Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

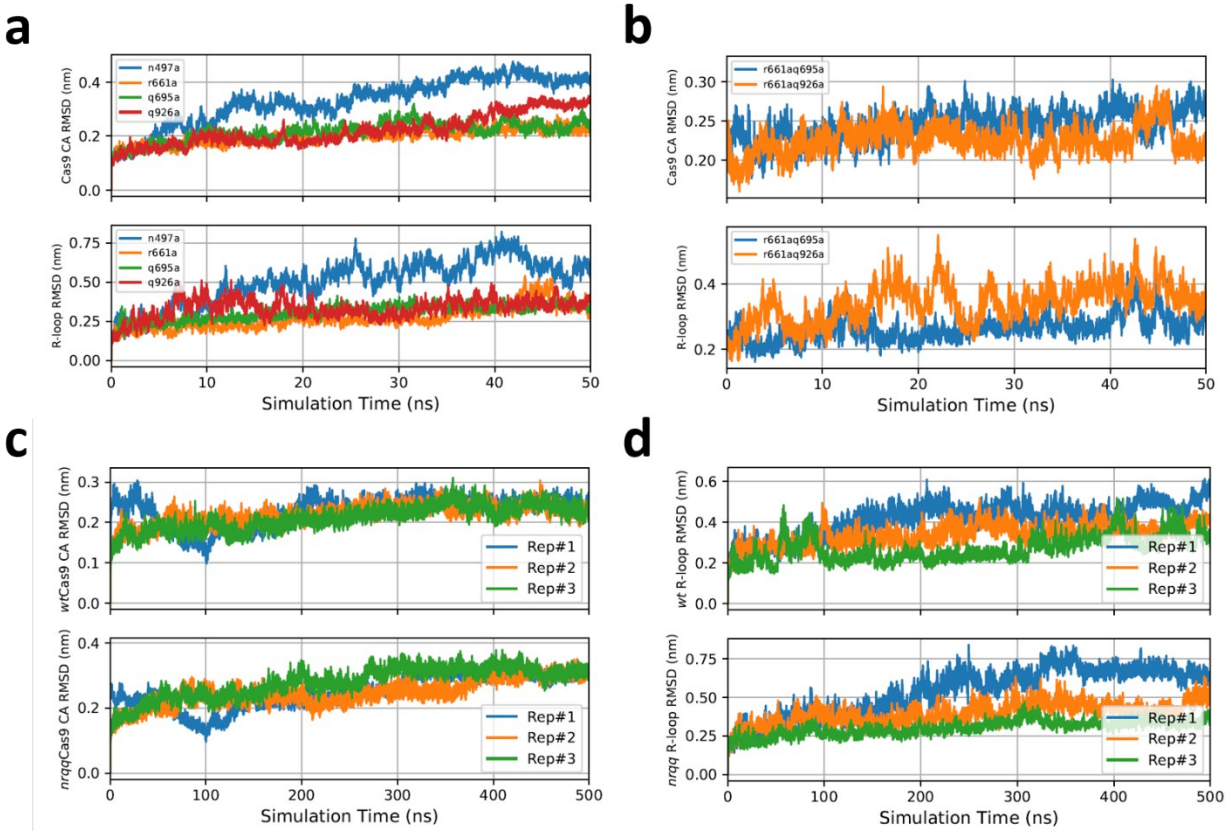

# Dynamics changes of CRISPR-Cas9 system induced by high fidelity mutations

Liangzhen Zheng[1], Jiahai Shi[2,3], Yuguang Mu[1,*]

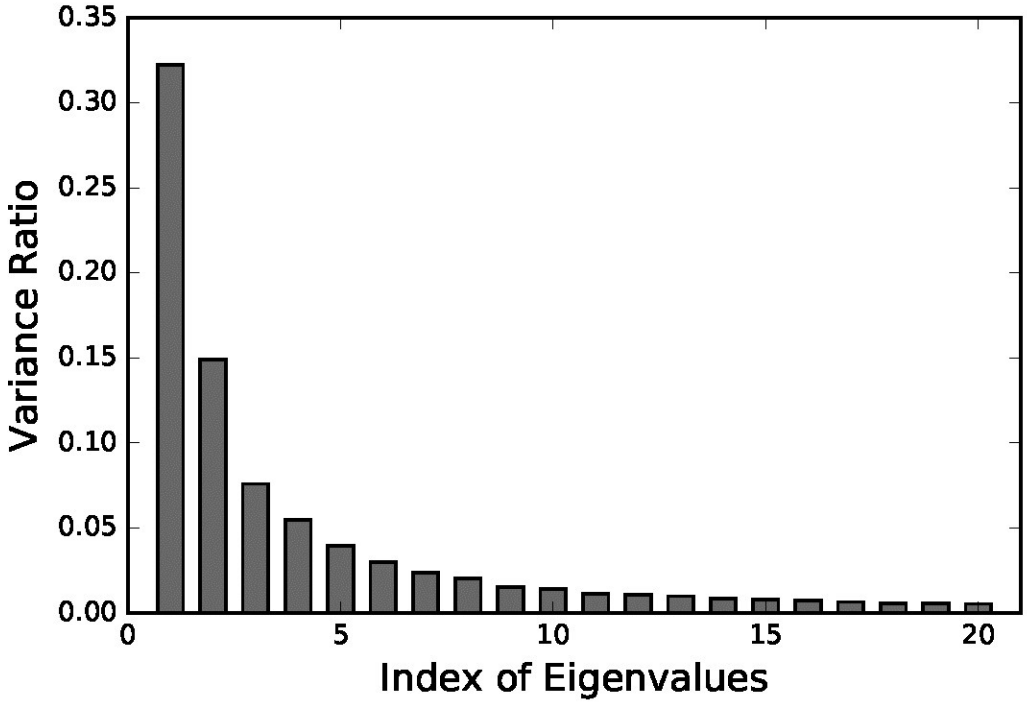
1. School of Biological Sciences, Nanyang Technological University, Singapore
2. Department of Biomedical Sciences, College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Hong Kong SAR
3. City University of Hong Kong Shenzhen Research Institute, Shenzhen, P. R. China

* Corresponding author, ygmu@ntu.edu.sg

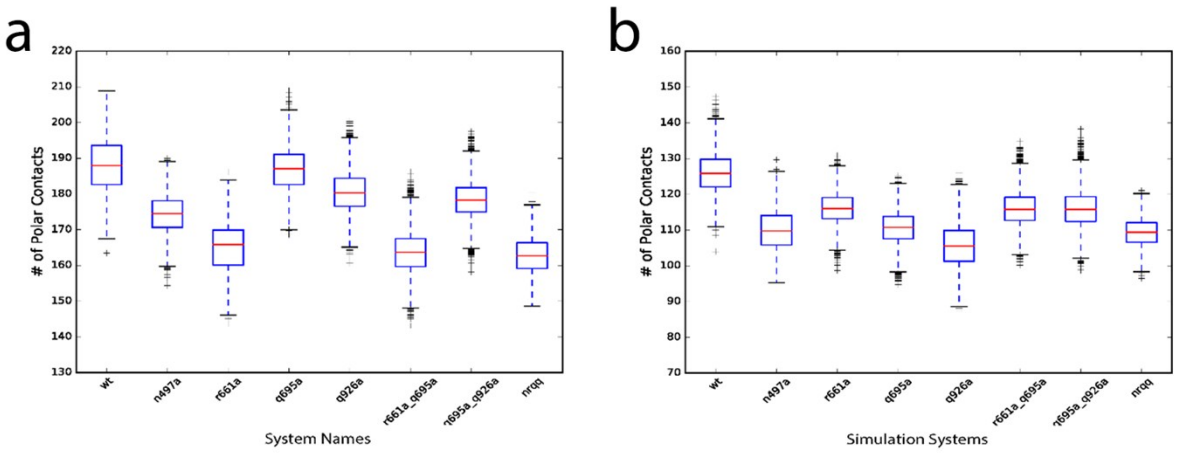
## Support Information



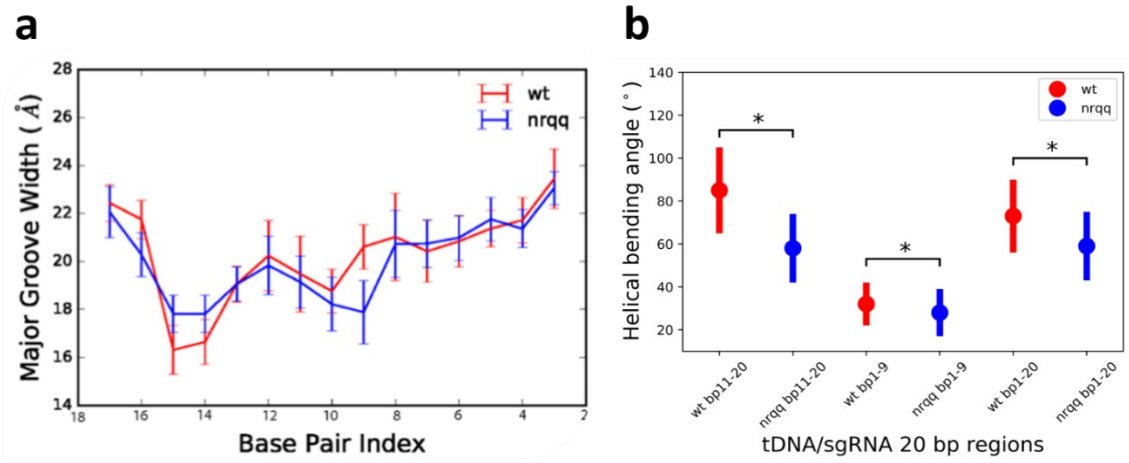

Support Figure 1. The RMSDs of all simulation systems. a) the Cas9 alpha-carbon atoms RMSDs and the R-loop RMSDs of the 4 single mutation variants (simulation systems n497a, r661a, q695a, q926a). b) the Cas9 alpha-carbon atoms RMSDs and the R-loop RMSDs of the 2 double mutation variants (simulation systems r661aq695a and r661aq926a). c) the Cas9 alpha-carbon atoms RMSDs and the R-loop RMSDs of the 3 repeats of *wt* systems. d) the Cas9 alpha-carbon atoms RMSDs and the R-loop RMSDs of the 3 repeats of *nrqq* systems.



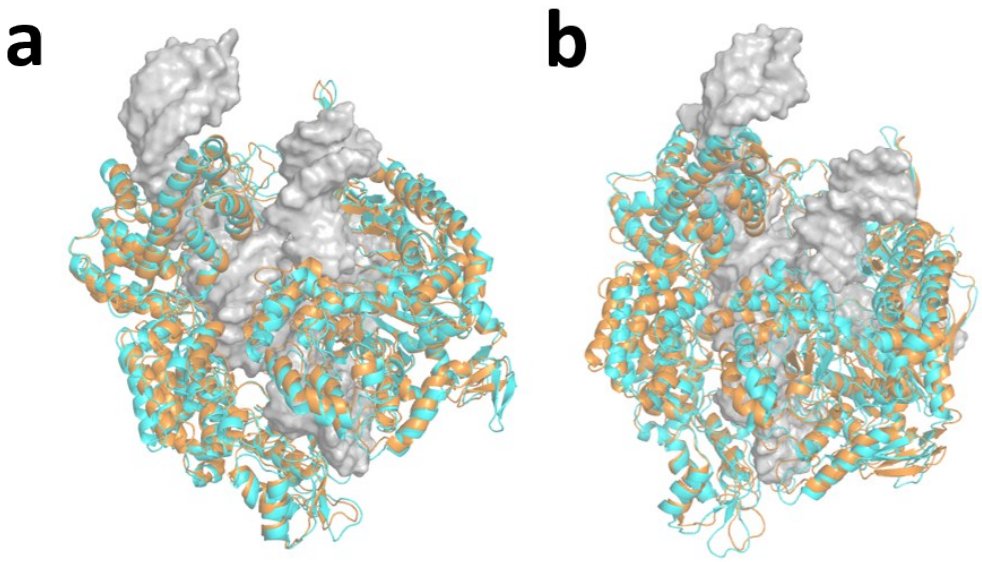

Support Figure 2. The variance of the first 20 PCs in αC atoms' coordinates based PCA analysis.



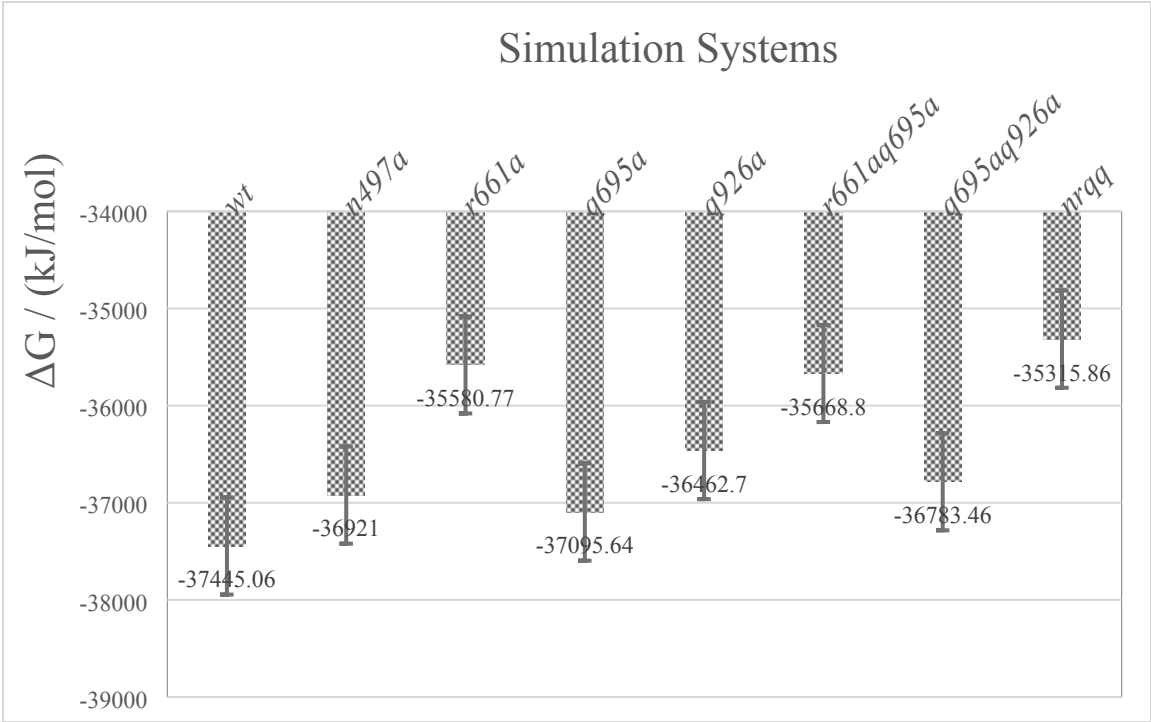

Support Figure 3. The total number of polar contacts between sgRNA and different Cas9 or mutants (a) and tDNA and different Cas9 or mutants (b).




Support Figure 4, a). Major groove width of tDNA/sgRNA heteroduplex in *wt* and *nrqq* simulations. b). Helical bending angles for different regions in 20 bp tDNA/sgRNA heteroduplex. *wt* and *nrqq* simulations are in red and blue respectively. The asterisks indicate the independent two sample t-test single tail p-values between groups are smaller than 0.01.




Support Figure 5. The superimpose of the Cas9 conformations. a) the representative conformations of *wt*Cas9 when PC1 (see Figure 4a) is smaller than 3 (cyan) and when PC1 is large than 5 (orange). b) the representative conformations of *wt*Cas9 when PC1 is larger than -3 (cyan) and when PC1 is smaller than -5 (orange). The R-loops in both panel a and b are shown in surface style, and are grey in color. The conformations of Cas9 were extracted given their respective PC1 values, and they were then subjected to clustering analysis using g_cluster in Gromacs with gromos method and a 0.2 nm RMSD cutoff. The representative structures are the center conformation of the largest populated cluster.




Support Figure 6. Binding free energy and number of polar contacts between Cas9 and R-loop. The standard errors were the standard deviation of the binding free energy (ΔG) of the snapshots selected for calculations.