

Research Article

Comparative Effects of ALA and EPA on the Conformational Stability of Wild-Type and Mutant SARS-CoV-2 Spike-ACE2

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ABSTRACT

The evolution of SARS-CoV-2 variants through mutations in the Spike (S) protein in the receptor-binding domain (RBD) significantly influences its binding affinity to human Angiotensin-Converting Enzyme 2 (ACE2). While polyunsaturated fatty acids (PUFAs), specifically alpha-linolenic acid (ALA) and eicosapentaenoic acid (EPA), are recognised for their anti-inflammatory properties, their molecular impact on the structural stability and mechanics of the S-ACE2 complex is less understood. This study utilised 100 ns molecular dynamics (MD) simulations to evaluate the modulatory effects of ALA and EPA on both wild-type and mutant S-ACE2 complexes in the presence of the 13 most frequently occurring S mutations in Malaysian patients from August 2023 to January 2024. Analyses of Root Mean Square Deviation (RMSD) and Radius of Gyration (Rg) indicated that the mutant S-ACE2 naturally adopts a more compact yet structurally divergent conformation compared to the wild-type. PUFA binding alters these dynamics; notably, EPA stabilised the mutant S-ACE2 into a conformation resembling the wild-type and markedly decreased per-residue fluctuations (RMSF) in critical interface regions (residues 474–486). Principal Component Analysis (PCA) confirmed that EPA binding restricts the conformational space of both mutant and wild-type S-ACE2. Snapshots at different time points show that the longer carbon chain of EPA binds R408S in mutated S-ACE2, underlying the stable conformation of the complex, whereas ALA, with a shorter carbon chain, does not do so. These findings suggest that ALA and EPA function as structural modulators of the S-ACE2 binding affinities, potentially interfering with the conformational stability required for optimal viral-host interaction. This research provides a molecular basis for the variable impacts of dietary PUFAs to act as structural inhibitors against evolving SARS-CoV-2 variants by disrupting the binding mechanics of the S-protein.

Key words: Alpha-linolenic acid, COVID-19, eicosapentaenoic acid, polyunsaturated fatty acids, spike protein, single nucleotide polymorphisms

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the global COVID-19 pandemic, which presents with a wide range of respiratory and systemic symptoms (Baj *et al.*, 2020). The primary mechanism of viral pathogenesis involves the binding of the viral Spike (S) glycoprotein to the human angiotensin-converting enzyme 2 (ACE2), thereby facilitating viral entry into host cells (Kumar *et al.*, 2023). This interaction represents the initial step in the infection process. The evolutionary trajectory of SARS-CoV-2 has been shaped by ongoing point mutations within the S protein, particularly in its receptor-binding motifs (RBM) of the receptor-binding domain (RBD). These structural changes ultimately induce significant alterations

in ACE2 binding affinities, affecting host-pathogen interaction and viral infectivity. Changes in S-ACE2 interaction in the long run induce changes in prophylactic and therapeutic strategies (Kumar *et al.*, 2023; McVoy & Kummarapurugu, 2025). The continuous emergence of SARS-CoV-2 variants has reduced the effectiveness of vaccine-induced neutralising antibodies, resulting in breakthrough infections (Harvey *et al.*, 2021). This has prompted the search for alternative therapeutic strategies that are less susceptible to viral mutations. One such strategy involves targeting host factors that are required for viral entry rather than the virus itself (Kaufmann *et al.*, 2018). Among these, dietary lipids have gained increasing attention due to their ability to modulate host membrane properties

and influence the structural conformation of viral proteins (Toelzer *et al.*, 2020), thereby potentially reducing viral entry into host cells.

In addition to the development of targeted vaccines and antiviral therapeutics, there has been a growing emphasis on the modulatory role of host nutrition, particularly on lipid metabolism in the pathogenesis of COVID-19. Following cellular entry, the virus remodels intracellular membranes to form double-membrane replication organelles and promotes lipid droplet accumulation, providing a favourable environment for viral replication and assembly (D'Avila *et al.*, 2024). These metabolic alterations further contribute to cellular dysfunction and excessive inflammatory responses during infection (Caterino *et al.*, 2021). Polyunsaturated fatty acids (PUFAs), particularly the essential omega-3 fatty acids alpha-linolenic acid (ALA) and its 20-carbon fatty acid derivative, eicosapentaenoic acid (EPA), are widely recognised for their potent anti-inflammatory properties. Mechanistically, EPA competes with arachidonic acid (AA) for incorporation into membrane phospholipids and as a substrate for cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, thereby reducing the production of pro-inflammatory lipid mediators (Das, 2020). EPA metabolism further promotes the formation of less inflammatory 3-series prostaglandins and 5-series leukotrienes, and it also serves as a precursor for specialised pro-resolving mediators, including resolvins and protectins, which suppress NF- κ B activation and reduce the production of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF- α) (Calder, 2013; Hathaway *et al.*, 2020). As ALA is the metabolic precursor of EPA, it may exert similar anti-inflammatory effects following its conversion to longer-chain omega-3 fatty acids. By modulating eicosanoid production, these PUFAs are hypothesised to mitigate the hyperinflammatory responses ("cytokine storms") commonly associated with severe COVID-19 outcomes (Baral *et al.*, 2022).

In addition, recent *in silico* and *in vitro* studies suggest that EPA and ALA possess direct, receptor-level antiviral properties against SARS-CoV-2. A previous study has shown that EPA and ALA inhibit the interaction between the SARS-CoV-2 Spike protein and the human ACE2 receptor in an *in vitro* target-based assay, reducing S-ACE2 binding by 67.5% and 54.3%, respectively (Goc *et al.*, 2021). Molecular docking models have demonstrated that these specific omega-3 PUFAs bind directly to the fatty acid binding pocket (FABP) within the RBD of the S protein, stabilising it in a "closed" inactive conformation and thereby preventing its attachment to host cells (Vivar-Sierra *et al.*, 2021). Furthermore, emerging evidence suggests that dietary supplementation with long-chain omega-3 PUFAs, specifically EPA, may reduce viral infectivity by downregulating both the absolute protein expression and the glycosylation of ACE2 in host tissues. An *in vitro* study by Huang *et al.* (2022) demonstrated that treatment of HEK293 cells with 80 μ M and 125 μ M DHA reduced both the non-glycosylated (p100) and glycosylated (p130) forms of ACE2. The reduction in ACE2 protein levels was accompanied by a significant decrease in SARS-CoV-2 pseudovirus entry, suggesting that DHA may reduce cellular susceptibility to SARS-CoV-2 infection by lowering ACE2 availability and glycosylation.

Consequently, exploring the dual action of EPA and ALA, both as modulators of host ACE2 expression and as direct structural disruptors of the S protein-ACE2 interaction, presents a promising and accessible avenue for mitigating viral entry and understanding host-viral lipid dynamics in the ongoing management of SARS-CoV-2. Therefore, this study employed an *in silico* approach to investigate the effects of EPA and ALA on the conformational stability and binding behaviour of the S-ACE2 complex through

computational approaches. Furthermore, the rapid mutations targeting the S-proteins are of interest to determine whether these mutational changes might have any effects in the presence of these two PUFAs. To extend the relevance of this study to evolving SARS-CoV-2 variants, the effects of EPA and ALA were also investigated in the mutated S-ACE2 complex to evaluate how the structural implications of both PUFAs may also be modulated by spike mutations.

MATERIALS AND METHODS

Structural selection and model preparation

The wild-type (ancestral Wuhan-Hu-1) initial structure of the SARS-CoV-2 S protein in complex with the human Angiotensin-Converting Enzyme 2 (ACE2) (herewith referred to as S-ACE2) at 3.60 Å resolution was retrieved from the Protein Data Bank (PDB ID: 7KJ2; engineered with stabilising K986P/V987P & GSAS furin-site mutations). S-ACE2 used in this project involved one protomer bound to ACE-2 in an 'up' conformation with the remaining two protomers in 'down' conformation (Xiao *et al.*, 2021). System preparation involved the removal of water molecules and heteroatoms, followed by residue renumbering and chain renaming using the PyMOL command-line interface to ensure consistency across trimeric protomers.

To model the mutated S protein, 13 of the most frequent S mutations identified from a dataset of 2,981 Malaysian patient sequences dated from August 2023 to January 2024 were extracted from the global GISAID database as tabulated in Table 1 (Khare *et al.*, 2021). These mutations were then simultaneously modelled onto the baseline S-ACE2 structure using the Mutagenesis Wizard tool in PyMOL. Although the 13 spike mutations do not necessarily coexist within a single viral isolate, these mutations are characteristic of circulating Omicron-descendant lineages, hence representing regional evolutionary pressure.

Molecular docking of dietary lipids and model preparation

The dietary PUFAs, EPA and ALA (PubChem ID: 446284 and 5280934, respectively), were prepared for docking using the Dock Prep tool in UCSF Chimera (Pettersen *et al.*, 2004). These ligands were docked into the FABP identified within each protomer of the S trimer. Docking simulations and scoring were performed using AutoDock Vina (version 1.2.5) (Morris *et al.*, 2009), with the resulting complexes selected based on binding affinity scores and the formation of key stabilising interactions.

Molecular dynamics simulation protocol

All atomistic molecular dynamics (MD) simulations were performed using the GROMACS (version 2023.1) (Pronk *et al.*, 2013) software package. The systems were parameterised using the CHARMM36m force field. Each complex was solvated in a dodecahedron box and neutralised with the addition of counterions. Energy minimisation was performed to remove steric clashes, followed by equilibration in the NVT ensemble, where the temperature was maintained at 310 K using a Berendsen thermostat. Production MD runs were conducted for 100 ns for each of the 6 complex models to evaluate the dynamic stability of the S-ACE2 interface under different genetic and nutritional conditions.

Trajectory analysis and binding free energy estimation

Post-simulation analysis was performed using GROMACS utilities and Visual Molecular Dynamics (VMD) (Humphrey *et al.*, 1996). Structural stability and flexibility were quantified through Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and Radius of Gyration (Rg). Principal Component Analysis (PCA) was employed to characterise large-scale conformational shifts and essential dynamics of the complex. To quantify the thermodynamic impact of SNPs and PUFAs on viral attachment, binding free energies were calculated using the Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) method. This analysis included contributions from Van der

Waals, electrostatic, and polar solvation energies to provide a comprehensive profile of the interaction affinity.

RESULTS AND DISCUSSION

Global stability and compactness

Previous studies have established the potential of PUFAs to inhibit SARS-CoV-2 entry (Vivar-Sierra *et al.*, 2021). They demonstrated through an *in vitro* study that ALA and EPA directly bind to the SARS-CoV-2 RBD, blocking its attachment to the ACE2 receptor by altering host protease activity like cathepsin L and TMPRSS2. While their work relied on *in vitro* assays against the wild-type virus, the present study utilised an *in silico* approach to evaluate the interactions of EPA and ALA against both the wild-type and mutated S-ACE2 complexes. To evaluate the structural interactions between PUFAs and the viral entry mechanism, EPA and ALA were docked into the S-ACE2 wild-type (WT) complex interface (Figure 1). Docking simulations successfully localised the PUFAs FABP defined by five Phenylalanine residues (Phe338, Phe342, Phe377, and Phe392) as previously described by Toelzer *et al.* (2020) and Vivar-Sierra (2021), providing a baseline for comparing the binding affinities of the wild-type complex against subsequent mutant models.

To elucidate the real-time structural transitions of the S-ACE2 complex upon PUFA binding, molecular dynamics (MD) simulations were conducted to monitor the root-mean-square deviation (RMSD) and radius of gyration (Rg) of the wild-type (WT) complex bound to eicosapentaenoic acid (EPA) and alpha-linolenic acid (ALA) (Figure 2). Over the 100 ns trajectory, both ligands exhibited minimal impact on the global stability of the WT S-ACE2 complex, maintaining a consistent fold.

Given that specific S mutations are associated with enhanced stability and heightened infectivity, we sought to determine if these regional Malaysian variants exhibited distinct *in silico* signatures. Rg analysis of the mutated S-ACE2 complex revealed a structural shift characterised by increased compactness. This was evidenced by a significant reduction in the mean Rg from 6.25 nm in the WT to 5.88 nm in the mutant system (Figure 3). While the mutated complex reached a stable RMSD plateau approximately 30 ns post-simulation—paralleling the WT—it exhibited higher overall deviation. This suggests that while mutations rigidify the core complex, distal

regions, such as the flexible C-terminal tails of the S protomers, continue to contribute to significant conformational fluctuations.

The integration of PUFAs into the mutated system introduced divergent stability profiles (Figure 4). Unlike the WT and apo-mutant systems, the ALA-bound mutant complex exhibited a transient RMSD spike between 45–80 ns before slowly lowering in fluctuations. Conversely, the EPA-mutant system showed a gradual, incremental rise in RMSD through the end of the 100 ns run, suggesting a prolonged adaptive phase. For both systems, longer simulation timescales are warranted to confirm the final equilibrated states.

Notably, the Rg for the EPA-mutant complex remained the most stable among all mutated systems, closely resembling the WT profile despite the underlying mutations. In contrast, the ALA-mutant complex displayed marked Rg fluctuations, indicating a loss of structural cohesion. These observations suggest that while EPA maintains global compactness in the presence of mutations, ALA fails to provide similar stabilisation.

Thermodynamic Reversal (MMPBSA)

To evaluate how the regional mutations change the thermodynamic stability between the spike protein and ACE2, we employed Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) calculations. Consistent with the observed structural compactness, the mutated S-ACE2 complex exhibited a heightened interfacial affinity compared to the WT, with total binding free energies shifting from −41.28 kcal/mol to −43.11 kcal/mol (Table 2). This corroborates previous findings that the specific Malaysian mutational profile enhances the pre-fusion stability of the complex (Li & Buck, 2022; Abdalla *et al.*, 2022). However, the introduction of PUFAs into the mutated environment revealed a distinct “reversal effect” in lipid binding preferences. While EPA demonstrated a significant enhancement in binding affinity within the mutated system (−40.71 kcal/mol) compared to the WT (−30.44 kcal/mol), the affinity for ALA was markedly compromised, rising from −45.76 kcal/mol to −35.25 kcal/mol. This divergent thermodynamic response suggests that the mutational landscape selectively optimises the fatty acid binding pocket (FABP) for the two different PUFAs.

To elucidate the residue-level dynamics underlying the reported thermodynamic reversal, we performed root-mean-

Table 1. Top 13 spike protein mutations detected in SARS-CoV-2 sequences from Malaysia (August 2023-January 2024). Frequency denotes the number of sequences carrying the mutation (n = 2981); percentage frequency indicates its proportion in the dataset.

Spike Mutations	Frequency	Frequency (%)
D614G	1967	65.99
S373P	1937	64.99
K417N	1935	64.92
D405N	1935	64.92
T376A	1932	64.82
R408S	1932	64.82
S375F	1931	64.79
G142D	1929	64.72
Y505H	1927	64.65
N501Y	1924	64.55
S477N	1921	64.45
N460K	1914	64.22
G446S	1913	64.19

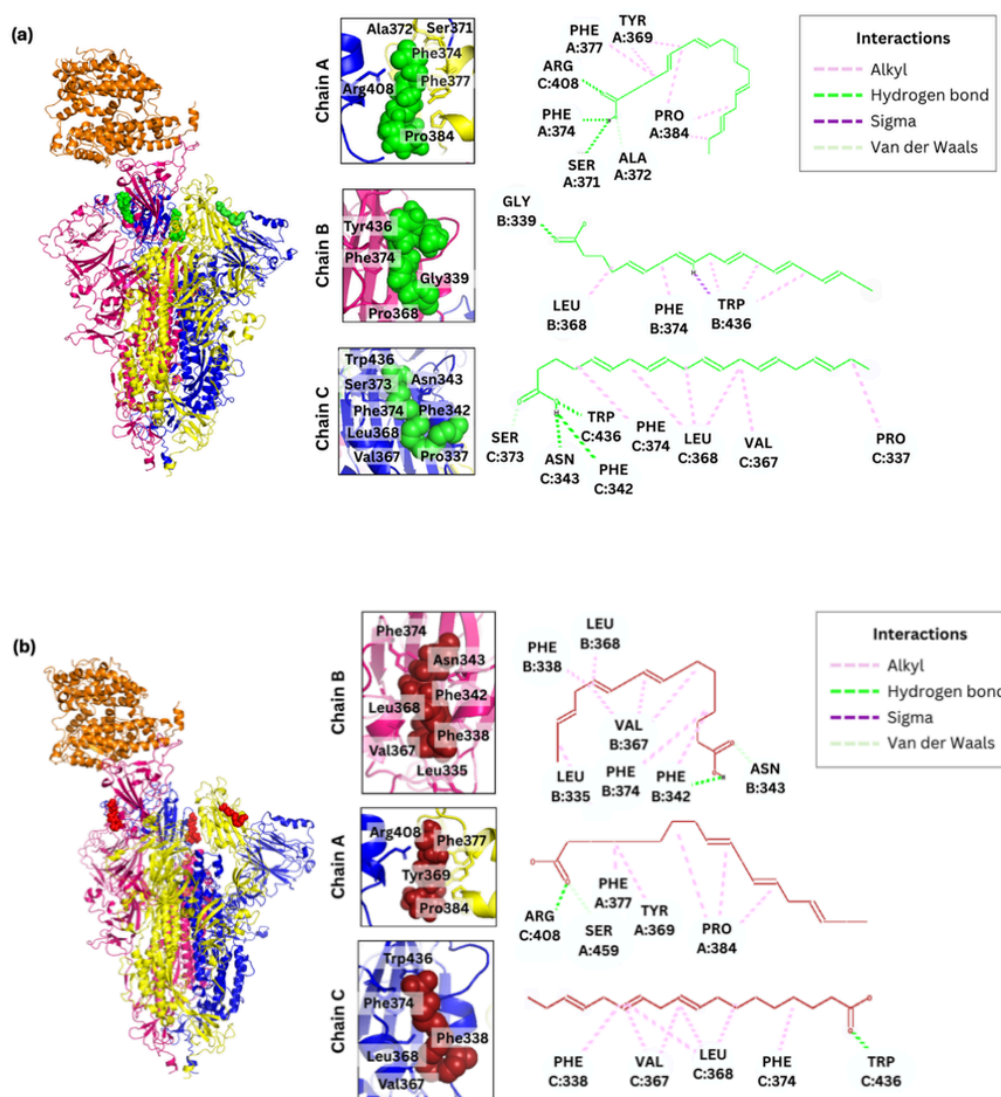


Fig. 1. S-ACE2 complex with EPA (a) and ALA (b) at the three binding sites. Chain B (pink) in 'up' conformation and interacts with ACE2. Chain A and C (blue) in the 'down' conformation. All chains are bound to a single EPA (green sphere)/ALA (red sphere) molecule, respectively. A zoomed-in view of EPA and ALA docked into each chain shows interacting residues (left) and their 2D interactions (right).

square fluctuation (RMSF) analysis (Figure 5) across the complex. Localising our analysis to the S receptor-binding motif (RBM; residues 430–510), we sought to quantify localised volatility changes within this critical attachment interface. RMSF showed that ALA-mutant S-ACE2 mirrored the fluctuation profile of the mutant S-ACE2, exhibiting a significant volatility peak between residues 474–488. This peak denotes a high degree of structural flexibility, suggesting that ALA fails to stabilise the mutation-induced fluctuations in the RBM region. In contrast, the EPA-mutant complex exhibited a suppressed, plateaued profile throughout this interface, effectively dampening the conformational fluctuations. This localised rigidification provides a structural rationale for the favourable binding affinities observed in the MMPBSA calculations, confirming that EPA—unlike ALA—effectively “locks” the RBM interface despite the presence of regional mutations (Table 2).

RBM interface locking

To evaluate whether the structural stabilisation provided by S mutations is maintained upon PUFA binding, we characterised the essential dynamics of the complex through Principal Component Analysis (PCA). We focused on the ALA-bound systems to

determine if this lipid could enforce a conserved dynamic signature across different genetic backgrounds. PCA revealed divergent and variable conformational space occupancies for the ALA complexes in both WT and mutated states (Figure 6). This lack of a consolidated cluster indicates that the structural trajectory of the ALA-bound complex remains highly sensitive to the mutations in S, failing to impose a uniform ‘locked’ state. This dynamic variance of trajectories is a stark contrast to the EPA-bound systems (Figure 6).

While the ALA-bound complexes showed high conformational sensitivity to mutational pressures, the EPA-bound systems demonstrated constant and stable trajectories. PCA revealed that both WT and mutated EPA complexes occupy a consolidated dynamics space, indicating that EPA enforces a conserved structural state regardless of the genetic background (Figure 7). This global stabilisation is directly supported by our localised fluctuation data (Figure 5); the EPA-bound systems effectively suppressed the volatility peaks at the RBM interface (residues 474–488) that were otherwise prominent in the mutated S-ACE2 and ALA complexes. By maintaining a uniform ‘locked’ conformation across both genotypes, EPA demonstrates superior allosteric resilience,

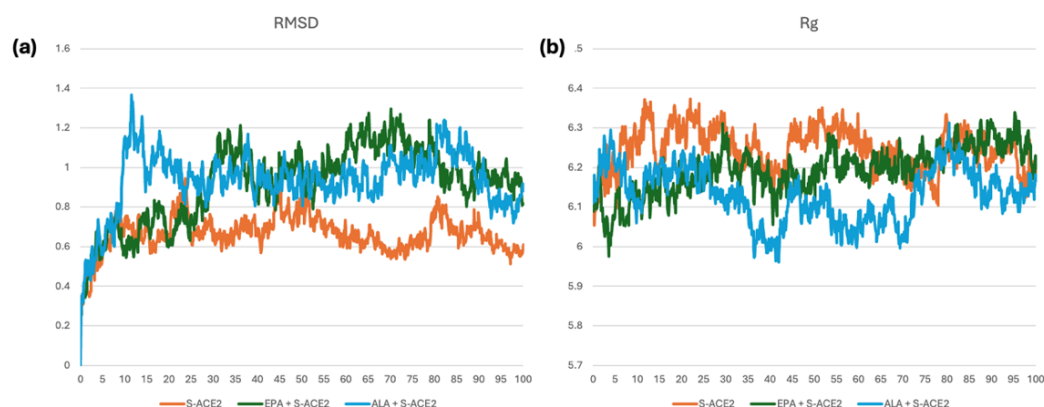


Fig. 2. RMSD (a) and Rg (b) for three systems: S-ACE2 (orange), EPA + S-ACE2 (green), and ALA + S-ACE2 (blue). Both plots are presented against simulation time on the x-axis, ranging from 0 to 100 ns.

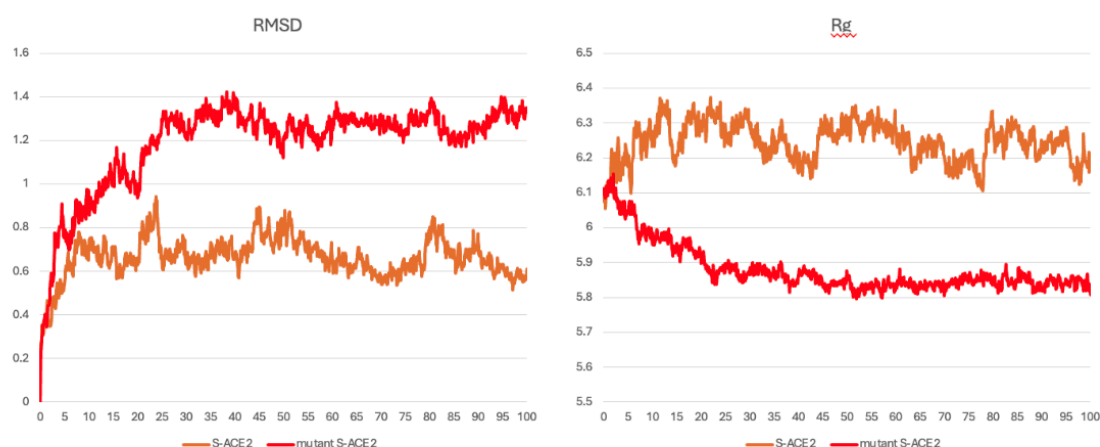


Fig. 3. RMSD (a) and Rg (b) of wild-type S-ACE2 (orange) and mutant S-ACE2 (red) over 100 ns. RMSD plot indicates greater structural deviation from the initial state for the mutant protein compared to the WT. (b) Rg plot showing the mutant S-ACE2 achieves a more compact and stable configuration (5.8 nm) relative to the higher and more fluctuating values of the WT.

suggesting the neutralisation of the structural instability induced by regional Malaysian variants.

The divergent dynamics observed in our study can be traced to the structural reconfiguration of the Fatty Acid Binding Pocket (FABP) induced by the R408S mutation. In the wild-type spike protein, Arg408 forms electrostatic interactions with the carboxylate head group of the bound fatty acid, as illustrated by the hydrogen-bonding interaction in Figure 1 (Chain A), contributing to stabilisation of the locked spike conformation (Toelzer *et al.*, 2020). Substitution of Arg408 with the neutral Ser408 removes this positively charged interaction, which may reduce the ability of PUFAs to stabilise the locked conformation through the FABP, thereby altering the structural dynamics of the S-ACE2 complex. Time-resolved structural snapshots at 0, 50, and 100 ns (Figure 8) demonstrate that the R408S substitution disrupts the critical electrostatic anchor—the “cationic bridge”—required for the stabilisation of the PUFA headgroup. In the ALA-bound complex,

the loss of this positive charge leads to increased ligand mobility and a subsequent loss of allosteric tension, and the distancing of ALA from 50 to 100 ns. Conversely, EPA’s extended 20-carbon eicosanoid chain allows for deeper penetration into the hydrophobic core of the FABP. This facilitates a compensatory hydrophobic fit (U-shaped conformational change) that anchors the ligand independently of the R408 site, effectively maintaining allosteric control over the ACE2 interface. These findings suggest that while regional mutations may compromise the efficacy of shorter-chain lipids like ALA, EPA’s unique structural geometry provides a resilient “molecular lock” against the evolutionary pressures found in Malaysian SARS-CoV-2 variants.

Structurally, the difference in stabilities and binding affinities of S-ACE2 in EPA and ALA is a basis for the broader antiviral properties of omega-3 PUFAs previously reported in this work. While previous studies suggest that EPA and ALA competitively interfere with the S-ACE2 interface by stabilising the S in an inactive state (Vivar-

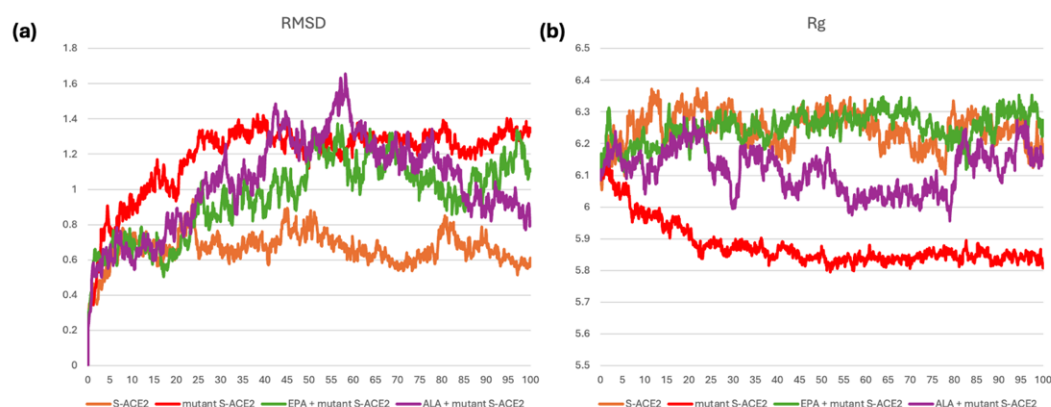


Fig. 4. RMSD (a) and Rg (b) of WT S-ACE2 (orange), mutant S-ACE2 (red), EPA + mutant S-ACE2 (green), and ALA + mutant S-ACE2 (purple) over 100 ns. (a) RMSD plot, showing the effect of ligand binding on the mutant protein's structural deviation compared to both the wild-type and the unbound mutant, with ALA binding resulting in the highest peak deviation. (b) Rg plot, demonstrating how EPA (green) stabilises the mutant S-ACE2 in a less compact, more 'open' conformation, similar to the WT, while ALA (purple) and the unbound mutant (red) achieve more compact and stable, 'closed' configurations (5.8-6.1 nm).

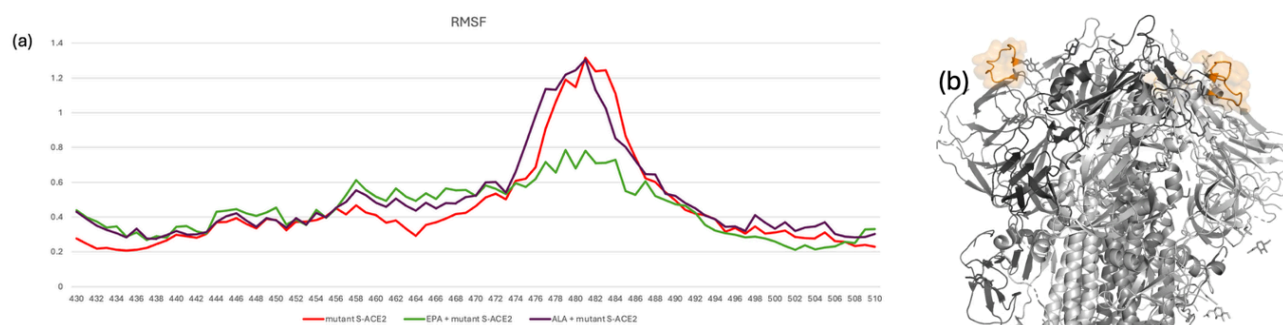


Fig. 5. Flexibility regions in S-ACE2 cause different binding stabilities to EPA and ALA. (a) RMSF of the mutant S-ACE2. The plot compares the per-residue flexibility of mutant S-ACE2 (red), EPA + mutant S-ACE2 (green) and ALA + mutant S-ACE2 (purple). (b) 3D structure of S-ACE2 modelled using PyMOL. Flexible region 474-488 (orange) highlighted with a semi-opaque orange shadow. Binding of EPA is shown to significantly reduce the flexibility of key residue regions, particularly between residues 474 and 486, compared to the unbound mutant and the ALA-bound complex, indicating a potential stabilising effect on specific protein loops.

Sierra *et al.*, 2021), our work shows that the efficacy of this interaction is contingent upon the structural aspect of the PUFA binding to the FABP. Evidently, the 'molecular lock' provided by the longer chain of EPA offers a better therapeutic and/or preventive measure against SARS-CoV-2 variants to reduce lipid-mediated stabilisation of S-ACE2.

Limitations

A structural limitation of this study is that our mutated spike model incorporated a combination of the 13 most prevalent spike mutations, rather than a single, naturally occurring viral sequence. While these 13 spike mutations heavily overlap with dominant circulating Omicron lineages during the study period, combining

Table 2. Comparative binding free energies of EPA and ALA across WT and Mutated systems.

System	MMPBSA (kcal/mol)
S-ACE2 (WT)	-41.28
Mutant S-ACE2	-43.11
EPA + S-ACE2	-30.44
EPA + Mutant S-ACE2	-40.71
ALA + S-ACE2	-45.76
ALA + Mutant S-ACE2	-35.25

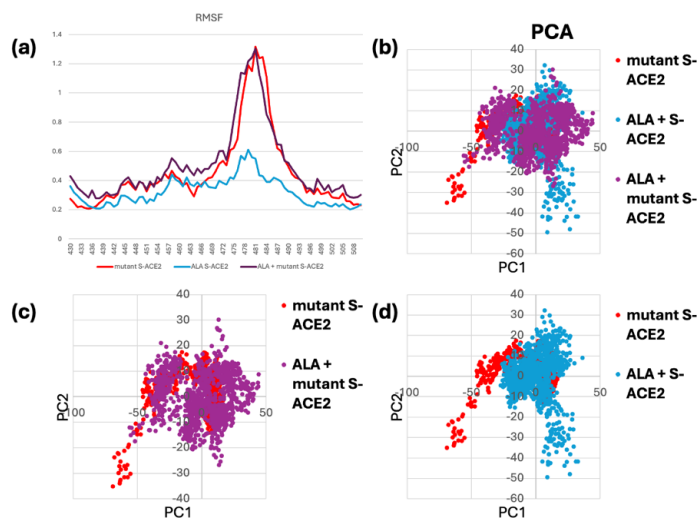


Fig. 6. RMSF (a) per-residue plot of mutant S-ACE2 (red), ALA + mutant S-ACE2 (cyan), and ALA + S-ACE2 (purple). (b-d) PCA projection onto the first two principal components (PC1 vs. PC2), comparing the explored conformational space. (b) All three systems plotted together, showing the overlapping space. (c) Comparison between mutant S-ACE2 (red) and ALA + mutant S-ACE2 (cyan). (d) Comparison between mutant S-ACE2 (red) and ALA + S-ACE2 (purple).

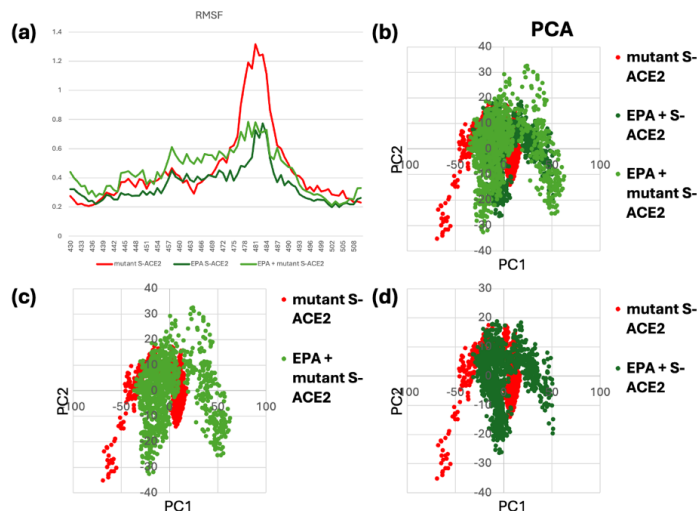


Fig. 7. RMSF (a) per-residue plot of mutant S-ACE2 (red), EPA + mutant S-ACE2 (light green), and EPA + S-ACE2 (dark green). (b-d) PCA projection onto the first two principal components (PC1 vs. PC2), comparing the explored conformational space. (b) All three systems plotted together, showing the overlapping space. (c) Comparison between mutant S-ACE2 (red) and EPA + mutant S-ACE2 (light green). (d) Comparison between mutant S-ACE2 (red) and EPA + S-ACE2 (dark green). Across all PCA plots, EPA binding is seen to constrain the mutant S-ACE2 dynamics.

them into a single rigid model lacks the natural evolutionary tuning and compensatory mutations often found in native viral strains (Frutos *et al.*, 2022). In nature, co-evolving residues frequently emerge to balance structural stability and maintain optimal viral fitness. Consequently, the deviations and altered binding behaviours observed in our molecular dynamics trajectories may reflect the cumulative threshold impact of these spike mutations

in an artificial arrangement, rather than the biologically optimized behavior of a specific circulating variant.

This study is further limited by simulation time and computational demand. Molecular dynamics simulations are highly dependent on the size of the biomolecular system, with larger complexes requiring greater computational power and longer processing time. The S-ACE2 complex used in this study is relatively large, with an approximate molecular weight of 496 kDa, and each full

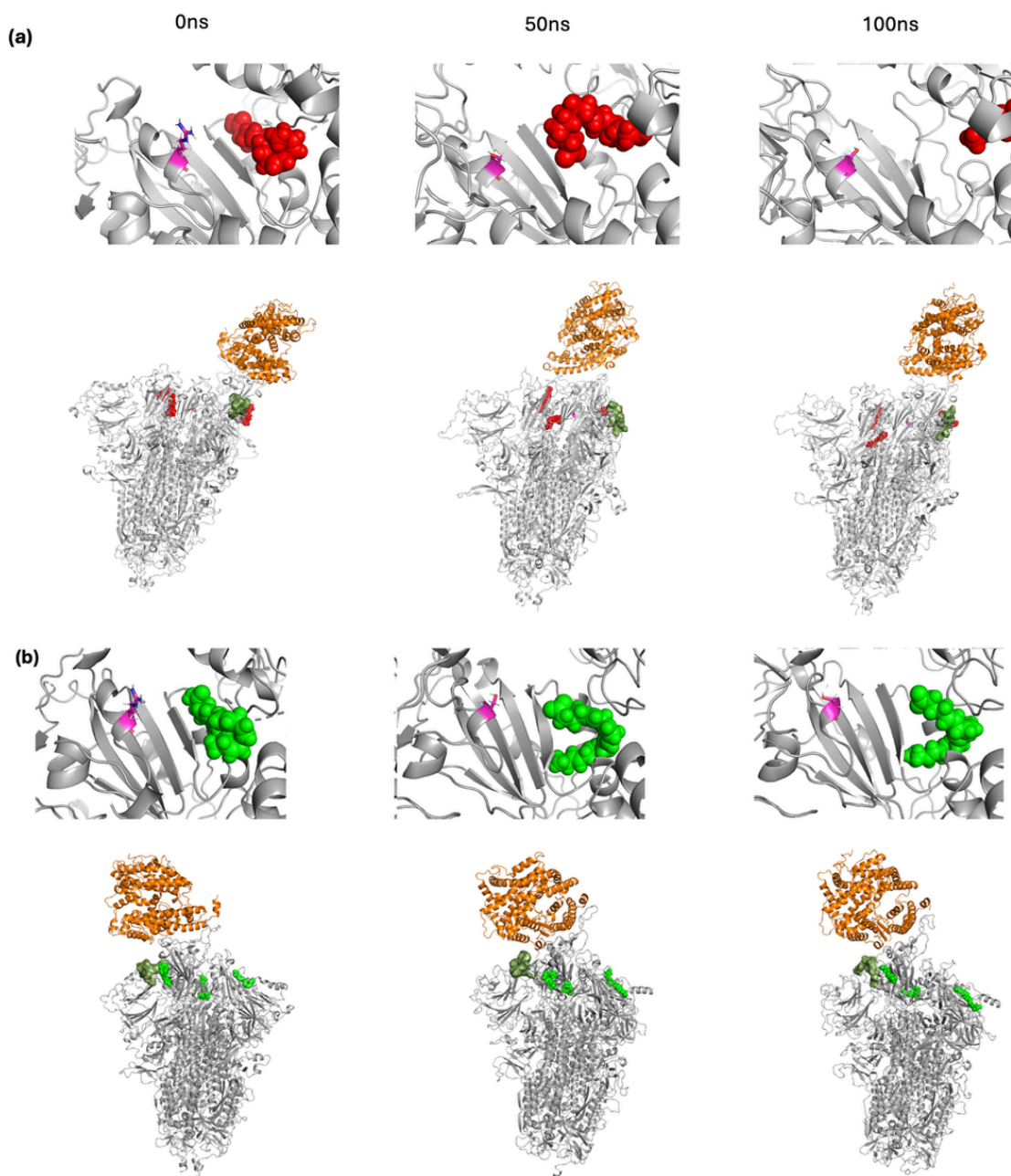


Fig. 8. Time-lapse snapshots of (a) ALA (red spheres) and (b) EPA (green spheres) within the mutant S-ACE2 complex (gray/orange) at 0 ns, 50 ns, and 100 ns. Top panels provide a zoomed-in view of the ligand-binding pocket, highlighting the relative positioning of the ligands near R408S (pink stick). Bottom panels illustrate the global orientation of the ligands within the full spike protein-ACE2 interface. The series demonstrates the progressive conformational adjustments and sustained occupancy of both ALA and EPA within their respective binding sites over the course of the 100 ns simulation.

100 ns simulation required a relatively long time to complete. Due to these constraints, the high computational time and cost prevented the use of simulation replicates, which could have strengthened the robustness of the findings, although replicates are not a strict requirement in MD studies. Additionally, due to time constraints, longer simulation time was not feasible. Nevertheless, MD simulations typically run from 10 ns to hundreds of ns, therefore 100 ns is widely used to capture meaningful motions.

Lastly, *in silico* studies on their own may not translate into clinical outcomes or actual COVID-19 susceptibility. This is because MD captures molecular behaviour in a controlled computational environment, whereas real COVID-19 outcomes are influenced by broader physiological factors. Consequently, results from *in silico* studies should be treated as structural and descriptive trends rather

than predictors of clinical risk. This study requires experimental validation such as biochemical binding assays, pseudovirus entry assays or cell-based infectivity models, and clinical data to further validate the findings and help bridge the gap between computational findings and clinical relevance.

CONCLUSION

Ultimately, the divergence between EPA and ALA in the presence of the R408S mutation highlights a critical intersection between nutrition and viral evolution. While both PUFAs have historically been grouped together as beneficial interventions, our data show that certain mutations in the spike region can selectively compromise the binding efficacy of different types of lipids, thus underscoring the necessity of moving beyond a 'one-size-fits-all' nutritional strategy in the context of COVID-19.

Furthermore, as viral evolution is often influenced by geographical pressures, our findings provide a basis for future studies to investigate the potential for nutritional recommendations to be tailored to account for localised mutational profiles and dominant regional genotypes. However, these observations require further experimental validation before any nutritional or clinical implications can be established.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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