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Evaluating SARS-CoV-2 RBD helices stability throughout the lens of molecular dynamics simulation

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Opening

- **Study aims:** evaluate the stability of SARS-CoV-2 S-protein RBD helices.

- Five helices with variable stability rates in SARS-CoV-2 RBD.
- The most stable SARS-CoV-2 RBD helix is $RBD_{417–421}$=KIADY, K(Lysine) at position 417.

POSITIVE CORRELATION between

SARS-CoV-2 immune escape properties due to K417 mutations. AND $RBD_{417–421}$=KIADY helix destabilization.

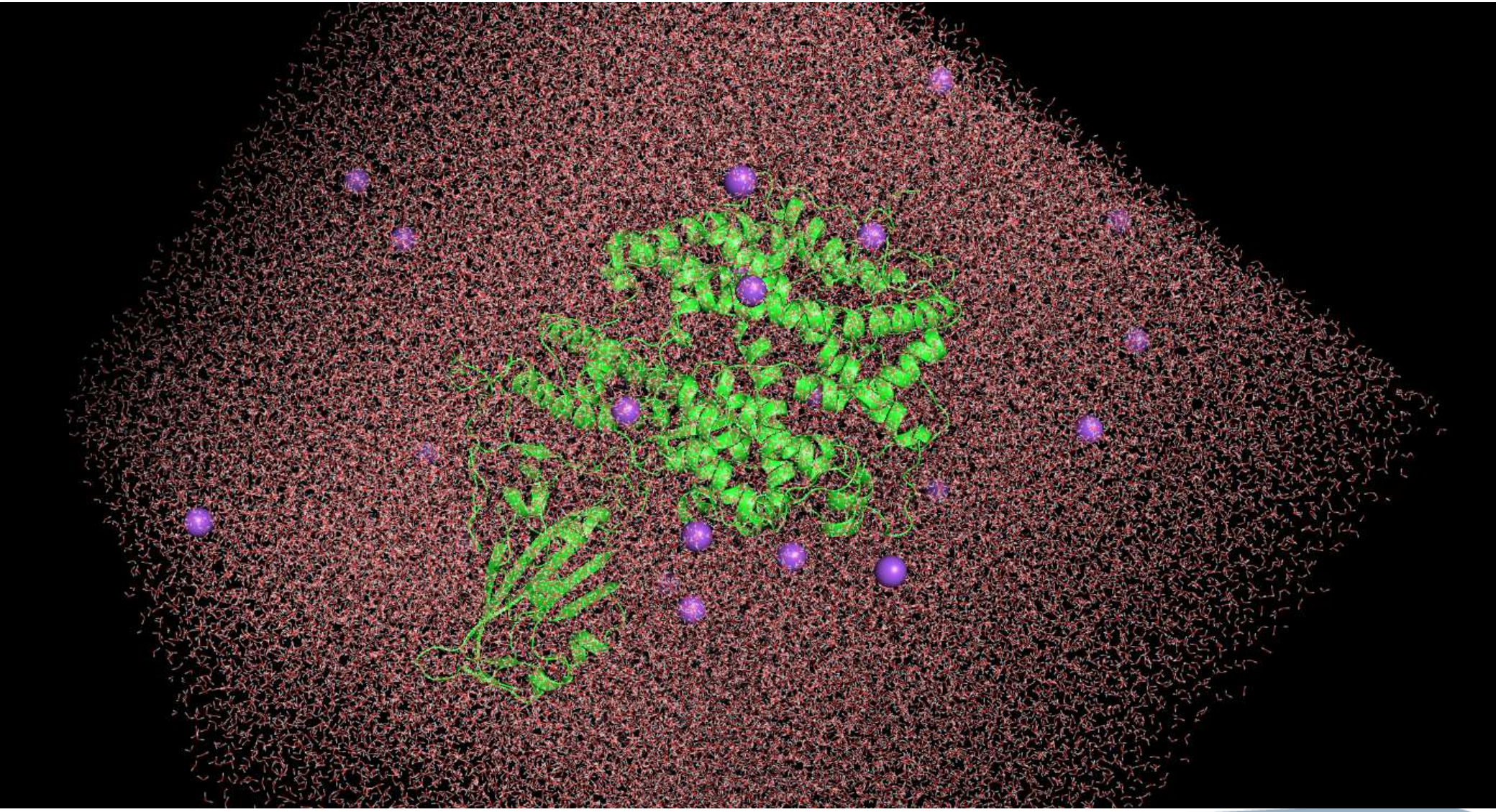
#Experimentally proved
Immune escape properties of SARS-CoV-2 RBD K417 mutations, Dupont et al., 2021. (K417N, K417T).

#Our findings
K417 mutations are likely to induce RBD417–421=KIADY helix destabilization.

1. Experimental Settings

1. 6m0j record retrieved from the Protein Data Bank (https://www.rcsb.org/): “The Crystal structure of the SARS-CoV-2 spike receptor-binding domain bound with ACE2”.
2. Put the molecule in the center of a cubic solvent box (1nm x 1nm x 1nm), dissolved under the SPC/E (simple point-charge/extended) water model.
3. Brought to the neutral net charge. *The initial net charge of the system was −25.0 *e*, then changed to 0.0 *e* (neutral net charge) by substituting 25 SPC/E water molecules with 25 Na^+ ions.
4. System potential energy gradually decreased/optimized towards the plateau $-10^5 \frac{kJ}{mol}$.
5. Apply V-rescale thermostat and Berendsen barostat to bring the system up to 310 K temperature and 1 bar pressure.
6. Run 50-ns molecular dynamics simulation in Gromacs.
7. Use simulation output files for DSSP secondary structure analysis.

1. Experimental Settings

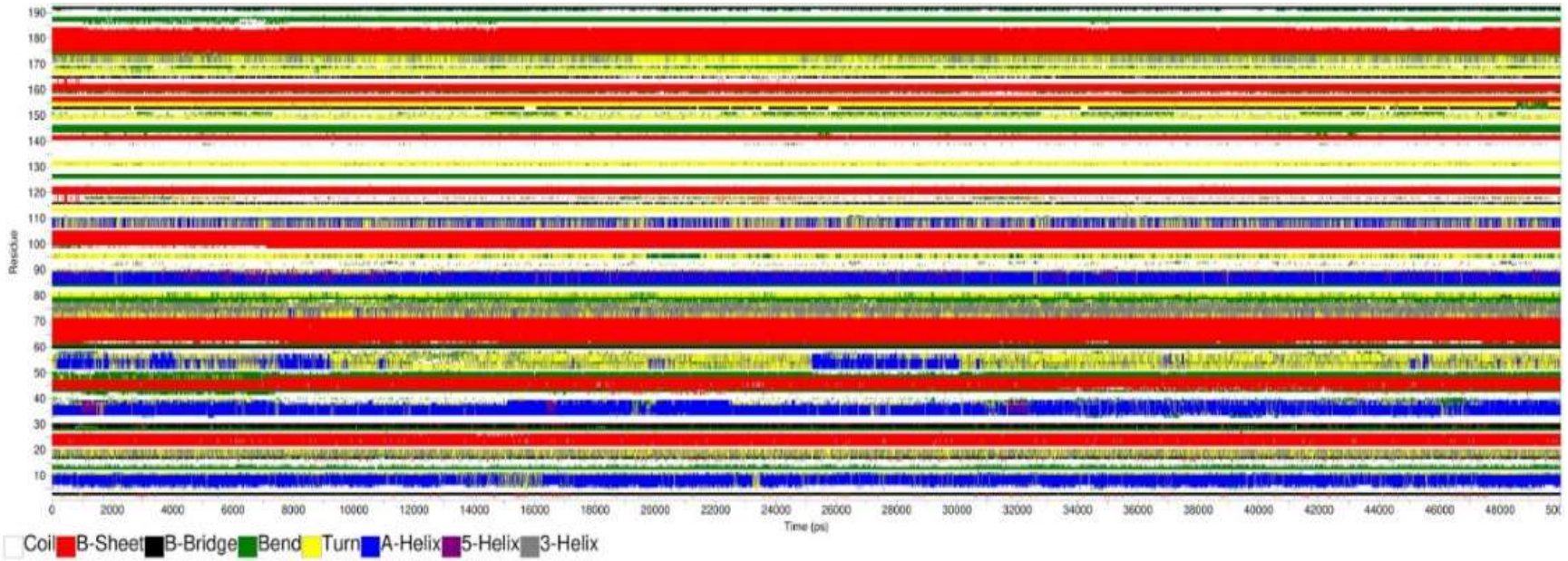


2. Dictionary of Secondary Structure in Proteins

- Per-residue assignment of the secondary structure of a protein in DSSP (Dictionary of Secondary Structure in Proteins).
- Elements prediction based on known hydrogen bonding patterns found in many secondary motifs.
- Hydrogen bond detection is based on a calculation of inter-residual electrostatic energy.
- The main chain amino group is the universal hydrogen bond donor, and the main chain oxygen atom is the universal hydrogen bond acceptor.
- $E = f q_{C,O} q_{N,H} \left(\frac{1}{r_{NO}} + \frac{1}{r_{CH}} - \frac{1}{r_{OH}} - \frac{1}{r_{CN}}\right) \left[\frac{kcal}{mol}\right]$
- $q_{C,O} = -0.42\ e$ and $q_{N,H} = +0{,}2\ e$, and $f = 332$ dimensionality constant.
- Types of secondary structure elements in DSSP:

H = alpha helix, I = π-helix, G = 3_{10}-helix, T = hydrogen-bonded turn, S = bend, B = β-bridge, E = β-sheet, P = kappa-helix (poly-proline II helix), ~ = loop.

3. Results



Helix	Sequence	Stability rate (%)
$RBD_{340–343}$	GEVF	94.6
$RBD_{366–369}$	SVLY	93.04
$RBD_{384–389}$	PTKLND	23.09
$RBD_{417–421}$	KIADY	98.12
$RBD_{439–442}$	NNLD	57.45

4. Discussion & Concluding remarks

1. There are 3 (three) stable helices in the SARS-CoV-2 receptor-binding domain: $RBD_{417–421}$=KIADY (stability rate=98.12%), $RBD_{340–343}$=GEVF (stability rate=94.6%) and $RBD_{366–369}$=SVLY (stability rate=93.04%).
2. $RBD_{384–389}$=PTKLND holds the premise for the least stable motif (stability rate=23.09%). *Although P_{384} may act as a helix breaker and K_{386}, and L_{387} have helix-forming propensities, the last two residues in the motif N_{388}, and D_{389} do not allow stable helix formation.
3. There is no specific secondary structure annotation of $RBD_{439–442}$=NNLD. *Frequent helix-to-turn oscillations are present.

- **Key insight**: Given the current knowledge of the antigenic properties of K417 mutations and the current findings for stable $RBD_{417–421}$=KIADY helix formation, in addition to K417, we assume that K417 mutations may lead to $RBD_{417–421}$=KIADY helix destabilization – a structural change that might be associated with SARS-CoV-2 immune escape strategies.

5. Related COVID-19 Research

- **Journal:** Meta Gene, Elsevier
- **Article:** Stojanov, D. (2021). Phylogenicity of B. 1.1. 7 surface glycoprotein, novel distance function and first report of V90T missense mutation in SARS-CoV-2 surface glycoprotein.

Main findings:

- B.1.1.7 spike protein lost some of the key mutations associated to higher infectivity.
- ΔI68 is supposed to compensate for missing ΔV70 in B.1.1.7 spike variant.
- First report of V90T missense mutation (Spanish isolate).
- NTD mutation V90T escapes 2–51 neutralizing antibody.
- Novel formula for computing genetic distance, why not try something new instead of the old-fashioned Jaccard and Sorensen-Dice distance functions?

$$d(A,B)=\frac{|A\backslash B||A|+|B\backslash A||B|}{|A|^2+|B|^2}$$

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- **Journal:** Biotechnology & Biotechnological Equipment, Taylor and Francis
- **Article:** Stojanov, D. (2023). Structural implications of SARS-CoV-2 Surface Glycoprotein N501Y mutation within receptor-binding domain [499-505]–computational analysis of the most frequent Asn501 polar uncharged amino acid mutations. Biotechnology & Biotechnological Equipment, 37(1), 2206492.

Novel and original biomolecular findings related to the consequences of N501Y mutation:

- Two S-protein variants considered: wild type (bearing N501) and mutant (bearing Y501).
- The hydrogen bond <RBD> Gly502 N-H…O=C Lys353 <hACE2> is of temporary charater in N501 variant, chnaged to a permanent polar contact in Y501 mutant.
- Energy calculations: E(Gly502 N-H…O=C Lys353) in N501Y=4.816210332kcal/mol

  E(Gly502 N-H…O=C Lys353) in N501=3.909532232kcal/mol
- N501Y mutation favors enhanced electrostatic interaction between Tyr505 (RBD) phenolic -OH group and Glu37 (hACE2) side chain oxygen atoms.
- I prove the presence of a Y501 binding pocket, based on RBD [499-505]: PTYGVGY Cα's RMSF peak formation.

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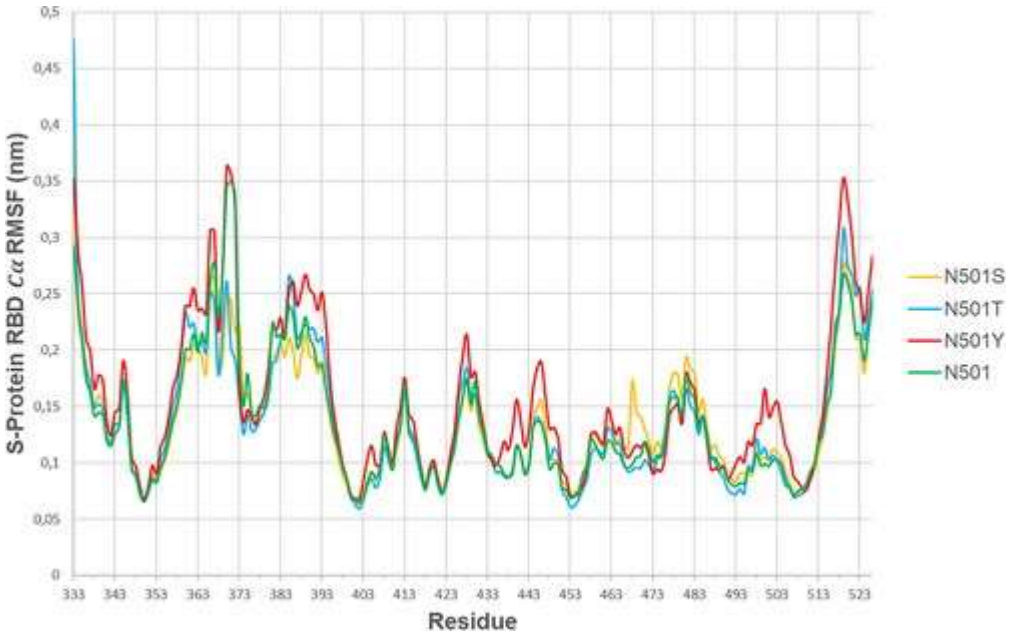

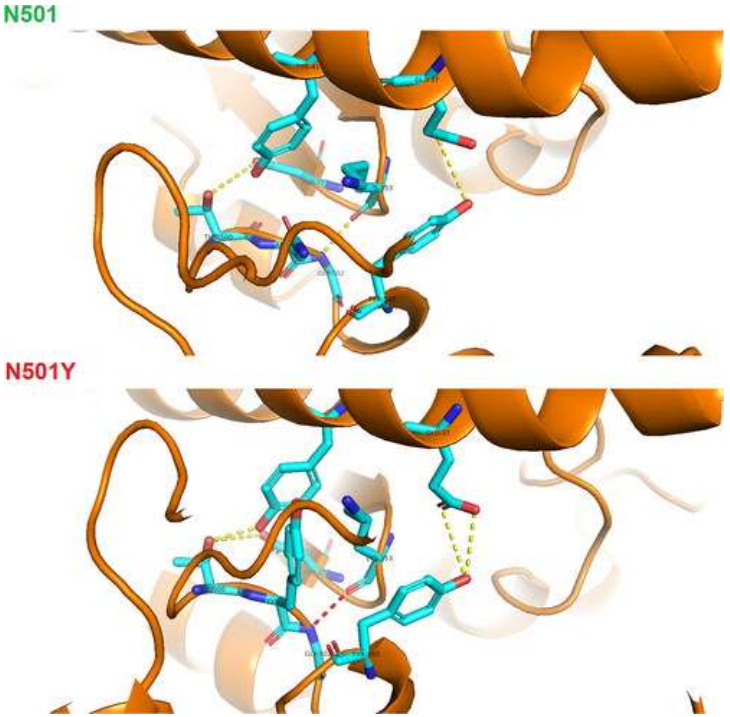

5. Related COVID-19 Research

- **Journal:** Gene & Protein in Disease
- **Article:** On the in silico application of the center-of-mass distance method

Novel in silico method for estimating binding affinity change between molecules in heterodimers, induced by one or more mutations:

- Two heterodimers considered: **W** (wild type) and **M** (mutant, a variant of W, including one or more mutations in one of the units).
- The idea:

1. Simulate W and M's molecular dynamics in GROMACS and monitor CoM (Center of Mass) distance between monomers in W and M.
2. Find the starting point of common convergence in both systems or stable CoM distance amplitudes in both systems.
3. Apply the following rule:

If M_CoM-distance(during convergence)>W_CoM-distance(during convergence)

induced mutations decrease binding affinity between monomers;

If M_CoM-distance(during convergence)<W_CoM-distance(during convergence)

induced mutations increase binding affinity between monomers;

If M_CoM-distance(during convergence)=W_CoM-distance(during convergence)

induced mutations have no impact on binding affinity;

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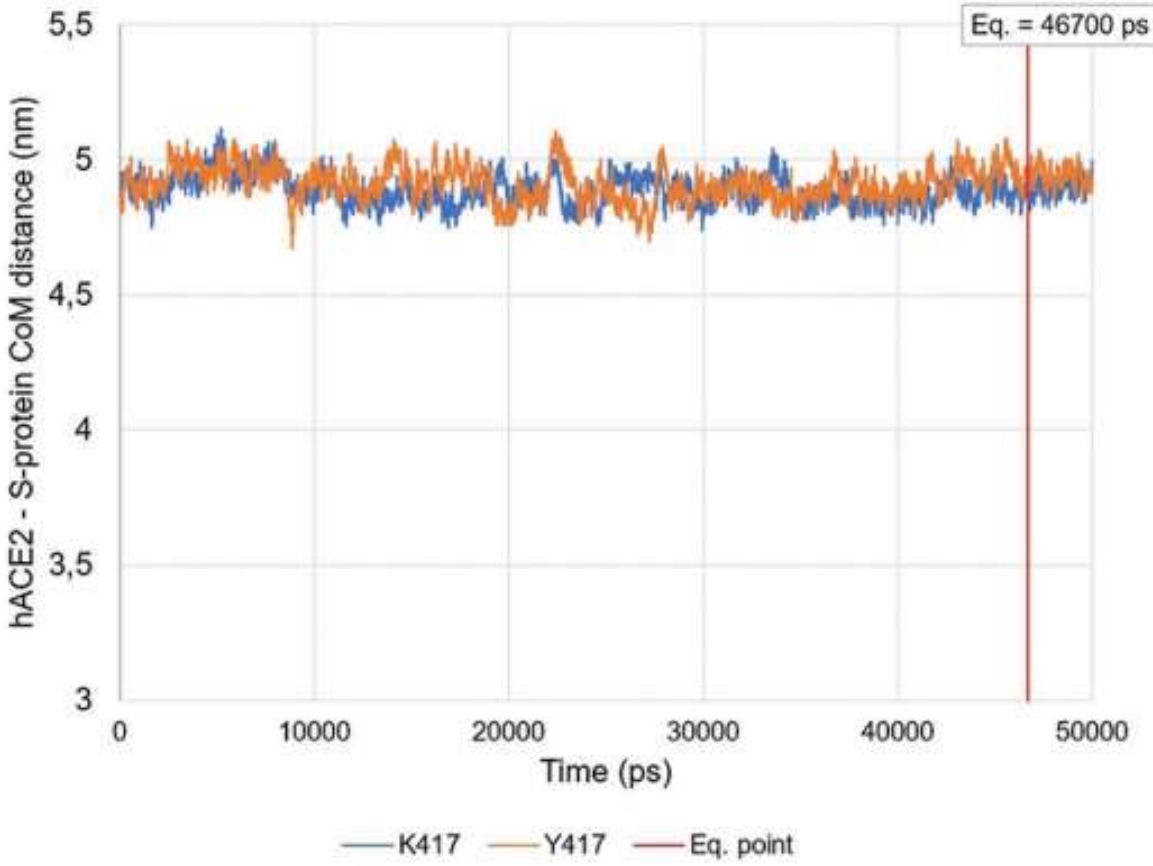

Thank you!

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