Supporting Information for

Dynamic Asymmetry Exposes 2019-nCoV Prefusion Spike

*Susmita Roy, Akhilesh Jaiswar and Raju Sarkar

Department of Chemical Sciences, Indian Institute of Science Education and Research Kolkata, Campus Road, Mohanpur, West Bengal 741246, India

Correspondence to: susmita.roy@iiserkol.ac.in

This PDF file includes:

Supporting Information:
- Method Pipeline of Building Super-Symmetric Contact Map of SARS-CoV-2 Prefusion Spike Protein
- Detailed Methodology for Super-Symmetric Contact Map Generation
- Development of Structure-Based Hamiltonian of Trimeric Spike Protein Simulation
- Equilibrium Simulation Details
- Temperature-Dependent Simulations and Analysis
- General Free Energy Calculation Method
- Umbrella Sampling Method for Free Energy Calculations
- Mutagenic Analysis Method
- Table S1: Values of Parameter Set
- Figures S1 to S12
- Table S2: Inter-Chain NTD-RBD Interfacial Contact Information for SARS-CoV-2 S.
- Table S3, S4: Mutagenic Analysis for SARS-CoV-2 S
- Captions for Movies S1, S2
- References for Supporting Information

Other Supporting Information for this manuscript includes the following:

Movies S1, S2
Computational Methods

Method Pipeline of Building Super-Symmetric Contact Map of SARS-CoV-2 Prefusion Spike Protein.

Detailed Methodology for Super-symmetric Contact Map Generation:
Coarse-grained structure-based simulations have been performed for full-length trimetric SARS-CoV-2 spike protein. The structure-based Hamiltonians for different simulations were derived after processing the recent Cryo-EM structure (pdb:6vsb) thorough the Swiss Model to complete missing loops present in the structure \(^1\). This generates a homo-trimeric SARS-CoV-2 spike where this initial structure has important components in terms of intra and inter-chain contacts (interaction) leading to an 'S1-head-up' and an 'S1-head-down' conformation for each protomer. In this prevalent trimeric variant, only one monomer adopts 'S1-head-up' and the same of the
other two adopts the 'S1-head-down' conformation. Few characteristic intra-chain contacts cause the receptor-binding domain to perform a hinge-motion resulting 'RBD-up' and 'RBD-down' conformations for each monomer. Intra-chain contact-pair list is generated separately from ChainA which is a representative of the RBD-up conformation as well as from ChainB which is a representative of the RBD-down conformation. To avoid over-counting the contact-pairs of ChainA and ChainB are compared. Only unique contacts related to RBD-up \( C_{\text{up}} \) and RBD-down \( C_{\text{down}} \) conformations and a set of shared contacts \( C_{\text{shared}} \) are considered as shown in the pipeline method. This set of \( \{ C_{\text{up}}, C_{\text{down}}, C_{\text{shared}} \} \) is symmetrically distributed over all chains making each of them dynamically capable of sampling both RBD-up and RBD-down conformations driven by \( C_{\text{intra}} \) as defined in the pipeline method. Contact calculation is performed using the Shadow criterion 3.

Interesting components are inter-chain contacts residing at the interface of the dimer. Now, two categories of interactive dimeric interfaces are there: asymmetric-dimer interface and symmetric-dimer interface. ChainA (S1-head-up) and the adjacent ChainB (S1-head-down) represent an asymmetric dimer unit. Similarly, ChainB (S1-head-down) and the adjacent ChainC (S1-head-down) represent a symmetric dimer unit. At the asymmetric-dimer interface, the RBD-domain of ChainA forms a few unique contacts with the NTD domain of the adjacent ChainB as shown in Figure 2D mostly ensuring the S1-head-up arrangement. This asymmetric-dimer also includes a relatively remote RBD(B)-S2(A) contacts (Figure 2E) that can bend down the S1-head of a chain only partially. All these dimeric interfacial contacts are unique and identified as \( C_{\text{AB}} \). Similarly, in the asymmetric-dimer interface, the RBD-domain of ChainB forms a few unique contacts with the S2 domain of the adjacent ChainC as shown in Figure 2E. These contacts can bend down the S1-head of a chain only partially. In trimeric spike, RBD(B)-S2(A) is equivalent to RBD(A)-S2(C) and RBD(C)-S2(B) following the cyclic rule. Our analysis shows that the complete bent-down of S1-head resulting in the S1-head-down conformation is collectively determined by RBD(A)-S2(B), RBD(A)-S2(C) type of contact elements along with intra-chain hinge contact. Finally, interface-related contact set \( \{ C_{\text{AB}}, C_{\text{BC}} \} \) has been cycled over all the interfaces making each of interfaces dynamically capable of inducing S1-head movement.
Similar approach has been followed up to develop the super-symmetric contact map of MERS-CoV S.

**Development of Structure-Based Hamiltonian for Trimeric Spike Protein Simulation:**

A structure-based Hamiltonian of trimeric spike protein for SAR-CoV 2 is derived using the super-symmetric contact map. For MERS-CoV S, the same methodical approach has been adopted. In the current structure-based model amino acids are represented by single beads at the location of the C-α atom 4-7. The coarse-grained structure-based model, a well-established model, comprehends a novel way to investigate the mechanisms associated with protein folding and function 8-17. In the current context of decoding virus entry mechanism, this model successfully characterized Class-I viral fusion protein dynamics including conformational rearrangement of a viral surface glycoprotein, influenza hemagglutinin (HA) during its prefusion and postfusion states 18-19.

As described in the pipeline method, the complete Hamiltonian comprises of two terms: 

\[ H_{\text{intra}}, H_{\text{inter}} \]

The complete Hamiltonian as a function of a set of position coordinates \( \{ r_i \} \) has the following simple form:

\[
H \{ r_i \} = H_{\text{intra}} + H_{\text{inter}} \quad \text{(Eq. S1)}
\]

Where \( H_{\text{intra}} \) contains three symmetric terms applied over chain A, Chain B and Chain C to maintain their internal local and non-local interactions.

\[
H_{\text{intra}} = H_{\text{inter}}^A + H_{\text{inter}}^B + H_{\text{inter}}^C \quad \text{(Eq. S2)}
\]

The local part of \( H_{\text{intra}}^{A/B/C} \) includes harmonic potentials that restrain bonds (\( r \)), angles (\( \theta \)). Dihedral angles (\( \phi \)) are treated with a cosine term, as shown in Eq. S4. The initial geometric parameters \( (r_i^0, \theta_i^0, \phi_i^0, r_y^0) \) are obtained from the initial trimeric spike structure as shown in **Figure 1B**.
\[ H_{\text{intra}}^{A/B/C} = \sum_{i} \frac{E_r}{2} (r_i - r_i^0)^2 + \sum_{i} \frac{E_\theta}{2} (\theta_i - \theta_i^0)^2 + \sum_{i} E_\phi^{(n)} F_D (\phi_i - \phi_i^0) \]
\[ + \sum_{i<j-3} E_c \left( 5 \left( \frac{r_i^0}{r_j^0} \right)^{12} - 6 \left( \frac{r_i^0}{r_j^0} \right)^{10} \right) \Delta_{ij} \]
\[ + \sum_{i<j-3} E_N C \left( \frac{\sigma}{r_{ij}} \right)^{12} (1-\Delta_{ij}) \]

(Eq. S3)

Where,

Where, \[ F_D (\phi) = \left[ 1 - \cos(\phi) \right] + \frac{1}{2} \left[ 1 - \cos(3\phi) \right] \]

(Eq. S4)

And, \[ C_{\text{intra}} = C_{\text{up}} + C_{\text{down}} + C_{\text{shared}} \]

(Eq. S5)

The first non-local term of the Hamiltonian used in \( H_{\text{intra}}^{A/B/C} \) represents non-bonded interaction potential in the form of 10–12 Lennard-Jones potential that is used to describe the interactions that stabilize the native contacts\(^4\). A native contact is defined for a pair of residues \((i\) and \(j)\) present in the native state using shadow criteria and when \((i-j)>3\). \( \Delta_{ij} \) is defined in such a way that if any \(i\) and \(j\) residues belong to \( C_{\text{intra}} \), \( \Delta_{ij} = 1 \) turning on 10–12 Lennard-Jones potential; otherwise \( \Delta_{ij} = 0 \). For all non-native pairs for which \( \Delta_{ij} = 0 \), a repulsive potential with \( \sigma = 4\AA \) is used. All the interaction coefficients used in this potential are given in Table S1.

As described in the method pipeline, \( H_{\text{inter}} \) will include only the non-local inter-chain contacts residing at the interface of the dimer which comprises of accounting for asymmetric-dimer interfacial contacts \( C_{\text{asym}} \) and symmetric-dimer interfacial contacts \( C_{\text{sym}} \) those are symmetrized for all AB, BC, CA dimeric interfaces.

\[ H_{\text{intra}} = H_{\text{int}}^{AB} + H_{\text{int}}^{BC} + H_{\text{int}}^{CA} \]

(Eq. S6)
\[
H_{\text{inter}}^{AB/BC/CA} = \sum_{ij} \varepsilon_{c} \left( 5 \left( \frac{r_{ij}^{0}}{r_{ij}} \right)^{12} - 6 \left( \frac{r_{ij}^{0}}{r_{ij}} \right)^{10} \right) \Delta_{ij} + \sum_{i} \varepsilon_{NC} \left( \frac{\sigma}{r_{ij}} \right) (1-\Delta_{ij}) \quad \text{(Eq. S7)}
\]

Similar to our early approach, \( \Delta_{ij} \) is such defined that if any \( i \) and \( j \) residues belong to \( C_{\text{inter}} \), \( \Delta_{ij} = 1 \), turning on 10–12 Lennard-Jones potential; otherwise \( \Delta_{ij} = 0 \). Here, \( C_{\text{inter}} = C_{AB} + C_{BC} + C_{CA} \)

**Equilibrium Simulation Details:**

To begin every simulation an initial structure is energetically minimized under the structure-based Hamiltonian using the steepest descent algorithm. Atomic coordinates of the energy minimized structure have been evolved using Langevin dynamics with a time step of 0.0005 \( \tau_{R} \). We used an underdamped condition for rapid sampling\(^{20} \). For explicit particles, reduced mass of \( 1 \mu_{R} \) and a drag coefficient \( 1 \tau_{R}^{-1} \) are used to efficiently sample the conformational space\(^{4,21} \) where \( \tau_{R} = \left( \frac{m \sigma^{2}}{\varepsilon_{c}} \right)^{0.5} \). Standard structure-based models implemented in Gromacs (SMOG) were used for all the simulations\(^{22} \).
Table S1: Values of parameter set used for current structure-based spike simulation

| Reduced parameter set     |                |
|---------------------------|----------------|
| $\mu_R$                   | 1 amu          |
| $\varepsilon_R$           | 1 kJ/mol       |
| $T_R$                     | 120 K          |

| SBM potential             |                |
|---------------------------|----------------|
| $\varepsilon_r$           | $2 \times 10^2 \, \varepsilon_R / $nm$^2$ |
| $\varepsilon_0$           | $20.0 \, \varepsilon_R / $rad$^2$ |
| $\varepsilon_{\phi (1)}$  | $1.0 \, \varepsilon_R$ |
| $\varepsilon_{\phi (3)}$  | $0.5 \, \varepsilon_R$ |
| $\sigma$                  | 0.4 nm         |
| $\varepsilon_{NC}$        | 1.0 kJ/mol     |
| $\varepsilon_C$           | 1.0 kJ/mol     |

Temperature-Dependent Simulations and Analyses

All temperatures mentioned here are in reduced units. Temperature dependence of the conformational transition has been performed over several temperatures. Three representative reduced temperature-dependent ($T^*=0.50T_R$, $T^*=0.58T_R$, and $T^*=0.83T_R$) analyses are shown for clarity in Figure S4. Population distribution as a function of the fraction of native inter-chain contacts formed in the S1-head-down state is monitored over these temperatures. Four states emerge as indicated in Figure 3B and Figure S4. As the temperature increases the population shifts more towards the S1-head-up state. At $T^*=0.58T_R$ the population of 1up-2down state appears as a predominant population in the conformational landscape which correlates well with the recent Cryo-EM data \cite{1}. We have performed all our simulations being consistent with this
selected temperature. The RMSD analyses ensure the correctness of the simulation progress and the emergence of the correct structure (Figure S3).

The population shifts more towards the S1-head-up state conformations as the temperature increases. It suggests that the S1-head-up states are more dynamic and entropically stable. Note that the dynamical transition between 1up-2down and 2up-1down states may tolerate a wide range of temperatures by a population shift mechanism. So far, we have examined that it tolerates the temperature range from $T^*=0.50T_R$ to $T^*=1.67T_R$. Temperature dependence of RBD hinge motion has also been studied (Figure S4). Population distribution as a function of the fraction of native intra-chain hinge-region contacts formed by the RBD at different temperatures has been monitored. A bimodal distribution reflects the population of the 'RBD-up' and 'RBD-down' states for any individual chain being in trimeric spike. As temperature increases, the RBD-up states start to enhance their populations.

**General Free Energy Calculation Approach:**

In a system, if a state “A” described by its reaction coordinate, $X_A$ (which in our case is the fraction of native contact) is separated from another state “B” described by its reaction coordinate, $X_B$, by a finite barrier, the free energy of transition from A to B can be expressed as,

$$F(X_B) - F(X_A) = -k_B T \ln \frac{\langle P(X_B) \rangle}{\langle P(X_A) \rangle}$$

(Eq. S8)

where, $\langle P(X_B) \rangle$ is the probability to find the system in state B at the reaction coordinate, $Q_B$. the same holds for $\langle P(X_A) \rangle$. From a finite set of unbiased simulations of trimeric spike protein, a complete thermodynamic description is obtained. Probability distributions are obtained by sampling the configurational space running 50 Molecular Dynamics simulation sets.
Umbrella-Sampling for Free Energy Calculations along Up-Down Transition Reaction-Coordinate:

For free energy calculations, we used the Umbrella Sampling method. It is an efficient technique which helps to surmount the barrier by effectively sampling near the barrier region along the reaction coordinate with the help of an artificial biasing umbrella potential, \( V \). The form of \( V \) over the reaction coordinate, \( N \) is expressed as, \( V = \frac{1}{2}k(N - N_0)^2 \), where \( k \) is the harmonic force constant. In the case of SARS-CoV 2 S, we have chosen our reaction coordinate as the distance between a lysine residue (residue id: 360) on the RBD and an aspartate (residue id: 2079) on the S2 stalk head region of the adjacent chain. This is a potential salt-bridge interaction maneuvering the inter-chain RBD-S2 subunit closure. Similarly, in the case of MERS-CoV S, the distance between a tyrosine (residue id: 2938) on RBD and an arginine (residue id: 1040) (potential π-cation interaction) residing on the S2 stalk head region is chosen as a reaction coordinate. A force constant of 500 kJ/mol·nm\(^2\) was used to restrain the RBD domain at the respective distance from its reference S2 stalk position. We have performed 10 ns long simulations for each umbrella window. A total of 30 windows have been generated to cover the total distance from its up conformation to down conformation. The average free energy of S1-head up-down transition for any given chain was calculated while S1-domains of other two chains are restrained to remain in the down conformation as it was in its prevalent cryo-EM structure. The restraining was done by enhancing the contact strength of S1 and S2 stalk. Finally, the corresponding probabilities of each simulation at each window have been computed and WHAM (weighted histogram analysis) has been applied to estimate the unbiased free energy. Four repeats were performed to check the consistency of the results with errors estimated. The error bars for the present study show the standard deviation from the mean, which is given by: 

\[
\frac{\sum_{i=1}^{4}(x_i - \langle x \rangle)^2}{(N-1)^2}
\]
Mutagenic Analysis: Predicting the Effect of Mutation on Protein Dynamics Using DynaMut Webserver

Normal modes analysis (NMA) is one of the efficient approaches to study protein dynamics at an atomistic length scale. NMA is a computational tool that approximates the system dynamics around the local minima through harmonic motion. Molecular Dynamics approach gives us the single molecule trajectory over time now conformational fluctuations can be evaluated by NMA using superposition of Eigenvectors (modes) and Eigenvalues (frequencies). However there are limited methods to study effects of mutation on protein dynamics. DynaMut, a webserver interface that uses the well-established NMA and other structure-based approaches like Bio3D, ENCoM, mCSM-PPI2 to study the mutation effects. DynaMut integrates these methods to calculate folding free energy to evaluate the stability of mutation. Bio3D is a R package which basically does the protein evolutionary dynamics and flexibility etc. based on NMA, freely available source code for Bio3D is https://bitbucket.org/Grantlab/bio3d/. In other words ENCoM is an elastic network contact model that basically applies a potential energy function and non-bonded atom-type interaction term to add an extra layer of information about the dynamics of specific amino acid. ENCoM predicts the stability of mutation by calculating $\Delta (\Delta G)$ through the estimation of vibrational entropy changes of Wild-Type and Mutant protein. The $\Delta S$ between the two conformations (A,B) is defined by $\Delta S_{vlb,A\rightarrow B} = \ln \left( \prod_{n=1}^{N} \frac{\lambda_{n,A}}{\lambda_{n,B}} \right)$ where $\lambda_{n,i}$ represents the n’th sets of eigenvalues and $\Delta (\Delta G) = \Delta G_{Wild-Type} - \Delta G_{Mutant}$. Publically available source code for ENCoM is https://github.com/NRGlab/ENCoM. The Structure-Based Prediction by mCSM-PPI2 uses graph-based structural signatures (where atoms are considered as nodes and their interactions are as edges). mCSM-PPI2 is a publically available webserver at http://biosig.unimelb.edu.au/mcsm_ppi2/. Now if the calculated Gibb’s free energy/Folding free energy $\Delta (\Delta G) < 0$ then DynaMut says that mutation destabilizes and $\Delta (\Delta G) \geq 0$ then mutation will stabilize the macromolecule. The mutagenic results are given in Table S3 and S4.
Figure S1: Inter-chain interaction from the 'S1-head-up' and the 'S1-head-down' states of SARS-CoV-2 spike. A. Inter-chain RBD-NTD domain closure in the S1-head-up state. The domain closure is mediated by double hydrogen bonds connecting an arginine of ChainA with asparagine and cysteine residues of ChainB. B. Inter-chain RBD-S2 domain closure in the S1-head-down state. The S2 stalk connection with RBD is mediated by a proline residue of ChainA with the formation of a CH-π type interaction with tyrosine and hydrophobic interaction with another proline of ChainB (residue index are discussed in the main text).
Figure S2: The structural alignment of two chains in the S1-head-down state. ChainB (orange) and ChainC (green) in the S1-head-down state extracted from the Cryo-EM structure (pdb:6vsb) of trimeric spike. Low RMSD between these two chains suggests that contact information extracted from any of these chains will be equivalent. This supports our contact map generation shown in the method pipeline.
Figure S3. RMS deviation of each chain from their initial state during a typical simulation progress. A. The initial state of Chain A in the trimeric spike was in 'S1-head-up' state and Chain B/C was in 'S1-head-down' state. B. The lower RMSD for Chain A corresponds to Chain A's S1-head-up state as the initial coordination of Chain-A is taken from its up-state (see 6vsb). C. The lower RMSD for chain B corresponds to Chain B’s head-down state as the initial coordination of Chain B is taken from its down-state. D. The lower RMSD for Chain C corresponds to Chain C’s head-down state for the same reason as discussed for Chain B. The RMSD analyses ensure the correctness of the simulation progress and the emergence of the correct structure.
Figure S4. Temperature dependence of S1-head up-down transition and RBD open-close breathing transition. A. Population distribution as a function of the native inter-chain contacts formed in the S1-head-down state as shown in Figure S1. Four states emerge as shown in Figure 3B. As temperature increases the population shifts more towards the S1-head-up state conformations indicating that S1-head-up states are more dynamic and entropically stable. Note that the dynamical transition between 1up-2down and 2up-1down states may tolerate a wide range of temperatures by a population shift mechanism. B. Population distribution as a function of the fraction of native intra-chain hinge-region contacts formed by the RBD. A bimodal distribution reflects the 'RBD-up' and the 'RBD-down' states for any individual chain being in the trimeric spike. As the temperature increases, RBD-up started populating more. Temperature analysis helps to choose an intermediate temperature to obtain correct population distribution.
Figure S5. Sequence alignment of SARS-CoV-2 spike (pdb: 6vsb) with that of SARS-CoV spike(pdb:5x5b), MERS-CoV spike (pdb: 5x5f) and RaTG13 spike. Only the RBD is highlighted in green. The unique histidine residue (highlighted in yellow) of the RBD of SARS-CoV-2 is noted. Identical residues are denoted by an “*” beneath the consensus position. The multiple sequence alignment is continued over the next page.
Figure S6. Building a super-symmetric contact map of the homotrimeric MERS-CoV spike protein. A. Amino acid sequence ranges of NTD, RBD, and S2-subunit are only highlighted. B. Side view of the homo-trimeric structure of MERS-CoV spike protein with one RBD of the S1 subunit head rotated in its up conformation. This all-atomistic conformation is taken from the pdb id: 5x5f. Residue-residue native contact map identifying unique intra and inter-chain contact-pairs formed by any single monomer in its S1-head up and S1-head down states. Inter-chain RBD-down conformation controlling (RBD-S2 stalk connection) contacts maneuvering its S1-head up-down movements are also highlighted by magenta circles.
Figure S7. 2D landscape pathway of up-down conformational transition of the S1-domain of MERS-CoV S trimer. A. The transition pathway has been traced along the up and down reaction-coordinates where a characteristic RBD-S2 stalk distance represents the down-reaction-coordinate and a characteristic RBD-NTD distance represents the up-reaction-coordinate. The following representative conformational states extracted along the pathway to monitor the up-down transition for MERS-CoV S: B. Up/flexi-up; C. Partially up; D. Down. Inter-chain RBD-S2 subunit closure has been characterized by the distance between a tyrosine (residue id: 2938) on RBD and an arginine (residue id: 1040) (potential n-cation interaction) marked in red. Inter-chain RBD-NTD closure has been characterized by the distance between a proline (residue id: 2748) on RBD and another proline (residue id: 268) on the next chain NTD marked in magenta.
Figure S8. Free energy profiles comparing the up-down transition of the S1-head of spike trimer in the presence and absence of RBD-NTD interactions. Free energy profiles calculated for A. SARS-CoV-2 S in the presence of all possible interactions; B. SARS-CoV-2 S, RBD-NTD interactions are manually deleted from its topological information; C. MERS-CoV S where RBD-NTD interactions are inherently absent in its relevant cryo-EM structure. Note that, the presence of the RBD-NTD association makes the S1-head-up state significantly stable than that of MERS-CoV S and modified SARS-CoV-2 S where RBD-NTD connections are missing.
Figure S9. 2D landscape pathway of up-down conformational transition of the S1-domain of A. modified SARS-CoV 2 S where RBD-NTD connections are manually deleted from the topological information and B. MERS CoV S where RBD-NTD interactions are inherently absent in its relevant cryo-EM structure. In both cases, the transition pathways appear scattered due to the high degree of flexibility of its up-conformations. The scattered pathway renders multiple possible routes for this transition to occur which is evident from the data-spread along RBD-NTD distance coordinate.
Figure S10. RBD up-down hinge dynamics triggered by inter-chain RBD-NTD domain interaction. A. For SARS-CoV-2 S where RBD-NTD inter-chain interactions exist. B. For modified SARS-CoV-2 S where the RBD-NTD inter-chain interactions are manually deleted from its topology. In this case, the hinge motion of RBD is hindered by populating more 'RBD-down' conformations and allows to sample 'RBD-up' conformation only rarely in a stochastic manner.
Figure S11. Mutagenic analysis at the interface of RBD and NTD of SARS-CoV-2 S spike trimer to understand the nature of RBD-NTD interaction. A. Predicted single point mutations are displayed that have the potential to destabilize the up conformation of the spike trimer affecting the interaction between NTD and RBD. Among all these mutations, the mutation of Pro 495 by ALA causes a severe destabilization effect. This indicates that this proline is a potential hotspot that regulates the RBD-NTD association. B. Comparison of RMSF between the unmuted spike and mutated (Pro495ALA) spike.
Figure S12. The free energy landscape of S1-head up-down transition of trimeric spike of SARS-CoV-2 S in the presence and in the absence of inter-chain RBD-S1 contacts. A. In the presence of inter-chain RBD-S1 contacts, the enhanced population of the 1up-2down is observed in comparison to 2up-1down state. B. In the absence of inter-chain RBD-S1 contacts, the population shifts from 1up-2down state 2up-1down state.
Table S2: The inter-chain NTD-RBD interfacial contact information for SARS-CoV-2 S. The number signifies the residual position at each chain. Inter-chain interactions are colored as follows: Pink: When RBD of chain A interacts with NTD of chain B; Cyan: When RBD of chain B interacts with NTD of chain C; green: When RBD of chain C interacts with NTD of chain A.

| Chain A | Chain B | Chain B | Chain C | Chain A | Chain C |
|---------|---------|---------|---------|---------|---------|
| 305     | 1135    | 1425    | 2255    | 15      | 2545    |
| 331     | 1259    | 1451    | 2379    | 139     | 2571    |
| 331     | 1260    | 1451    | 2380    | 140     | 2571    |
| 331     | 1261    | 1453    | 2381    | 141     | 2571    |
| 333     | 1261    | 1453    | 2381    | 141     | 2573    |
| 334     | 1261    | 1454    | 2381    | 141     | 2574    |
| 334     | 1262    | 1454    | 2382    | 142     | 2574    |
| 334     | 1324    | 1454    | 2444    | 204     | 2574    |
| 494     | 1325    | 1614    | 2445    | 205     | 2734    |
| 494     | 1326    | 1614    | 2446    | 206     | 2734    |
| 495     | 1292    | 1615    | 2412    | 172     | 2735    |
| 495     | 1293    | 1615    | 2413    | 173     | 2735    |
| 495     | 1294    | 1615    | 2414    | 174     | 2735    |
| 495     | 1325    | 1615    | 2444    | 204     | 2735    |
| 495     | 1326    | 1615    | 2446    | 206     | 2735    |

Table S3: In-silico mutagenic analysis for the RBD in its up-state (for SARS-CoV-2 S). This table enlists only those residues on the RBD that interact with the NTD of the next chain (Chain B) when its RBD is in the up-state (in this case, RBD of chain A in the cryo-EM structure (6vsb) is in the up-state). Only those residues are enlisted that show significant thermodynamic destabilizing effect upon alanine mutation.

| Chain ID | Wild-type Residue | Residue No | Mutant Residue | ∆ (ΔG) in kJ/mol | Binding Affinity |
|----------|-------------------|------------|----------------|------------------|-----------------|
| A        | PRO               | 495        | ALA            | -3.6036          | Decreasing      |
| A        | HIS               | 493        | ALA            | -1.6674          | Decreasing      |
| A        | ARG               | 331        | ALA            | -1.6044          | Decreasing      |
| A        | ASN               | 334        | ALA            | -1.5708          | Decreasing      |
| A        | ASN               | 305        | ALA            | -1.0374          | Decreasing      |
| A        | SER               | 333        | ALA            | -0.378           | Decreasing      |
Table S4: In-silico mutagenic analysis for the NTD when RBD is in the up-state (for SARS-CoV-2 S). This table enlists only those residues on the NTD (chain B) that interact with the RBD of next chain (chain A) when its RBD is in the up-state (in this case, RBD of chain A in the cryo-EM structure (6vsb) is in the up-state). Only those residues are enlisted that shows significant thermodynamic destabilizing effect upon alanine mutation.

| Chain ID | Wild-type Residue | Residue No | Mutant Residue | Δ(ΔG) in kJ/Mol | Binding Affinity |
|----------|-------------------|------------|----------------|-----------------|-----------------|
| B        | ILE               | 1325       | ALA            | -3.759          | Decreasing      |
| B        | TYR               | 1294       | ALA            | -3.4188         | Decreasing      |
| B        | LYS               | 1135       | ALA            | -2.7258         | Decreasing      |
| B        | PHE               | 1262       | ALA            | -2.688          | Decreasing      |
| B        | PRO               | 1324       | ALA            | -2.5872         | Decreasing      |
| B        | CYS               | 1260       | ALA            | -2.4948         | Decreasing      |
| B        | ASN               | 1259       | ALA            | -2.1546         | Decreasing      |
| B        | GLY               | 1293       | ALA            | -2.1336         | Decreasing      |
| B        | GLY               | 1326       | ALA            | -1.9782         | Decreasing      |
| B        | ASP               | 1292       | ALA            | -1.6464         | Decreasing      |
| B        | THR               | 1261       | ALA            | -1.3524         | Decreasing      |

Captions for movies:

**Movie S1:** Conformational dynamics of full-length trimeric SARS-CoV-2 spike protein showing rapid symmetry breaking.

**Movie S2:** Conformational dynamics of a monomer of the full-length SARS-CoV-2 showing RBD hinge motion.
References:

(1) Wrapp, D.; Wang, N.; Corbett, K. S.; Goldsmith, J. A.; Hsieh, C. L.; Abiona, O.; Graham, B. S.; McLellan, J. S. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* **2020**, *367* (6483), 1260-1263.

(2) Waterhouse, A.; Bertoni, M.; Bienert, S.; Studer, G.; Tauriello, G.; Gumienny, R.; Heer, F. T.; de Beer, T. A. P.; Rempfer, C.; Bordoli, L. et al. SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic acids research* **2018**, *46* (W1), W296-W303.

(3) Noel, J. K.; Whitford, P. C.; Onuchic, J. N. The shadow map: a general contact definition for capturing the dynamics of biomolecular folding and function. *The journal of physical chemistry. B*** **2012**, *116* (29), 8692-702.

(4) Clementi, C.; Nymeyer, H.; Onuchic, J. N. Topological and energetic factors: what determines the structural details of the transition state ensemble and "en-route" intermediates for protein folding? An investigation for small globular proteins. *Journal of molecular biology* **2000**, *298* (5), 937-53.

(5) Dutta, M.; Jana, B. Role of AAA3 Domain in Allosteric Communication of Dynein Motor Proteins. *ACS omega* **2019**, *4* (26), 21921-21930.

(6) Ghosh, C.; Jana, B. Intersubunit Assisted Folding of DNA Binding Domains in Dimeric Catabolite Activator Protein. *The journal of physical chemistry. B*** **2020**, *124* (8), 1411-1423.

(7) Jana, B.; Hyeon, C.; Onuchic, J. N. The origin of minus-end directionality and mechnanochemistry of Ncd motors. *PLoS computational biology* **2012**, *8* (11), e1002783.

(8) Dokholyan, N. V.; SpringerLink (Online service). *Computational Modeling of Biological Systems : From Molecules to Pathways*. p 1 online resource (VI, 366 p.).

(9) Brooks, C. L., 3rd; Gruebele, M.; Onuchic, J. N.; Wolynes, P. G. Chemical physics of protein folding. *Proceedings of the National Academy of Sciences of the United States of America* **1998**, *95* (19), 11037-8.

(10) Bryngelson, J. D.; Onuchic, J. N.; Socci, N. D.; Wolynes, P. G. Funnels, pathways, and the energy landscape of protein folding: a synthesis. *Proteins* **1995**, *21* (3), 167-95.

(11) Dill, K. A.; Chan, H. S. From Levinthal to pathways to funnels. *Nature structural biology* **1997**, *4* (1), 10-9.

(12) Jana, B.; Morcos, F.; Onuchic, J. N. From structure to function: the convergence of structure based models and co-evolutionary information. *Physical chemistry chemical physics : PCCP* **2014**, *16* (14), 6496-507.

(13) Leopold, P. E.; Montal, M.; Onuchic, J. N. Protein folding funnels: a kinetic approach to the sequence-structure relationship. *Proceedings of the National Academy of Sciences of the United States of America* **1992**, *89* (18), 8721-5.

(14) Socci, N. D.; Onuchic, J. N.; Wolynes, P. G. Protein folding mechanisms and the multidimensional folding funnel. *Proteins* **1998**, *32* (2), 136-58.

(15) Wolynes, P. G. Symmetry and the energy landscapes of biomolecules. *Proceedings of the National Academy of Sciences of the United States of America* **1996**, *93* (25), 14249-55.
(16) Wolynes, P. G.; Eaton, W. A.; Fersht, A. R. Chemical physics of protein folding. *Proceedings of the National Academy of Sciences of the United States of America* **2012**, *109* (44), 17770-1.

(17) Wolynes, P. G.; Onuchic, J. N.; Thirumalai, D. Navigating the folding routes. *Science* **1995**, *267* (5204), 1619-20.

(18) Eddy, N. R.; Onuchic, J. N. Rotation-Activated and Cooperative Zipping Characterize Class I Viral Fusion Protein Dynamics. *Biophysical journal* **2018**, *114* (8), 1878-1888.

(19) Lin, X.; Eddy, N. R.; Noel, J. K.; Whitford, P. C.; Wang, Q.; Ma, J.; Onuchic, J. N. Order and disorder control the functional rearrangement of influenza hemagglutinin. *Proceedings of the National Academy of Sciences of the United States of America* **2014**, *111* (33), 12049-54.

(20) Honeycutt, J. D.; Thirumalai, D. The nature of folded states of globular proteins. *Biopolymers* **1992**, *32* (6), 695-709.

(21) Jana, B.; Onuchic, J. N. Strain Mediated Adaptation Is Key for Myosin Mechanochemistry: Discovering General Rules for Motor Activity. *PLoS computational biology* **2016**, *12* (8), e1005035.

(22) Noel, J. K.; Levi, M.; Raghunathan, M.; Lammert, H.; Hayes, R. L.; Onuchic, J. N.; Whitford, P. C. SMOG 2: A Versatile Software Package for Generating Structure-Based Models. *PLoS computational biology* **2016**, *12* (3), e1004794.

(23) Torrie, G. M.; Valleau, J. P. Monte-Carlo Free-Energy Estimates Using Non-Boltzmann Sampling - Application to Subcritical Lennard-Jones Fluid. *Chem. Phys. Lett.* **1974**, *28* (4), 578-581.

(24) Rodrigues, C. H.; Pires, D. E.; Ascher, D. B. DynaMut: predicting the impact of mutations on protein conformation, flexibility and stability. *Nucleic acids research* **2018**, *46* (W1), W350-W355.

(25) Rodrigues, C. H. M.; Myung, Y.; Pires, D. E. V.; Ascher, D. B. mCSM-PPI2: predicting the effects of mutations on protein-protein interactions. *Nucleic acids research* **2019**, *47* (W1), W338-W344.