Structure, Volume *29*

Supplemental Information

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Supplemental Information (Table and Figures)

Conformational flexibility and structural variability of SARS-CoV2 S protein

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Table S1: **Data collection and refinement parameters of S protein,** Related to STAR*Methods and Figures 1-3

	pH 6.5				**pH 8.0**		**pH 7.4**				**pH 7.4**
Data collection and processing	**Class2**	**Class3**	**Class4**	**Class5**	**Class1**	**Class2**	**Class3**	**Class 5**	**Class 8**	**Class 9**	**C3 Symmetry 3-RBD Down**
Magnification	42000	42000	42000	42000	42000	42000	42000	42000	42000	42000	42000
Voltage (kV)	200	200	200	200	200	200	200	200	200	200	200
Electron exposure (e–/Å2)	80	80	80	80	80	80	80	80	80	80	80
Number of frames	20	20	20	20	20	20	20	20	20	20	20
Defocus range (μm)	-0.75 to -2.25	-0.75 to -2.25	-0.75 to -2.25	-0.75 to -2.25	-0.75 to -2.25	-0.75 to -2.25	-0.75 to -2.25	-0.75 to -2.25	-0.75 to -2.25	-0.75 to -2.25	-0.75 to -2.25
Pixel size (Å)	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17
Symmetry imposed	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C3
Number of movies	3166				2405		4504				
Number of particles in model	78742	49370	94237	87829	34904	54153	85701	217396	140411	132606	347752
Map resolution (Å)	4.75	4.9	5.44	4.53	5.76	5.34	4.6	4.16	4.47	4.34	3.88
FSC threshold	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143
Map sharpening B factor (Å2)	-150	-150	-150	-150	-100	-100	-100	-100	-70	-100	-150
EMRinger Score	1.33	0.66	0.24	1.81	0.37	0.45	0.85	2.08	0.59	1.64	1.64
RBD conformation	**3-RBD Down**	**1-RBD Up**	**1-RBD Up**	**3-RBD Down**	**1-RBD Up**	**3-RBD Down**	**3-RBD Down**	**1-RBD Up**	**1-RBD Up**	**3-RBD Down**	**3-RBD Down**

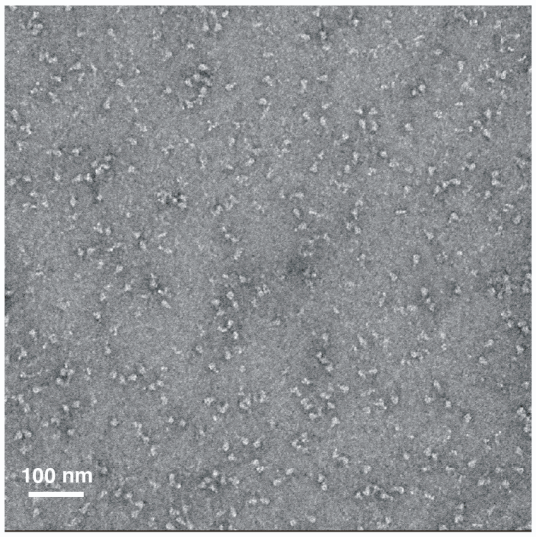
Table S2: **MolProbity score of atomic models of S protein,** Related to STAR*Methods and Figure 2-3

Validation	pH 6.5 Class2	pH 6.5 Class3	pH 6.5 Class4	pH 6.5 Class5	pH 7.4 Class3	pH 7.4 Class5	pH 7.4 Class8	pH 7.4 Class9	pH 8.0 Class1	pH 8.0 Class2
MolProbity score	2.28	2.62	2.91	2.37	2.34	2.26	3	2.38	2.59	2.45
Clash score	17.73	34.26	56.62	20.07	23.41	16.19	61.17	23.45	41.46	32.49
Poor rotamers (%)	0	0	0	0	0	0.04	0	0.04	0	0.04
Ramachandran plot										
Outlier (%)	0	0.11	0.07	0	0.07	0.18	0.54	0	0.07	0
Allowed (%)	9.45	12.19	16.41	10.86	7.69	9.93	19.7	8.89	8.22	7.17
Favored (%)	90.55	87.7	83.52	89.14	92.24	89.89	79.76	91.11	91.71	92.83

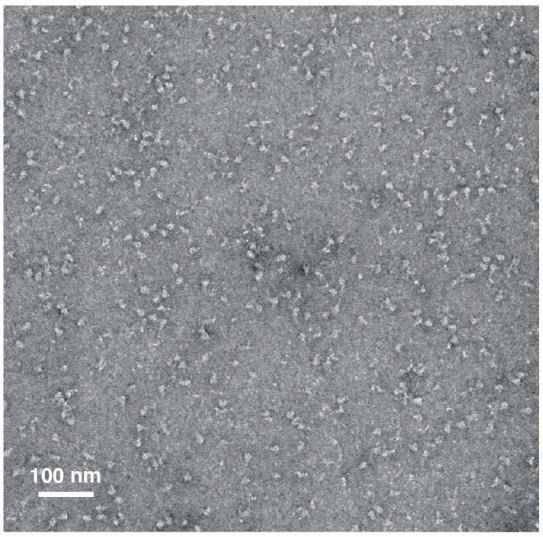
Table S3: **Pairwise RMSD calculation,** Related to Figures 2 and 3

Pairwise RMSD (Angstrom) calculation between different classes of different pH									
	Pairwise RMSD		**Atoms aligned**	**Residue number**		**Pairwise RMSD**		**Atoms aligned**	**Residue number**
		Class 8					**Class 4**		
pH 7.4	**Class 5**	**1.672**	189	27-291	**pH 6.5**	**Class 3**	**1.77**	189	27-291
	Class 5	**2.868**	161	331-528		**Class 3**	**3.017**	161	331-528
	Class 5	**1.756**	16	696-711					
	Class 5	**1.509**	84	1064-1147					
	Class 5	**1.693**	52	1096-1147					
	Pairwise RMSD		**Atoms aligned**	**Residue number**		**Pairwise RMSD**		**Atoms aligned**	**Residue number**
		Class 9					**Class 2**		
pH 7.4	**Class 3**	**4.408**	203	27-291	**pH 6.5**	**Class 5**	**1.877**	203	27-291
	Class 3	**1.744**	178	331-528		**Class 5**	**1.519**	178	331-528

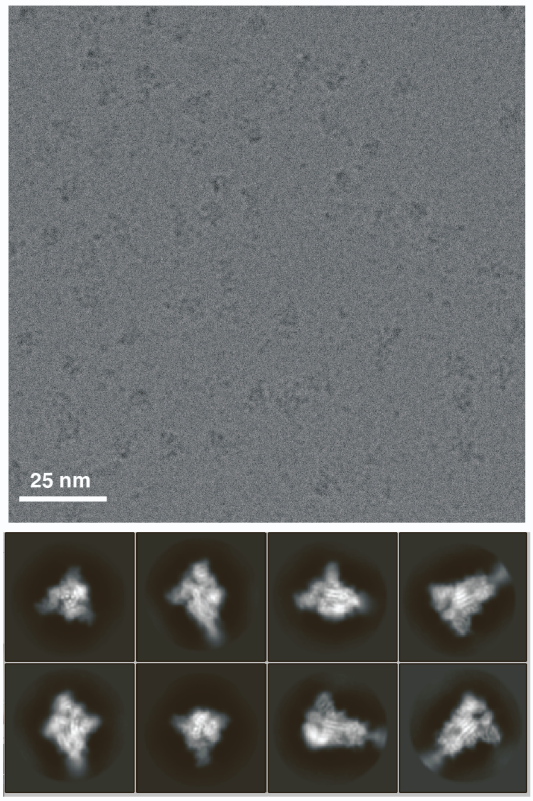
A. NS micrograph at pH 6.5



B. NS micrograph at pH 8.0



C. Cryo-EM micrograph and 2D Classification at pH 6.5



D. Cryo-EM micrograph and 2D Classification at pH 8.0

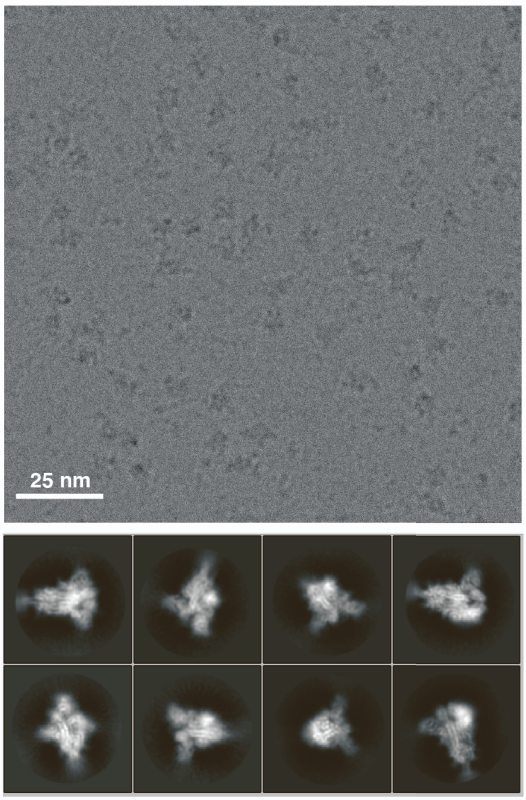


Figure S1: **Negative staining and cryo-Electron microscopy micrograph and 2D class averages of S protein at pH 6.5 and 8.0, Related to Figure 1:** A. Negative staining micrograph of S-protein at pH 6.5. B. Negative staining micrograph of S-protein at pH 8.0. C. Cryo-Electron microscopy micrograph and reference free 2D class averages of S-protein at pH 6.5. D. Cryo-Electron microscopy micrograph and reference free 2D class averages of S-protein at pH 8.0.

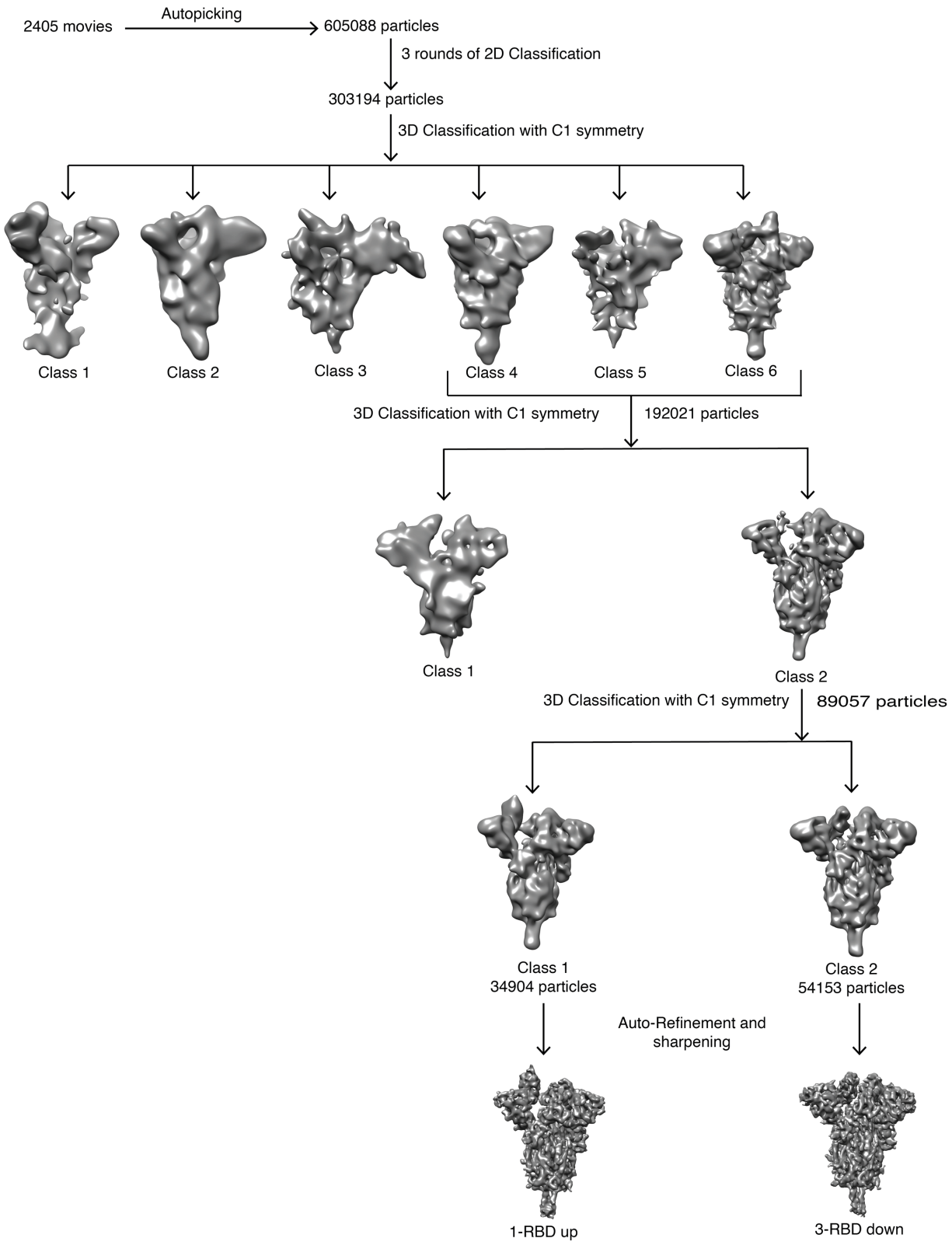

2405 movies
Autopicking
605088 particles
3 rounds of 2D Classification
303194 particles
3D Classification with C1 symmetry
Class 1
Class 2
Class 3
Class 4
Class 5
Class 6
3D Classification with C1 symmetry
192021 particles
Class 1
Class 2
3D Classification with C1 symmetry
89057 particles
Class 1
34904 particles
Class 2
54153 particles
Auto-Refinement and
sharpening
1-RBD up
3-RBD down

Figure S2: **Pipeline of data processing using single particle cryo-EM and 3D classification of S protein at pH 8.0, Related to STAR*Methods and Figure 1**: Cryo-EM data processing workflow and structure determination of S protein. Detailed procedures provided in method section. Different 3D classification models are marked as 1-RBD up and 3-RBD down conformations.

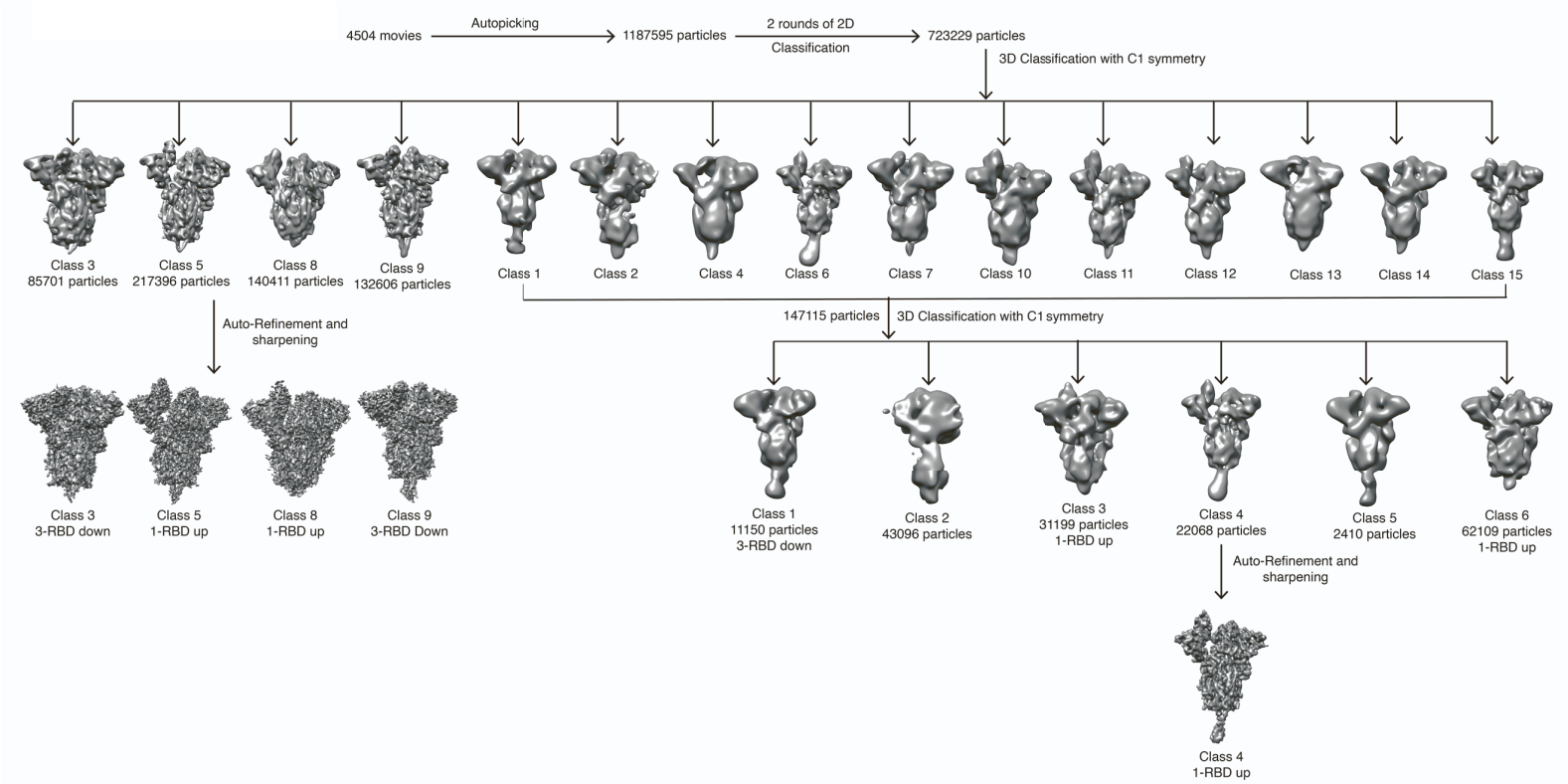


Figure S3: **Pipeline of data processing using single particle cryo-EM and 3D classification of S protein at pH 7.4, Related to STAR*Methods and Figure 2**: Cryo-EM data processing workflow and structure determination of S protein. Detailed procedures provided in method section. Different 3D classification models are marked as 1-RBD up and 3-RBD down conformations.

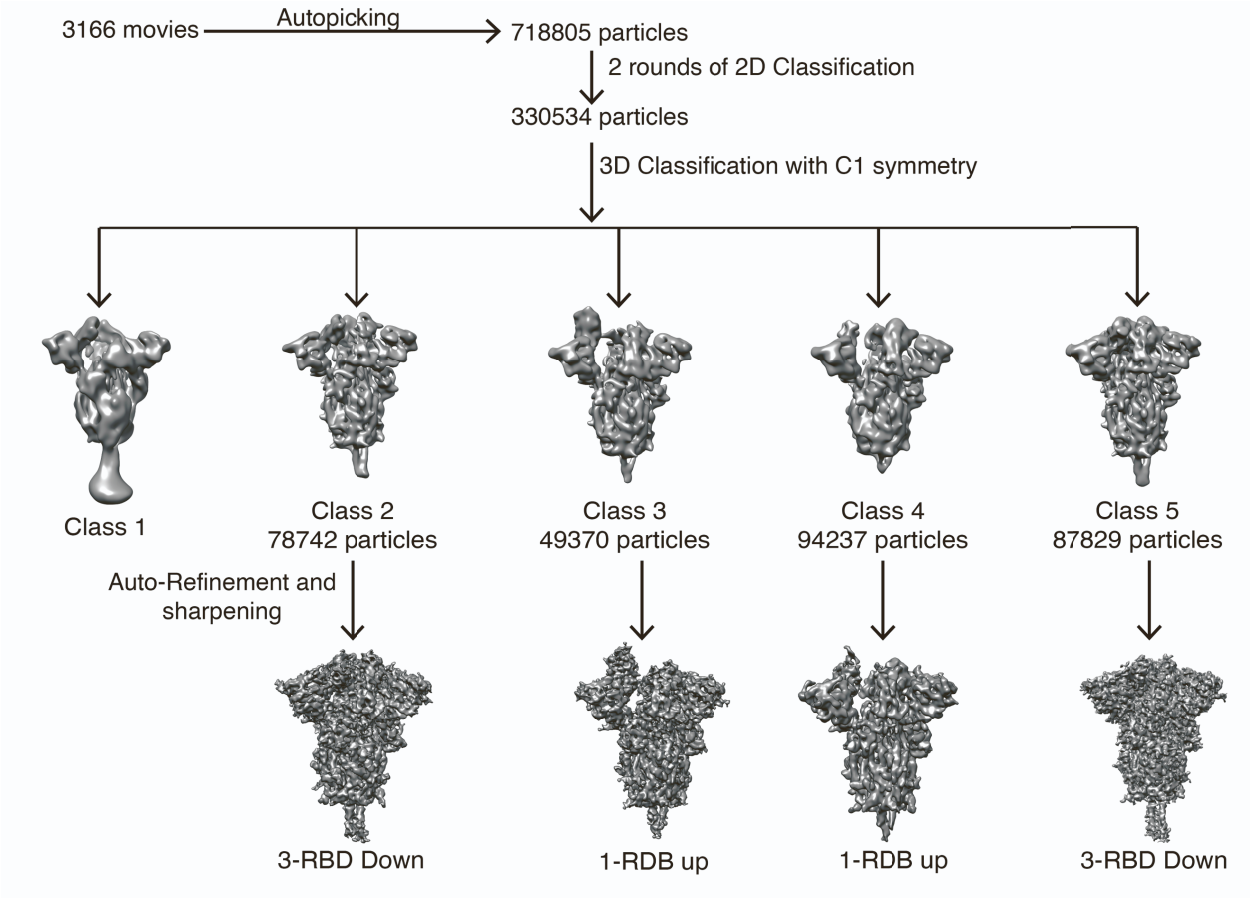


Figure S4: **Pipeline of data processing using single particle cryo-EM and 3D classification of S protein at pH 6.5, Related to STAR*Methods and Figure 3**: Cryo-EM data processing workflow and structure determination of S protein. Detailed procedures provided in method section. Different 3D classification models are marked as 1-RBD up and 3-RBD down conformations.

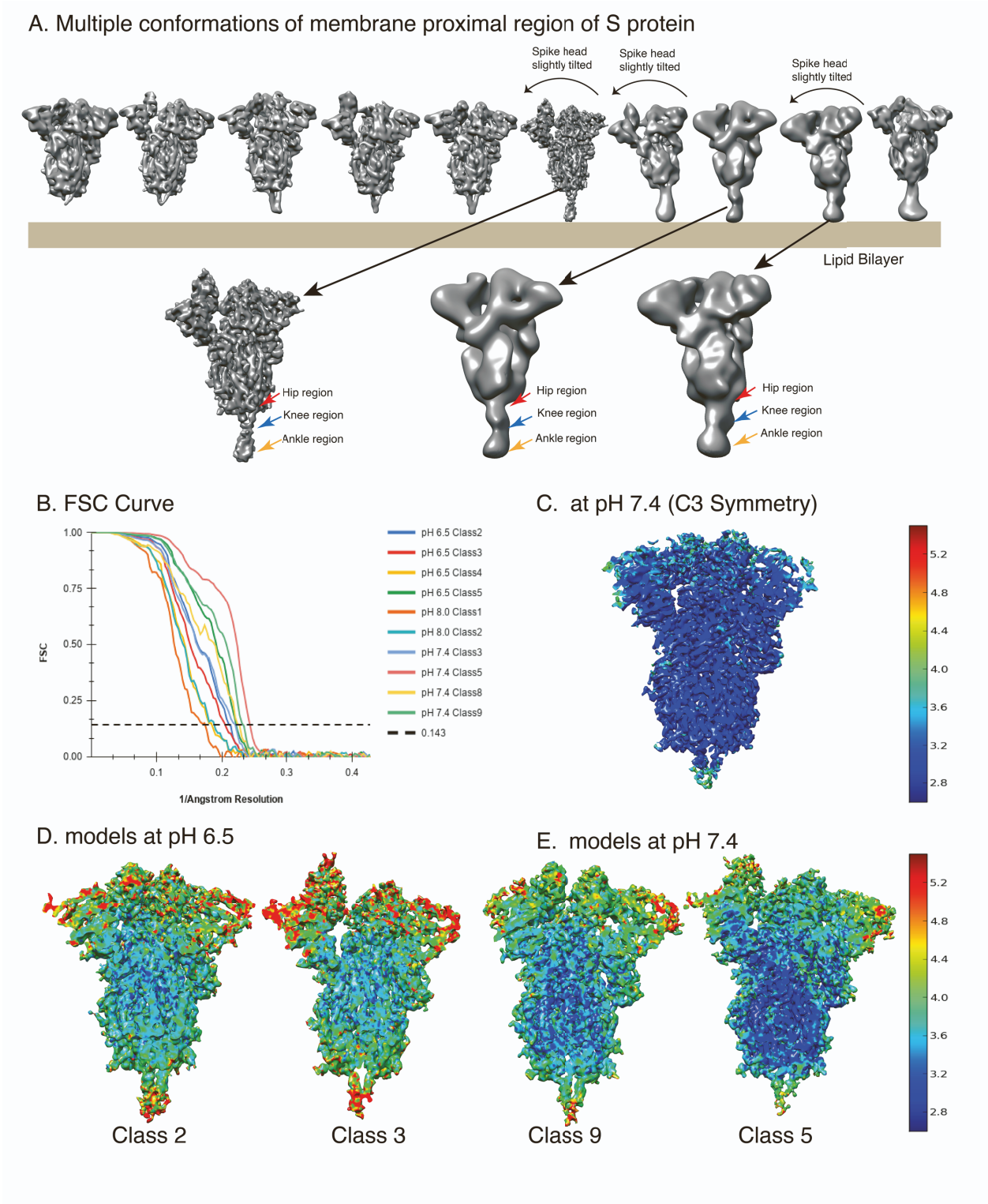

A. Multiple conformations of membrane proximal region of S protein
Spike head slightly tilted
Spike head slightly tilted
Spike head slightly tilted
Lipid Bilayer
Hip region
Knee region
Ankle region
Hip region
Knee region
Ankle region
Hip region
Knee region
Ankle region
B. FSC Curve
FSC
1/Angstrom Resolution
pH 6.5 Class2
pH 6.5 Class3
pH 6.5 Class4
pH 6.5 Class5
pH 8.0 Class1
pH 8.0 Class2
pH 7.4 Class3
pH 7.4 Class5
pH 7.4 Class8
pH 7.4 Class9
0.143
C. at pH 7.4 (C3 Symmetry)
D. models at pH 6.5
Class 2
Class 3
E. models at pH 7.4
Class 9
Class 5

Figure S5: **Multiple conformations of membrane proximal region of S protein, FSC calculation and local resolution estimation, A- Related to main text, B-E- Related to Figures 2 and 3**: A. Membrane proximal region (Stalk region) placed on a cartoon representation of lipid bilayer to identify the orientation of spike head. Hip, knee and ankle regions are marked with red arrows, blue arrows and yellow arrows respectively. B. Gold standard Fourier Shell Correlation (FSC) calculated for the high resolution cryo-EM models of S protein at pH 8.0, pH 7.4 and pH 6.5 using RELION 3.0. C. Local Resolution calculated for high resolution 3-RBD down model of S protein at pH 7.4 (imposing C3 Symmetry) using ResMap. D. Local Resolution calculated for the high resolution 3-RBD down and 1-RBD up models of S protein at pH 6.5 (imposing C1 symmetry) using ResMap E. Local Resolution calculated for the high resolution 3-RBD down and 1-RBD up models of S protein at pH 7.4 (imposing C1 symmetry) using ResMap.

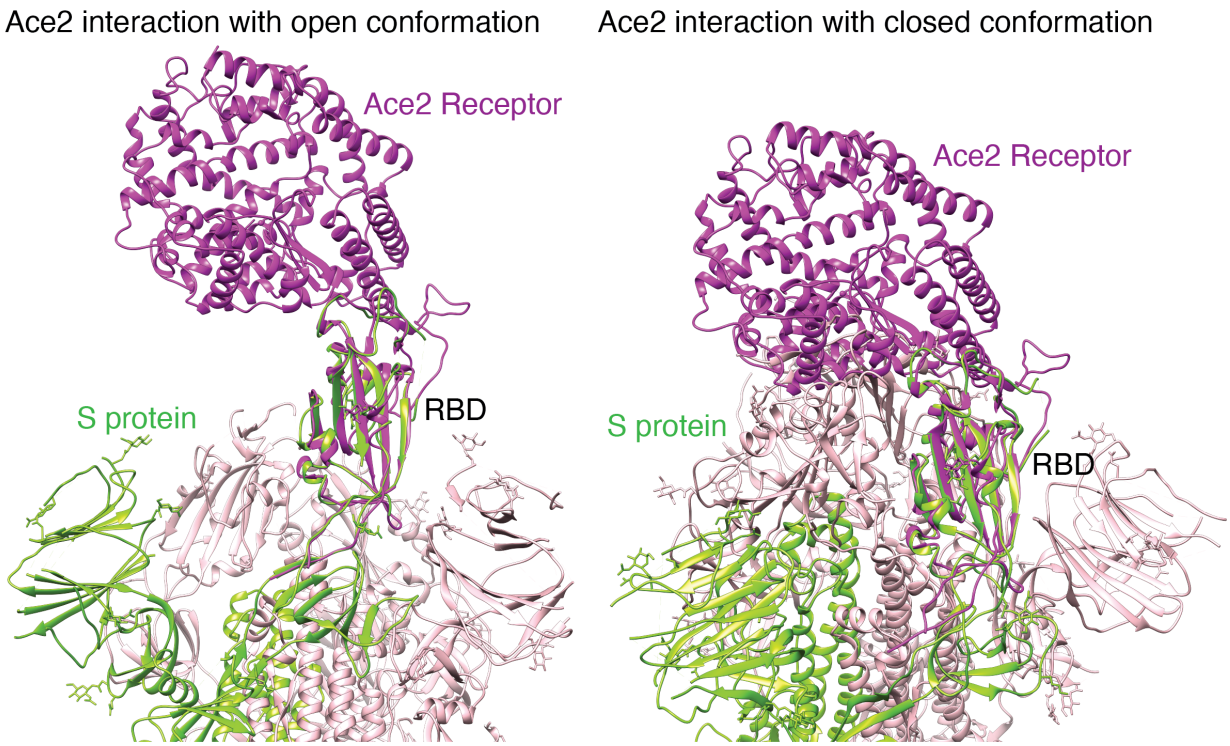


Figure S6: **ACE2 interaction with open and closed conformations of S protein, related to Figure 2**: hACE2 receptor (PDBID: 7A94.pdb) and RBD of SARS CoV-2 S protein superimposed with Class 5 (open state; left) and Class 3 (closed state; right) at pH 7.4. Steric clashes observed with adjacent RBDs in closed model illustrate the significance of open conformation for interaction with host cell ACE2 receptor.