Electronic Supplementary Material (ESI) for Chemical Science.

Determination of intermediate state structures in the opening pathway of SARS-CoV-2 spike using cryo-electron microscopy

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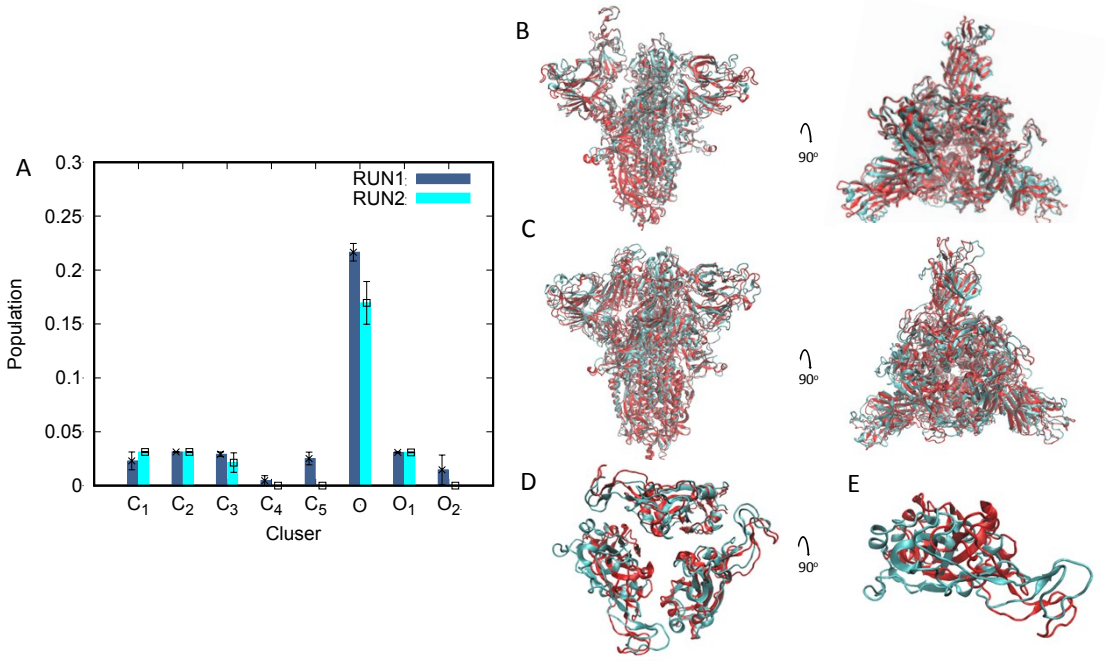

Figure S1. Analysis of the structural ensemble of spike determined in this study from the electron density map of the open state. **(A)** Convergence analysis of the structural ensemble, showing the populations of the intermediate states. **(B)** Structure comparison between a previously determined open state structure (6VSB) (*6*) (red) and the open state determined in the structural ensemble (cyan). **(C)** Structural comparison between a previously determined closed state (6VXX) (*6*) (red) and the most populated closed state C_1, which was determined in this study from the electron density map of the open state (cyan). **(D)** RBD structure comparison between the 6VXX structure of the closed state (*6*) (red) and the most populated closed state C_1 determined in this study (cyan). **(E)** RBD_1 structure comparison between the 6VXX structure of the closed state (*6*) (red) and the most populated closed state C_1 determined in this study (cyan).

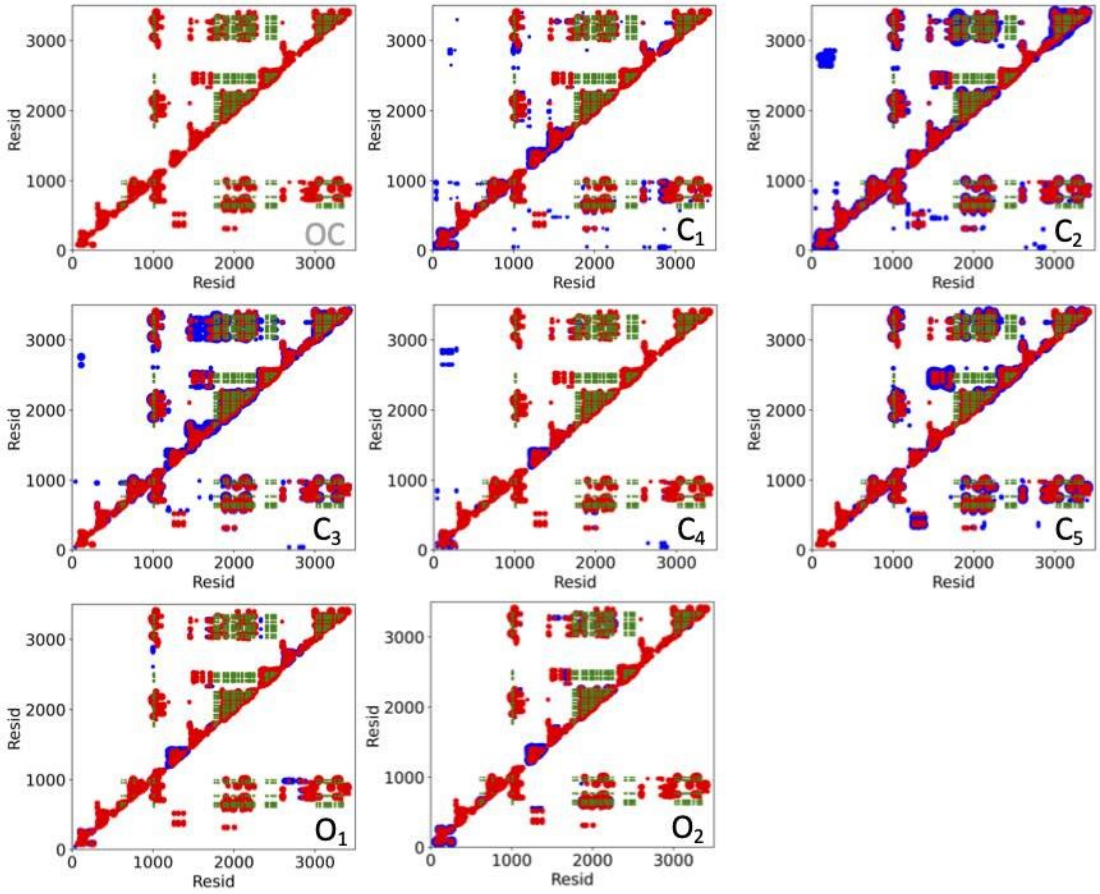

Figure S2. Contact map analysis for the residues in the cryptic pocket determined in this study. Residues pairs participating in the pocket in the closed and open states are shown in green and red, respectively. In all plots apart from the OC, we also overlay in blue the pocket residue map in the respective states, i.e. C_1, C_2, C_3, C_4, C_5, O_1 and O_2.

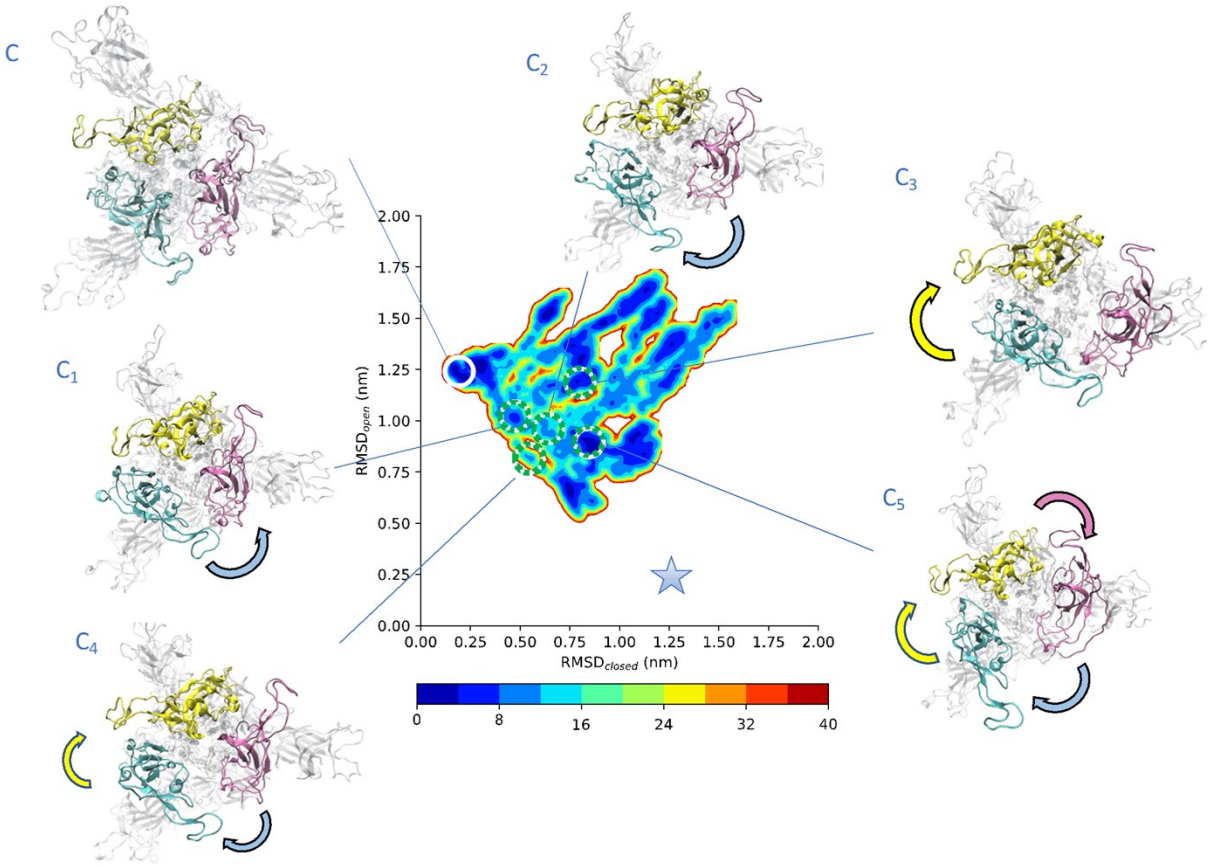

Figure S3. Determination of intermediate states in the closing transition pathways of spike. The structural ensemble of the closed state of spike is represented here as a free energy landscape as a function of the distances from previously reported closed state (PDB:6VXX) and open state (PDB:6VSB) structures. Cyan, purple and yellow arrows indicate the motions of RBD_1, RBD_2 and RBD_3, respectively. The common intermediate states (C_1, C_2, C_3, C_4 and C_5) in the structural ensemble calculations using the open and the closed EM maps are highlighted in the free energy landscape with dotted green-white circles. The closed state C is highlighted with solid white circle. The position of the open state structure (PDB:6VSB) is highlighted with an asterisk, as it is not sampled in the structural ensemble of the closed state. The energy is given in k_BT units.

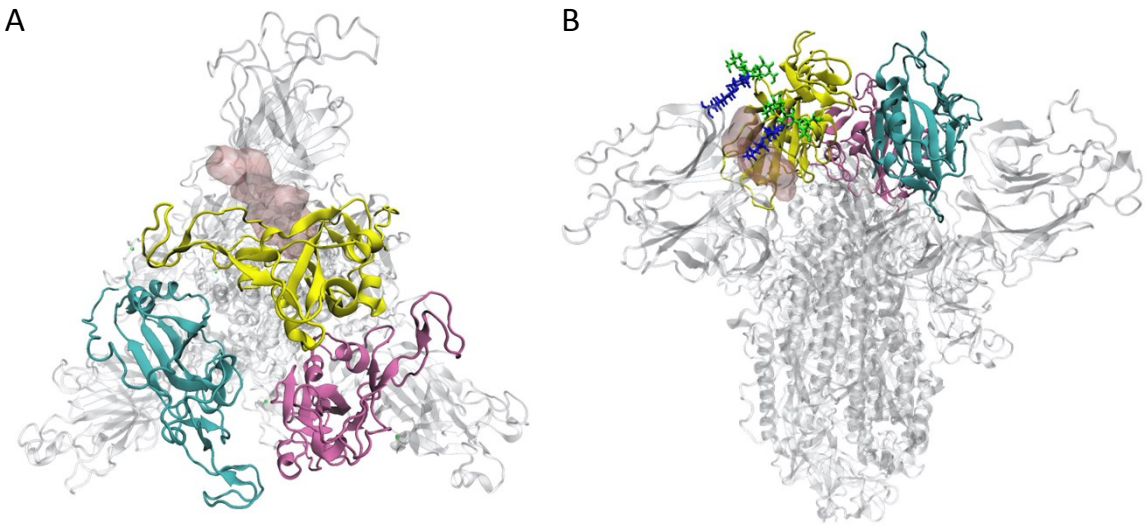

Figure S4. Identification of a cryptic pocket in an intermediate state populated in the closing transition of spike. **(A)** Position (shown in pink) of the cryptic pocket between the NTD and the RBD, which is present in the intermediate state C_1. **(B)** Structural of the glycans in the NTD and RBD in the intermediate state C_1, illustrating their protective role of the cryptic pocket.

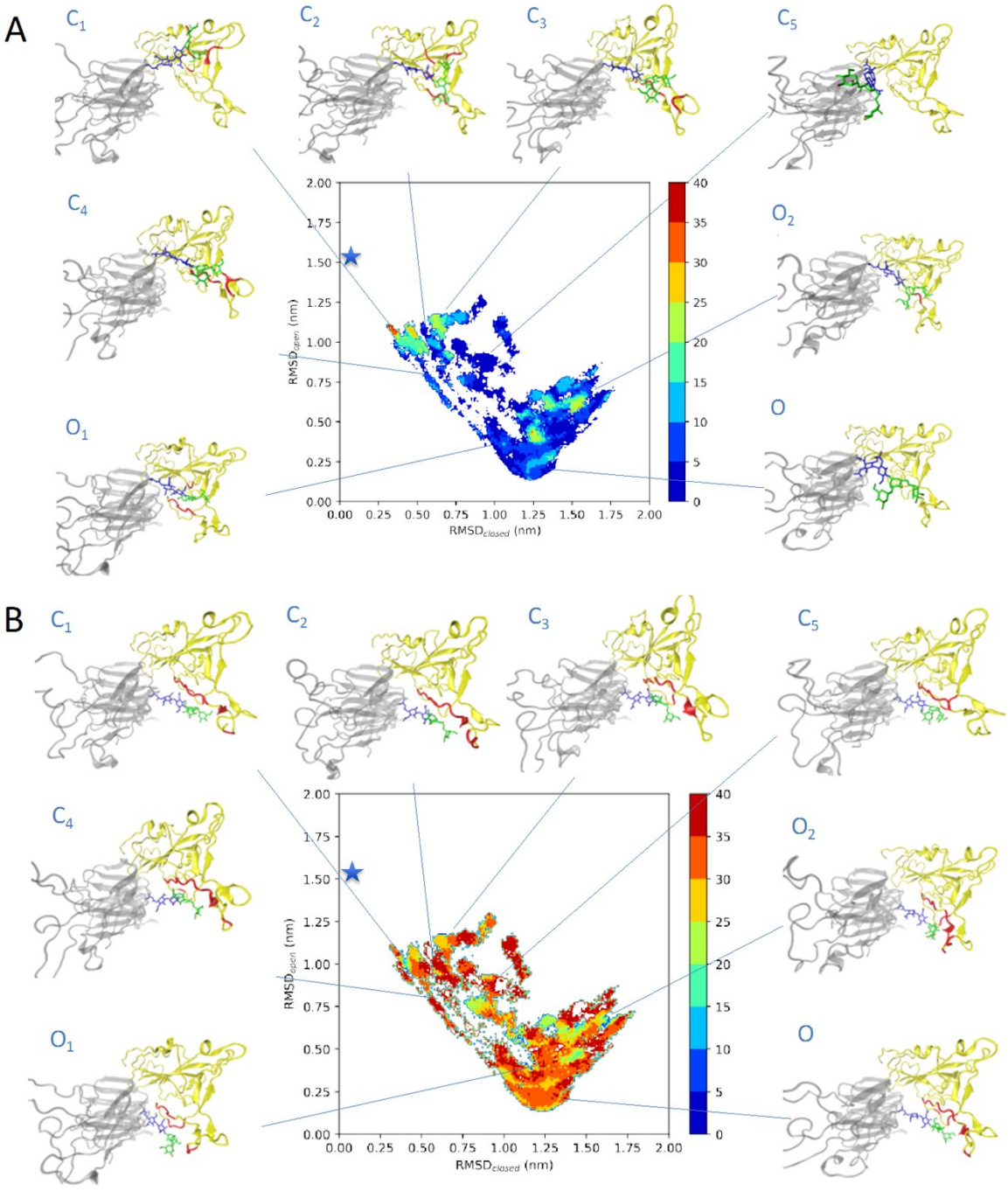

Figure S5. Role of the glycans N165 and N245 in protecting the cryptic binding pocket. Contacts between the down RBD_3 and N165 (A) and N245 (B), projected along the distance from the open and the closed state coordinates.

States	*C1*	*C2*	*C3*	*C4*	*C5*	*O1*	*O2*	*O*
#Pockets	7.80 ± 0.37	7.24 ±0.09	6.38± 1.87	6.21 ± 0.27	6.68 ± 0.98	5.44 ± 0.36	6.33 ± 0.28	6.78 ± 0.01

Table S1. Average number of binding pockets formed in each of the states identified in this work with druggability scores higher than 0.45.