Electronic Supplementary Material (ESI) for

Allosteric Regulation in SARS-CoV-2 Spike Protein

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Figures:

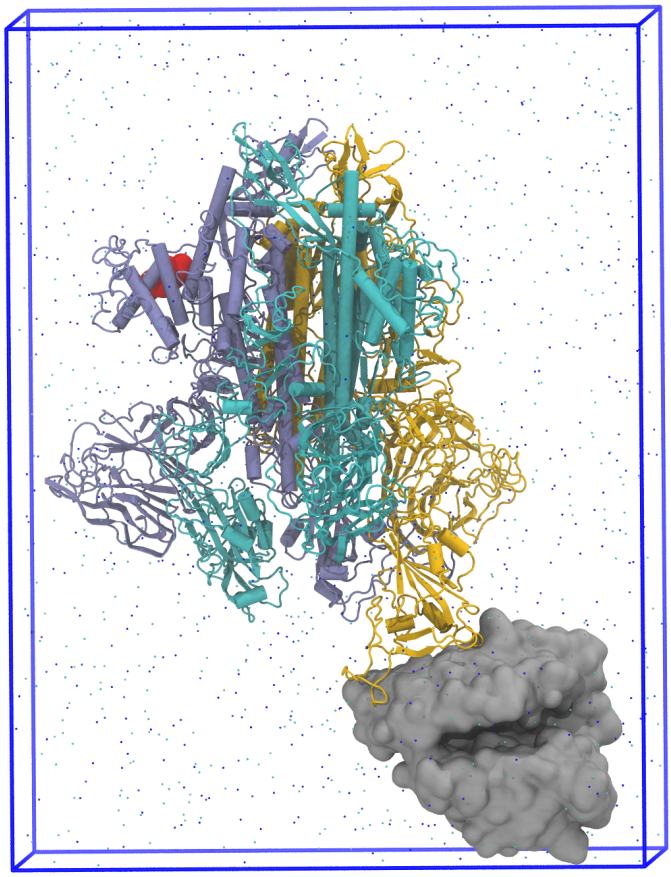

Fig. S1. Initial structure of the spike-ACE2 complex in the presence of the neutralizing tetrapeptides EELE. The three subunits of the trimeric spike protein are colored in ice blue/cyan/orange for the subunits A/B/C, respectively. The ACE2 receptor and the tetrapeptide EELE are colored silver and red, respectively. The blue/cyan dots denote Na^+/Cl^- ions, respectively. Water molecules are omitted from the display. The blue solid lines stand for the boundary of the simulation box with a size of 16 nm×18 nm×24 nm. The system has 692,370 atoms in total including water molecules.

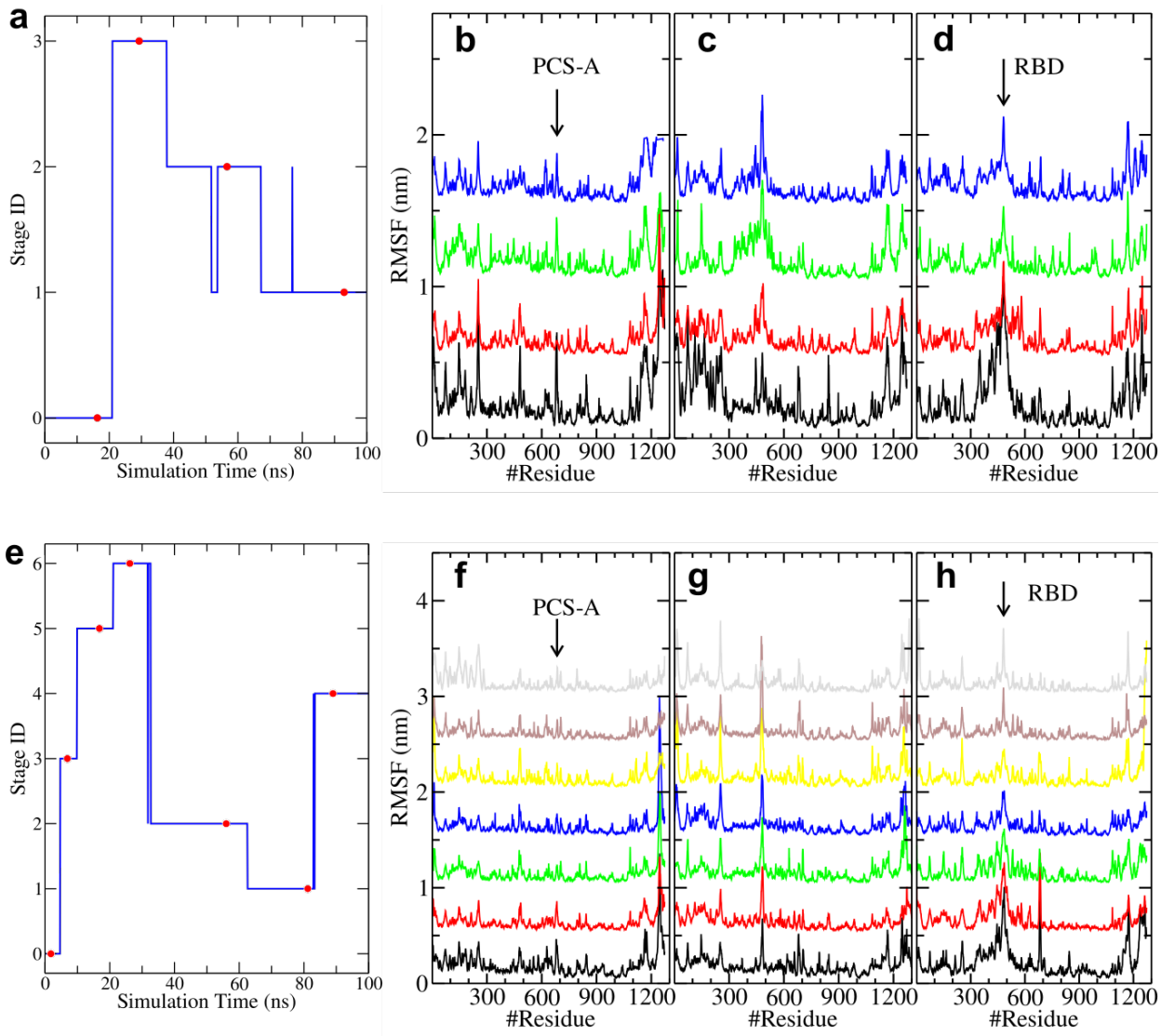

Fig. S2. Simulations and contrastive ML results for the parallel run 2 (a - d) and the parallel run 3 (e - h). (**a**) Four clustered stages of spike protein molecular structure transition in the process of the protein-ACE2 binding, in chronological order. The red dots are the positions of the contact map that are closest to the centroid of each cluster, respectively. Structural fluctuations of (**b**) subunit A, (**c**) subunit B, and (**d**) subunit C of the spike protein in the presence of a tetrapeptide EELE obtained from the atomistic MD simulation. The Cα RMSFs were calculated for the 4 stages derived from the contrastive ML, which are shifted for the display. (**e - h**) The corresponding results for the parallel run 3.

Table:

Table S1. A higher number of EELE peptides leads to a stronger destabilization of the RBD-ACE2 binding.

	#HBond (RBD-ACE2) [d]	**Energy (RBD-ACD2)** [e] **kJ/mol**
No EELE [a]	8 ± 1 [f]	-740 ± 70 [f]
One EELE (This work) [b]	5.0 ± 0.9 [g]	-560 ± 30 [g]
Three EELE peptides [c]	4.7 ± 0.8 [f]	−490 ± 50 [f]

a. Data from Ref. [1]. No peptide was present.
b. Only one peptide EELE was present, which was bound to PCS-A.
c. Data from Ref. [1]. Three peptide EELE chains were included, each binding to the PCS on one subunit of the spike trimer.
d. Number of hydrogen bonds between RBD and ACE2 using the structural criteria of hydrogen bond that the donor (D) – acceptor (A) distance $r_{DA} \leq 3.5$ Å and the hydrogen−donor−acceptor angle $\theta_{HDA} \leq 30°$.
e. The sum of the short-range Coulomb and LJ interaction energies between RBD and ACE2.
f. The error bars stand for the standard deviation from three parallel simulations.
g. The error bars stand for the standard deviation from five parallel simulations. [1]

Table S2. Ohm results on the allosteric regulation pathway from different start epitopes to the RBD on subunit C. [a]

Start [b]	Residues [c]	URL
PCS-A	$R_{682}RAR_{685}$ (subunit A)	https://dokhlab.med.psu.edu/ohm/#/Task/938672960 https://dokhlab.med.psu.edu/ohm/#/Task/210318842 https://dokhlab.med.psu.edu/ohm/#/Task/795108774 https://dokhlab.med.psu.edu/ohm/#/Task/394435553
PCS-B	$R_{682}RAR_{685}$ (subunit B)	https://dokhlab.med.psu.edu/ohm/#/Task/523806861 https://dokhlab.med.psu.edu/ohm/#/Task/105340982
PCS-C	$R_{682}RAR_{685}$ (subunit C)	https://dokhlab.med.psu.edu/ohm/#/Task/973057069 https://dokhlab.med.psu.edu/ohm/#/Task/143654006
NTD-A	L_{141}GVYYHKNNKSWMESE$_{156}$ (subunit A)	https://dokhlab.med.psu.edu/ohm/#/Task/310163365 https://dokhlab.med.psu.edu/ohm/#/Task/367688634
NTD-B	L_{141}GVYYHKNNKSWMESE$_{156}$ (subunit B)	https://dokhlab.med.psu.edu/ohm/#/Task/226180651 https://dokhlab.med.psu.edu/ohm/#/Task/44412515
NTD-C	L_{141}GVYYHKNNKSWMESE$_{156}$ (subunit C)	https://dokhlab.med.psu.edu/ohm/#/Task/898546126 https://dokhlab.med.psu.edu/ohm/#/Task/20432819

a. The calculations were carried out twice or four times using the recommended parameters of cut-off distance = 3.4 Å, alpha = 3, and 10,000 rounds of perturbation propagation. [2]
b. Start epitopes. The end epitope is always the RBD on the subunit C with the residues of L455 - Y505.
c. Residues of the start epitopes

Movies:

Movie S1. Rotation animation demonstrating the allosteric regulation route obtained via Ohm from (a) PCS-A, (b) PCS-B, and (c) PCS-C to the RBD on the subunit C. The three subunits of the trimeric spike protein are colored in ice blue/cyan/orange for the subunits A/B/C, respectively. PCSs are colored in blue and the RBD on subunit C in orange, which is in direct contact with ACE2 (in silver). The backbone atoms on the critical amino acids are colored in magenta.

Movie S2. Rotation animation demonstrating the allosteric regulation route obtained via Ohm from (a) NTD-A, (b) NTD-B, and (c) NTD-C to the RBD on the subunit C. NTDs are colored in dark blue. The other color codes are the same as those in ESI Movie S1.

Movie S3. Rotation animation demonstrating all allosteric regulation routes obtained via Ohm from PCSs/NTDs to the RBD on the subunit C. PCSs and NTDs are colored in blue and dark blue, respectively. The other color codes are the same as those in ESI Movie S1.

Notes and references

1. B. Qiao and M. Olvera de la Cruz, *ACS Nano*, 2020, **14**, 10616-10623.
2. J. Wang, A. Jain, L. R. McDonald, C. Gambogi, A. L. Lee and N. V. Dokholyan, *Nat. Commun.*, 2020, **11**, 3862.