Viral Capsid Equilibrium Dynamics Reveal Anisotropy in Elastic Properties

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Supporting Material

Materials and Methods

The structure of the CCMV capsid was obtained from VIPERdb (1) (id:1CWP). In this work, we are studying the wild-type unswollen form of CCMV, which is stable under low pH conditions around 5. To mimic the low pH environment we chose appropriate ionization states fro the titratable residues. We modeled all histidines as doubly protonated (type HSC in CHARMM19 FF), and the aspartic and glutamic acids were left unprotonated. Calcium ions were modeled into the structure at the pseudo-threefold axis site. The calcium ions are coordinated by side chain oxygen atoms on Glu81, Gln85, Glu148, Gln149*, Asp153*, where * indicates an adjacent subunit, both the side chain oxygens in Asp153 are coordinating atoms (2). The system, including the calcium ions, had a total net charge +1.0. The calcium ions were placed at the center of geometry of the six coordinating atoms, as shown in Fig. S1 below.

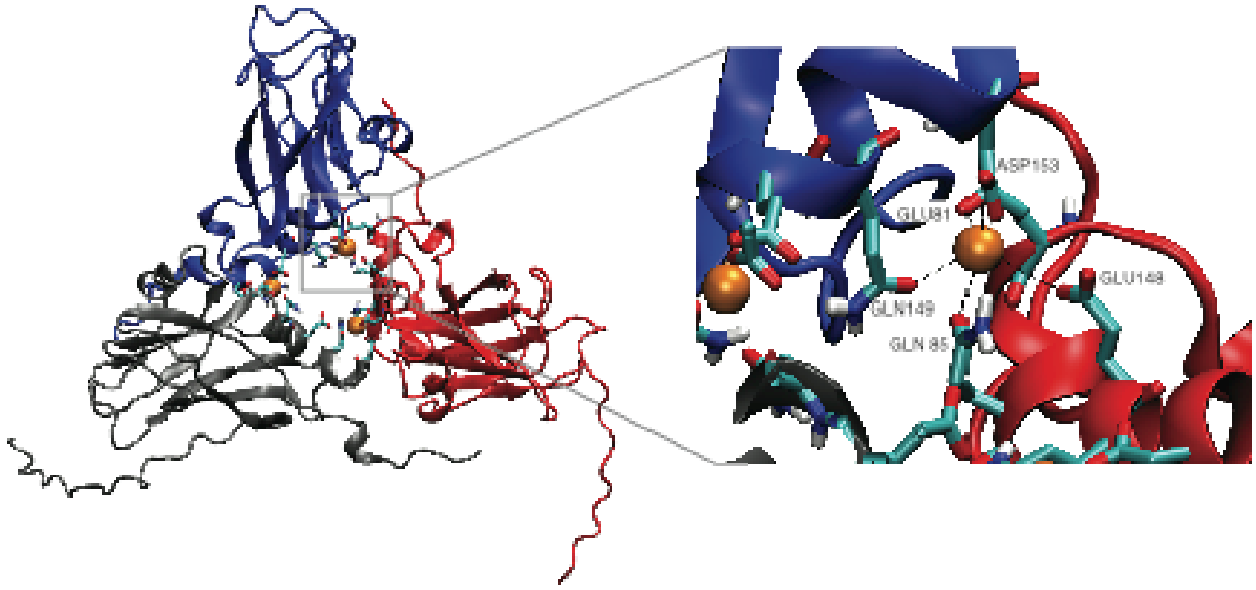

Figure S1. The 3-protien asymmetric unit of CCMV is shown on the left, including the 3 calcium ions (orange), and the coordinating residues. The image on right zooms in on the calcium ion bound at the A-B protein interface, the 5 coordinating residues are labeled.

Energy minimization and equilibration molecular dynamics (MD) were carried out on the system, all such calculations were performed in CHARMM (3, 4) using the CHARMM19 united-atom force field (3). The parameters for calcium were taken from the CHARMM22 force field (5)

and used without modification. The distance between the calciums and the coordinating oxygens were restrained to their original distances using a harmonic restraint with force constant of 10 kcal/(mol*Å^2). The GBSW implicit solvent model (6) was used in all calculations as were rotational-symmetry boundary conditions (7), effectively modeling the full capsid under an icosahedral symmetry constraint. Non-bonded forces were calculated between atom pairs separated by less than 14 Å. Minimization was performed for several cycles using various restraints, each cycle consisted of 1000 steps of steepest descents (SD), followed by 2000 steps of adopted basis Newton-Raphson (ABNR). In the first minimization cycle all non-hydrogen atoms were restrained by 100 kcal/(mol*Å^2) harmonic positional restraint, that restraint was released and a 100 kcal/(mol*Å^2) harmonic positional restraint was placed on the protein backbone atoms and another cycle of minimization was performed, then the force constant on the protein backbone atoms was reduced to 50 kcal/(mol*Å^2) and a final minimization cycle was performed. The restraints on the backbone atoms were further reduced to 20 kcal/(mol*Å^2) and MD were performed for 20 ps. The dynamics were carried out using a Langevin integrator with a time step of 2 fs, a friction factor of 5.0 ps^{-1} coupling the system to a heat bath at 300 K, and the SHAKE algorithm (9) was used to constrain all bonds involving hydrogen atoms. Production dynamics were computed for 28 ns by releasing the backbone restraints and using the same simulation parameters as the restrained dynamics.

The final 10 ns of the equilibrium dynamics were used to compute spherical harmonic mode magnitudes. This was done by first computing the capsid surface by binning atoms on an equiangular grid. All surfaces were computed on a grid with 24 grid points in both the polar and azimuthal directions, for a total of 576 grid points. The positions of the surface are given by the average radial position of all heavy atoms falling into each bin (non-mass weighted). The decomposition is then preformed using the SpharmonicKit software package (10).

The elastic network model was built from the average structure during the final 10 ns of the asymmetric unit MD simulation. The icosahedral symmetry operations were performed on the asymmetric unit to construct the complete capsid. All non-hydrogen atoms were included in the network, including the calcium ions; a harmonic bond was placed between any two atoms within 5 Å of each other. In our formulation of the elastic network, we assign a mass of 1 arbitrary mass unit and set each spring constant to 1 arbitrary energy unit/Å^2. By computing the second derivates of the network potential energy, a Hessian matrix is constructed, which can be diagonalized to obtain the normal modes and frequencies of the network. The diagonalization of the Hessian was performed using the rotation-translation of blocks method (8, 11), available through the Multiscale Modeling Tools in Structural Biology (12). In this method, segments of the structure can be treated as rigid "blocks"; we have chosen five consecutive residues as a rigid block.

The ENM trajectory was computed for 5000 steps with a time step of 0.5 time units (chosen based upon the frequencies of the modes) according to $\Delta\mathbf{r}_n(t) = \sum_{i=7}^{N} \frac{C_i}{\omega_i} \boldsymbol{\alpha}_n^i \cos(\omega_i t + \psi_i)$ where, $\Delta\mathbf{r}_n$ is the displacement vector for atom n, $\boldsymbol{\alpha}_n^i$ is the component of Eigenvector i projected onto atom n, ω is the mode frequency, ψ is a phase shift of the mode and C is an amplitude factor, the sum starts at 7 because first 6 modes correspond to rigid body translation and rotation, and goes to mode N, in practice we choose N=999. We choose to set the amplitude factor the same value for all modes and choose a value for C that will give atomic fluctuations typical of the thermal scale (300 K). The mean-squared fluctuation

(msf) of each atom is given by $\left\langle \left|\Delta r_n\right|^2 \right\rangle = \frac{C^2}{2}\sum_{i=7}^{N}\left|\frac{\alpha_n^i}{\omega_i}\right|^2$ and we can determine *C* by setting the average msf = 1 Å^2.

The spherical harmonic decomposition of the ENM trajectory is performed in the same manner as for the MD trajectory. The initial coefficients from the ENM ($\left\langle \left|\hat{a}_l\right|^2 \right\rangle_{ENM}$) are renormalized by the MD coefficients according to $\left\langle \left|\hat{a}_l\right|^2 \right\rangle_{ENM}^{\#} = \left(\frac{\sum_l \left\langle \left|\hat{a}_l\right|^2 \right\rangle_{MD}}{\sum_l \left\langle \left|\hat{a}_l\right|^2 \right\rangle_{ENM}}\right)\left\langle \left|\hat{a}_l\right|^2 \right\rangle_{ENM}$, where # indicates the renormalized values. This renormalization has the effect of conserving the sum of the mode magnitudes between the MD and ENM systems, which is a representation of the system temperature according to our elastic model (see Eq. 2 in main text).

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