS

Previously simulated MD trajectories were used to perform effective and accurate distance analysis of the wild-type and VOCs for both their open and closed conformations. Each simulated structure consists of approximately 51,000 atoms. Visualization Molecular Dynamics or VMD, software was used for all visual aspects as well as the distance analysis to allow frame by frame calculations between the implicitly applied CY3 and CY5 fluorescent dyes at residue positions 427 and 556. To obtain a FRET Efficiency, the calculated distances between the donor dye (cy3) and acceptor dye (cy5) along each position during the trajectory was converted using the appropriate efficiency formula (efficiency = / ()) that takes into account the distance between the dyes (R) and the distance at which 50% FRET efficiency (R_0) should, theoretically, occur (based on variables of the donor dye). Once these values were calculated for each frame, it was appropriate to convert the FRET efficiency into probability to acquire a histogram representation of the probability of FRET efficiency for each structure.

RESULTS

Figure 2



RESULTS CONTINUED

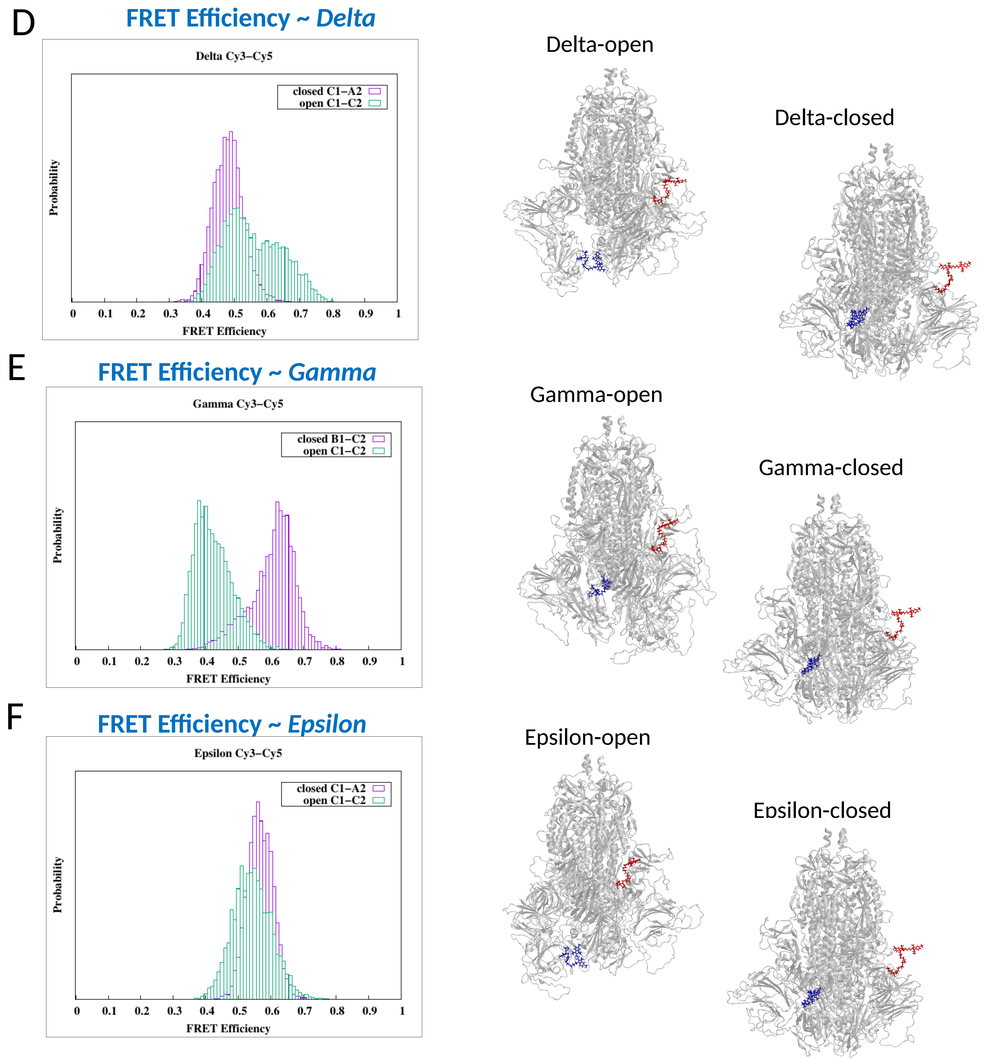


Fig 2. A-F) Each plot represents the highest FRET Efficiency distribution for the given system analyzed. To the right of each figure is the cartoon representation of the protein with dyes attached. Each plot contains the FRET Efficiency of closed and open structure of SARS-CoV2 S for the respective protomer combination, mentioned in legend.

CONCLUSION

It can be noted that the FRET efficiency of the open and closed conformation from the wild-type S with the implicitly applied cy3 and cy5 dye differ by nearly 0.2 with similar probability. The FRET efficiency between dyes when both are on protomer C (C1-C2) of the spike, was most common for having the greatest potential for energy transfer under smFRET conditions. This work has yet to be concluded, but the hope is that results will shed light on the structural heterogeneity of the spike protein in different variants of SARS-CoV-2.

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