Supplementary Material:
Rational Design of Dengue Vaccines: Integrating Mutagenesis, Structure Prediction, and Docking Simulations in *Nicotiana tabacum*

Table S1. Stakeholder Identification Table

No.	Stakeholder	Question
1.	Patient of Dengue Fever	**1. SYMPTOMS** a. What are the initial symptoms experienced when infected with dengue fever? b. How severe are these symptoms? c. How long do these symptoms persist before dengue fever is diagnosed? **2. TREATMENT** a. What is the procedure for handling dengue fever patients in the hospital? b. What care and treatments are provided by the hospital? c. Are there any traditional medicine that are family recipe to treat dengue fever? **3. AWARENESS PROGRAMS** a. What is the role of the government in addressing dengue fever cases? b. How does the community respond to government programs aimed at tackling dengue fever cases?
2.	Medical Center	**1. CASES** a. How many dengue fever (DF) cases are there in the area, in particular Sleman? b. How many people have been affected by DF and dengue hemorrhagic fever (DHF) approximately? c. How many deaths due to dengue fever have occurred in the area? **2. SYMPTOMS** a. What symptoms are commonly experienced by patients that lead to a diagnosis of DF and referral to the hospital? b. At what stage are most dengue fever patients when they visit the health center? **3. PUBLIC HEALTH**

		a. What activities have been carried out by the medical center to address dengue fever cases? b. How often are these activities conducted, and what are their objectives? c. Is dengue fever vaccination distributed at the health center? d. How is the distribution managed? e. Which dengue fever vaccine is being used? **4. TREATMENT AND AWARENESS** a. How does the community respond to dengue fever prevention activities? b. When dengue fever patients visit the health center, to what extent can the health center assist these patients?
3.	Sleman District Health Office	**CASES** a. How many dengue fever (DF) cases are there in the area? Is there a trend of increasing cases over the past 5 years? b. Approximately how many people have been affected by DF and dengue hemorrhagic fever (DHF), categorized by gender and age? c. How many deaths due to dengue fever have occurred in the area? d. What are considered the main factors behind the rise in DHF cases in Sleman compared to other regions? Are factors such as climate change, urbanization, or others contributing? e. How well does the community understand the dangers of DHF? What educational strategies are being implemented to raise awareness about DHF prevention, such as mosquito breeding eradication programs? f. What is the overall impact of dengue cases on society, considering all aspects? **VACCINE** a. How is the dengue vaccine obtained by the community, and what is the community's acceptance of it?

		b. Is the vaccine accessed independently by individuals, or is it facilitated by the government (Sleman District Health Office)? c. What type of dengue vaccine is used and how is it distributed? d. What is the perspective of Sleman District Health Office on the development of dengue vaccines, and to what extent do they see the potential of this vaccine in addressing current challenges in Sleman? **REGULATIONS AND HEALTH DEPARTMENT PROGRAMS** a. Are there any regulations governing the prevention and treatment of dengue fever cases? b. What measures has the Sleman District Health Office taken to control the spread of DHF, including prevention and treatment efforts for the community? c. How does the Sleman District Health Office coordinate with other healthcare institutions (health centers) in handling dengue fever cases? d. How prepared are healthcare facilities in Sleman to handle surges in DHF cases, including medicine supplies, the number of healthcare facilities (hospitals/health centers/clinics), and medical personnel? e. Are there specific challenges in implementing dengue fever management programs (e.g., with vaccines), such as regulations, budgets (noting that plant-based vaccines might be lower cost), or community involvement?
4.	Ministry of Health of the Republic of Indonesian	**DENGUE FEVER EPIDEMIC** 1. How have Dengue Fever (DF) cases in Indonesia trended over the past few years? Has there been an increase in cases? 2. How many DF cases in Indonesia have resulted in deaths, and has there been an increase in mortality rates over the past few years? 3. Which regions have reported the highest number of DF cases? What are the contributing factors? **GOVERNMENT PROGRAM**

		1. What initiatives has the government undertaken to address DF cases in Indonesia? 2. What preventive programs have been implemented by the government to minimize DF cases in the community? 3. What challenges does the government face in managing DF cases? Are there any instances of public resistance? 4. What strategies does the government employ to educate the public on DF prevention and control? **VACCINE DEVELOPMENT** 1. What is the progress of Dengue vaccine development in Indonesia? 2. What types of vaccines are currently being developed or have already been developed? 3. Is there collaboration between the government and researchers in developing the Dengue vaccine? 4. What challenges has the government encountered in vaccine development over the years? 5. What are the regulations governing the production and distribution of Dengue vaccines? **INNOVATION** 1. What is the government's stance on innovations such as plant-based bivalent Dengue vaccines? 2. Has the government supported similar research in the past? 3. Does the government have specific regulations or guidelines for vaccine research and production in Indonesia?
5.	Indonesian Food and Drug Monitoring Agency (BPOM)	**REQUIREMENTS** 1. What is BPOM's perspective on the innovation of plant-based DHF vaccines? Has any similar research received distribution approval from BPOM?

		2. What are BPOM's regulations regarding the approval of vaccines for distribution? 3. What are the basic requirements that vaccine manufacturers must meet in order to obtain approval from BPOM in Indonesia? 4. What documents are required by BPOM in the process of submitting an application for DHF vaccine approval, and what is the procedure for submission? 5. On average, how long does it take for BPOM to grant approval for a vaccine, especially for a new vaccine? 6. What criteria does BPOM use to assess the quality and safety of a vaccine before granting approval for distribution? 7. Does BPOM have specific rules regarding clinical trials for plant-based vaccines before approval for distribution can be granted? **MONITORING PROGRAM** 1. What steps does BPOM take to ensure that the supervision and monitoring of vaccines that have received approval are carried out effectively? 2. Does BPOM have regulations regarding the distribution of approved vaccines to ensure they reach the public in safe condition? 3. What challenges does BPOM often face when granting approval for new or innovative vaccines? 4. How is the environmental assessment of synthetic biology products implemented in Indonesia?

Table S2. Mutant Generation Result

No	DENV2		DENV3	
	Mutation Position	**Score**	**Mutation Position**	**Score**
1	C302E	2.895199537	P334T	2.600211143
2	Y326N	2.68605566	C300D	2.385468245
3	C333V	2.19573164	C331V	2.383506775
4	H346K	2.183547497	H345V	1.995705843
5	W391L	1.946171284	W389V	1.966603994
6	H317K	1.689182281	H315E	1.966442585
7	F392K	1.674343109	Y390K	1.813663125
8	M301G	1.662555695	P369V	1.691242933
9	N366T	1.626881123	Y297G	1.687600255
10	R345G	1.507603168	M299D	1.522553444
11	P356G	1.505821228	N364V	1.502995729
12	F373E	1.472773552	F371E	1.446895123
13	M340K	1.437977552	P330T	1.402588844
14	P371L	1.38369751	P354G	1.392884493
15	Y299G	1.31979394	E360G	1.33241272
16	T359L	1.266584277	Y324G	1.293160677
17	F306G	1.237601042	A367V	1.276085138

18	M297G	1.197107792	R348V	1.256219625
19	I380E	1.178946257	N346L	1.203291893
20	A369I	1.112708688	G342V	1.195405126
21	Q386K	1.061329365	L301G	1.171097517
22	N390K	1.034748793	P362V	1.127448559
23	P364V	1.012950659	Q341K	1.049582243
24	D362G	0.9753689766	L319T	1.049442649
25	Y377G	0.9656157494	T337V	0.9940526485
26	Q316E	0.9220318794	P370V	0.9813220501
27	A313K	0.9171237946	S374G	0.943883419
28	L351K	0.9108345509	Q314E	0.9405589104
29	P336E	0.910456419	N388K	0.9133369923
30	K344G	0.9036477804	F304K	0.9130589962
31	Q325E	0.8996145725	A344L	0.895660162
32	S376Y	0.880494833	G379K	0.8934106827
33	I378E	0.8764894009	T357V	0.8918185234
34	S300D	0.8368589878	A329V	0.8896474838
35	F337V	0.8271378279	N375E	0.8685064316
36	R323E	0.7745893002	K358V	0.8128187656

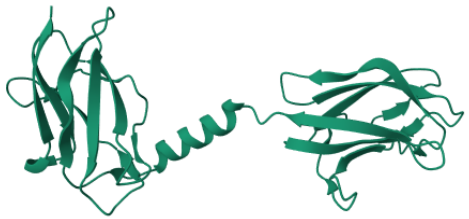
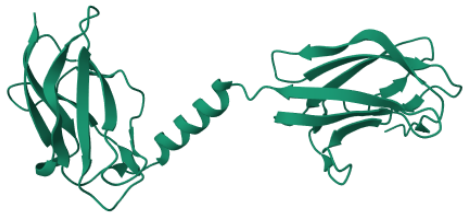
37	V321T	0.7683391571	N302G	0.8127007484
38	R350K	0.7606422901	K308G	0.8117909431
39	K307G	0.73808074	N353K	0.8022422791
40	N355K	0.7336175442	E368L	0.7932584286
41	S331K	0.7096738815	G347L	0.7898206711
42	S363K	0.6926679611	T303G	0.7848792076
43	G381E	0.6823232174	R391K	0.7783725262
44	P372V	0.6802537441	T351L	0.7777249813
45	T315G	0.6569824219	A384E	0.6831877232
46	K310G	0.6524817944	A352L	0.6772167683
47	E370L	0.6488497257	I380G	0.6702308655
48	V382K	0.6242008209	E338L	0.6313073635
49	P384D	0.6108925343	T313G	0.6311676502
50	L342K	0.6079423428	A298E	0.6310679913
51	V354G	0.6073713303	S311K	0.6289703846
52	I320V	0.583886385	K332T	0.611105442
53	I322V	0.5705845356	I320V	0.6037522554
54	T319K	0.566390276	K383G	0.6027984619
55	T303G	0.5525155067	D328E	0.5902431011

56	I379E	0.5520761013	F335V	0.550835371
57	E360L	0.433652401	D382G	0.5074424744
58	D329K	0.4307904243	V310G	0.4998047352
59	D375G	0.4207065105	E361V	0.4838256836
60	I357K	0.4182610512	I318V	0.4719281197
61	L387Y	0.4113459587	V305T	0.4582495689
62	G328K	0.4044499397	S336V	0.4540407658
63	S298D	0.3996334076	E312L	0.4447476864
64	T353G	0.3699355125	G372K	0.3674166203
65	P332S	0.3651528358	K343V	0.354912281
66	L348K	0.3610384464	T317K	0.3460049629
67	I312G	0.3510642052	E366T	0.3396923542
68	E368T	0.3459715843	E323T	0.3130187988
69	I352V	0.3014163971	I376V	0.2981741428
70	V324G	0.2978274822	I378V	0.2942016125
71	K334E	0.290040493	S296E	0.2941980362
72	G374K	0.2778439522	K307G	0.2904229164
73	V358K	0.2398791313	L306G	0.28926301
74	I339V	0.2247581482	I365V	0.2789404392

75	V309E	0.208368063	V377T	0.2716808319
76	V347L	0.1764419079	L385V	0.2579083443
77	V308E	0.1631295681	D339G	0.2462980747
78	E311G	0.1452379227	I387V	0.2432155609
79	E383K	0.1440289021	E327K	0.2348897457
80	I335V	0.1277112961	E373G	0.2244803905
81	L389V	0.1179652214	K359E	0.2147707939
82	E327K	0.054448843	L349V	0.1929109097
83	K388T	0.0456495285	K321E	0.1890814304
84	K361E	0.03825521469	I350L	0.1743357182
85	E343G	0.005762815475	G340L	0.0969080925
86	K305E	-0.006000518799	K386E	0.07787084579
87	E314K	-0.02287960052	E309G	0.07722640038
88	G349K	-0.02477407455	V356L	0.06389045715
89	I367V	-0.1093397141	K325E	0.06168055534
90	V365L	-0.1329960823	V355T	0.009061336517
91	G296D	-0.1455814838	I333L	-0.01590466499
92	G385K	-0.1478655338	G381K	-0.0233297348
93	E338T	-0.1845285892	V322G	-0.1459743977

94	G330K	-0.304756403	V363L	-0.2274887562
95	K393G	-0.4523117542	K392L	-0.2375872135
96	G304L	-0.4760243893	G316K	-0.4041819572
97	D341G	-0.4822888374	G326D	-0.7401046753
98	K394E	-0.4990010262	-	-
99	G318K	-0.6982964277	-	-

Table S3. Vaccine Sequence and Structure

Vaccine Construct ID	Sequence	3D Structure
D2Native_D3Native	GMSYSMCTGKFKVVKEIAETQHGTIVIRVQ YEGDGSPCKIPFEIMDLEKRHVLGRLITVNPI VTEKDSPVNIEAEPPFGDSYIIIGVEPGQLKL NWFKKEAAAKEAAAKEAAAKSYAMCLNTF VLKKEVSETQHGTILIKVEYKGEDAPCKIPFS TEDGQGKAHNGRLITANPVVTKKEEPVNIE AEPPFGESNIVIGIGDKALKINWYRK	
D2M1_D3M1	GMSYSMETGKFKVVKEIAETQHGTIVIRVQY EGDGSPCKIPFEIMDLEKRHVLGRLITVNPIV TEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLN WFKKEAAAKEAAAKEAAAKSYAMCLNTFV LKKEVSETQHGTILIKVEYKGEDAPCKITFST EDGQGKAHNGRLITANPVVTKKEEPVNIEAE PPFGESNIVIGIGDKALKINWYRK	

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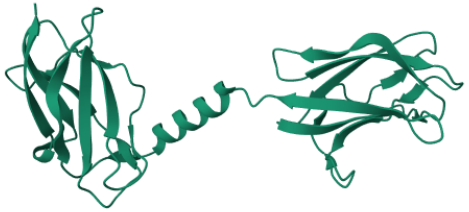
D2M1_D3M3	GMSYSMETGKFKVVKEIAETQHGTIVIRVQY EGDGSPCKIPFEIMDLEKRHVLGRLITVNPIV TEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLN WFKKEAAAKEAAAKEAAAKSYAMCLNTFV LKKEVSETQHGTILIKVEYKGEDAPVKIPFST EDGQGKAHNGRLITANPVVTKKEEPVNIEAE PPFGESNIVIGIGDKALKINWYRK	
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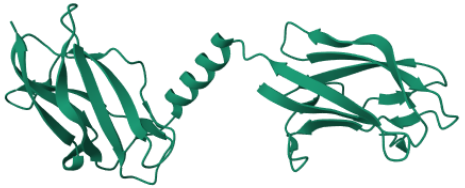
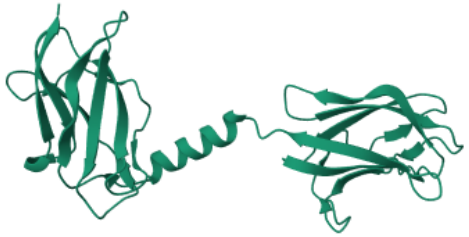
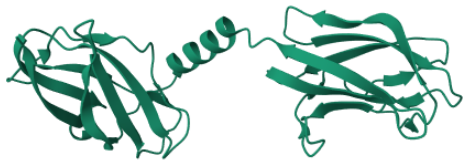
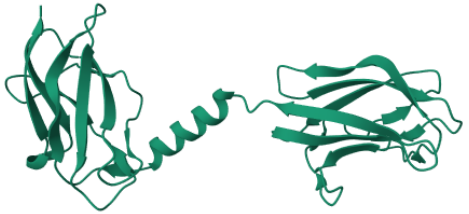
D2M1_D3M5	GMSYSMETGKFKVVKEIAETQHGTIVIRVQY EGDGSPCKIPFEIMDLEKRHVLGRLITVNPIV TEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLN WFKKEAAAKEAAAKEAAAKSYAMCLNTFV LKKEVSETQHGTILIKVEYKGEDAPCKIPFST EDGQGKAHNGRLITANPVVTKKEEPVNIEAE PPFGESNIVIGIGDKALKINVYRK	

D2M2_D3M1	GMSYSMCTGKFKVVKEIAETQHGTIVIRVQN EGDGSPCKIPFEIMDLEKRHVLGRLITVNPIV TEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLN WFKKEAAAKEAAAKEAAAKSYAMCLNTFV LKKEVSETQHGTILIKVEYKGEDAPCKITFST EDGQGKAHNGRLITANPVVTKKEEPVNIEAE PPFGESNIVIGIGDKALKINWYRK	
D2M2_D3M2	GMSYSMCTGKFKVVKEIAETQHGTIVIRVQN EGDGSPCKIPFEIMDLEKRHVLGRLITVNPIV TEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLN WFKKEAAAKEAAAKEAAAKSYAMDLNTFV LKKEVSETQHGTILIKVEYKGEDAPCKIPFST EDGQGKAHNGRLITANPVVTKKEEPVNIEAE PPFGESNIVIGIGDKALKINWYRK	
D2M2_D3M3	GMSYSMCTGKFKVVKEIAETQHGTIVIRVQN EGDGSPCKIPFEIMDLEKRHVLGRLITVNPIV TEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLN WFKKEAAAKEAAAKEAAAKSYAMCLNTFV LKKEVSETQHGTILIKVEYKGEDAPVKIPFST EDGQGKAHNGRLITANPVVTKKEEPVNIEAE PPFGESNIVIGIGDKALKINWYRK	
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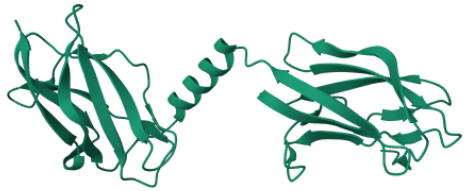
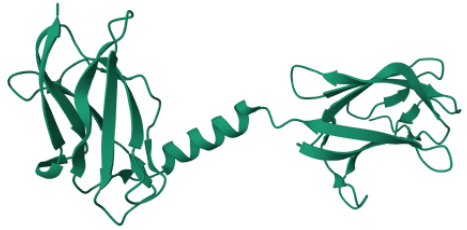
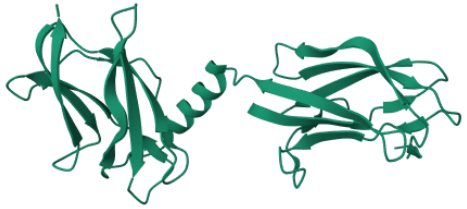
D2M2_D3M5	GMSYSMCTGKFKVVKEIAETQHGTIVIRVQN EGDGSPCKIPFEIMDLEKRHVLGRLITVNPIV TEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLN WFKKEAAAKEAAAKEAAAKSYAMCLNTFV LKKEVSETQHGTILIKVEYKGEDAPCKIPFST EDGQGKAHNGRLITANPVVTKKEEPVNIEAE PPFGESNIVIGIGDKALKINVYRK	
D2M3_D3M1	GMSYSMCTGKFKVVKEIAETQHGTIVIRVQY EGDGSPVKIPFEIMDLEKRHVLGRLITVNPIV TEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLN WFKKEAAAKEAAAKEAAAKSYAMCLNTFV LKKEVSETQHGTILIKVEYKGEDAPCKITFST EDGQGKAHNGRLITANPVVTKKEEPVNIEAE PPFGESNIVIGIGDKALKINWYRK	
D2M3_D3M2	GMSYSMCTGKFKVVKEIAETQHGTIVIRVQY EGDGSPVKIPFEIMDLEKRHVLGRLITVNPIV TEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLN WFKKEAAAKEAAAKEAAAKSYAMDLNTFV LKKEVSETQHGTILIKVEYKGEDAPCKIPFST EDGQGKAHNGRLITANPVVTKKEEPVNIEAE PPFGESNIVIGIGDKALKINWYRK	
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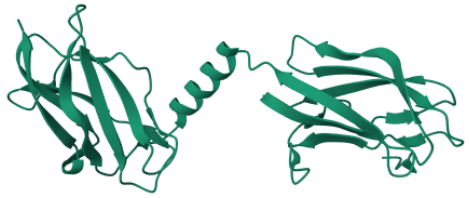
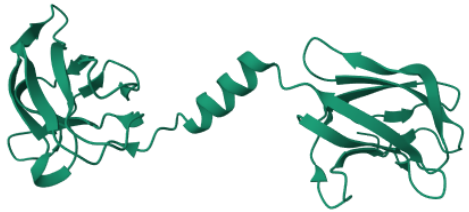
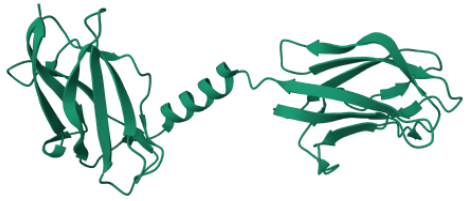
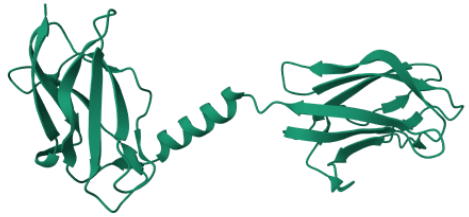
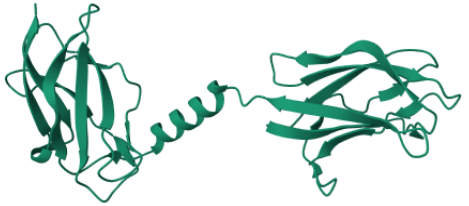
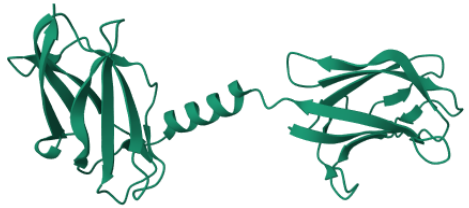
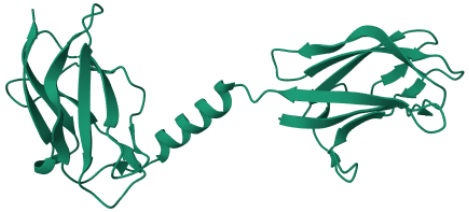
D2M3_D3M5	GMSYSMCTGKFKVVKEIAETQHGTIVIRVQY EGDGSPVKIPFEIMDLEKRHVLGRLITVNPIV TEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLN WFKKEAAAKEAAAKEAAAKSYAMCLNTFV LKKEVSETQHGTILIKVEYKGEDAPCKIPFST EDGQGKAHNGRLITANPVVTKKEEPVNIEAE PPFGESNIVIGIGDKALKINVYRK	
D2M4_D3M1	GMSYSMCTGKFKVVKEIAETQHGTIVIRVQY EGDGSPCKIPFEIMDLEKRKVLGRLITVNPIV TEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLN WFKKEAAAKEAAAKEAAAKSYAMCLNTFV LKKEVSETQHGTILIKVEYKGEDAPCKITFST EDGQGKAHNGRLITANPVVTKKEEPVNIEAE PPFGESNIVIGIGDKALKINWYRK	
D2M4_D3M2	GMSYSMCTGKFKVVKEIAETQHGTIVIRVQY EGDGSPCKIPFEIMDLEKRKVLGRLITVNPIV TEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLN WFKKEAAAKEAAAKEAAAKSYAMDLNTFV LKKEVSETQHGTILIKVEYKGEDAPCKIPFST EDGQGKAHNGRLITANPVVTKKEEPVNIEAE PPFGESNIVIGIGDKALKINWYRK	

D2M4_D3M3	GMSYSMCTGKFKVVKEIAETQHGTIVIRVQY EGDGSPCKIPFEIMDLEKRKVLGRLITVNPIV TEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLN WFKKEAAAKEAAAKEAAAKSYAMCLNTFV LKKEVSETQHGTILIKVEYKGEDAPVKIPFST EDGQGKAHNGRLITANPVVTKKEEPVNIEAE PPFGESNIVIGIGDKALKINWYRK	
D2M4_D3M4	GMSYSMCTGKFKVVKEIAETQHGTIVIRVQY EGDGSPCKIPFEIMDLEKRKVLGRLITVNPIV TEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLN WFKKEAAAKEAAAKEAAAKSYAMCLNTFV LKKEVSETQHGTILIKVEYKGEDAPCKIPFST EDGQGKAVNGRLITANPVVTKKEEPVNIEAE PPFGESNIVIGIGDKALKINWYRK	
D2M4_D3M5	GMSYSMCTGKFKVVKEIAETQHGTIVIRVQY EGDGSPCKIPFEIMDLEKRKVLGRLITVNPIV TEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLN WFKKEAAAKEAAAKEAAAKSYAMCLNTFV LKKEVSETQHGTILIKVEYKGEDAPCKIPFST EDGQGKAHNGRLITANPVVTKKEEPVNIEAE PPFGESNIVIGIGDKALKINVYRK	
D2M5_D3M1	GMSYSMCTGKFKVVKEIAETQHGTIVIRVQY EGDGSPCKIPFEIMDLEKRHVLGRLITVNPIV TEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLNL FKKEAAAKEAAAKEAAAKSYAMCLNTFVL KKEVSETQHGTILIKVEYKGEDAPCKITFSTE DGQGKAHNGRLITANPVVTKKEEPVNIEAEP PFGESNIVIGIGDKALKINWYRK	

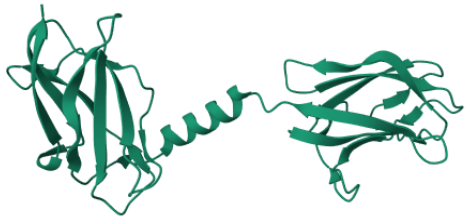
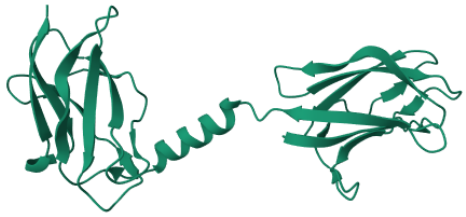
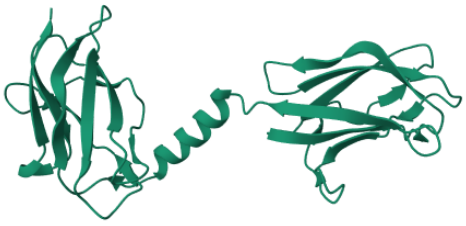
D2M5_D3M2	GMSYSMCTGKFKVVKEIAETQHGTIVIRVQY EGDGSPCKIPFEIMDLEKRHVLGRLITVNPIV TEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLNL FKKEAAAKEAAAKEAAAKSYAMDLNTFVL KKEVSETQHGTILIKVEYKGEDAPCKIPFSTE DGQGKAHNGRLITANPVVTKKEEPVNIEAEP PFGESNIVIGIGDKALKINWYRK	
D2M5_D3M3	GMSYSMCTGKFKVVKEIAETQHGTIVIRVQY EGDGSPCKIPFEIMDLEKRHVLGRLITVNPIV TEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLNL FKKEAAAKEAAAKEAAAKSYAMCLNTFVL KKEVSETQHGTILIKVEYKGEDAPVKIPFSTE DGQGKAHNGRLITANPVVTKKEEPVNIEAEP PFGESNIVIGIGDKALKINWYRK	
D2M5_D3M4	GMSYSMCTGKFKVVKEIAETQHGTIVIRVQY EGDGSPCKIPFEIMDLEKRHVLGRLITVNPIV TEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLNL FKKEAAAKEAAAKEAAAKSYAMCLNTFVL KKEVSETQHGTILIKVEYKGEDAPCKIPFSTE DGQGKAVNGRLITANPVVTKKEEPVNIEAEP PFGESNIVIGIGDKALKINWYRK	
D2M5_D3M5	GMSYSMCTGKFKVVKEIAETQHGTIVIRVQY EGDGSPCKIPFEIMDLEKRHVLGRLITVNPIV TEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLNL FKKEAAAKEAAAKEAAAKSYAMCLNTFVL KKEVSETQHGTILIKVEYKGEDAPCKIPFSTE DGQGKAHNGRLITANPVVTKKEEPVNIEAEP PFGESNIVIGIGDKALKINVYRK	

Table S4. Antigenicity and allergenicity result

Vaccine Construct ID	Antigenicity	Allergenicity
D2Native_D3Native	Antigen (0,5967)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M1_D3M1	Antigen (0,6263)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M1_D3M2	Antigen (0,6051)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M1_D3M3	Antigen (0,6147)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M1_D3M4	Antigen (0,6118)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M1_D3M5	Antigen (0,5699)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M2_D3M1	Antigen (0,6200)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN

D2M2_D3M2	Antigen (0,5988)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M2_D3M3	Antigen (0,6085)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M2_D3M4	Antigen (0,6055)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M2_D3M5	Antigen (0,5636)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M3_D3M1	Antigen (0,6236)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M3_D3M2	Antigen (0,6024)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M3_D3M3	Antigen (0,6121)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M3_D3M4	Antigen (0,6091)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M3_D3M5	Antigen (0,5672)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1

		Classification based on the most similar protein: Probable NON-ALLERGEN
D2M4_D3M1	Antigen (0,6099)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M4_D3M2	Antigen (0,5887)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M4_D3M3	Antigen (0,5983)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M4_D3M4	Antigen (0,5953)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M4_D3M5	Antigen (0,5534)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M5_D3M1	Antigen (0,5810)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M5_D3M2	Antigen (0,5597)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M5_D3M3	Antigen (0,5694)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN

D2M5_D3M4	Antigen (0,5664)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN
D2M5_D3M5	Antigen (0,5245)	Most similar protein: tr|B2M0R3|B2M0R3_SOLTU Starch-granule-bound R1 protein OS=Solanum tuberosum GN=R1 PE=1 SV=1 Classification based on the most similar protein: Probable NON-ALLERGEN

S5. Method

CollabFold

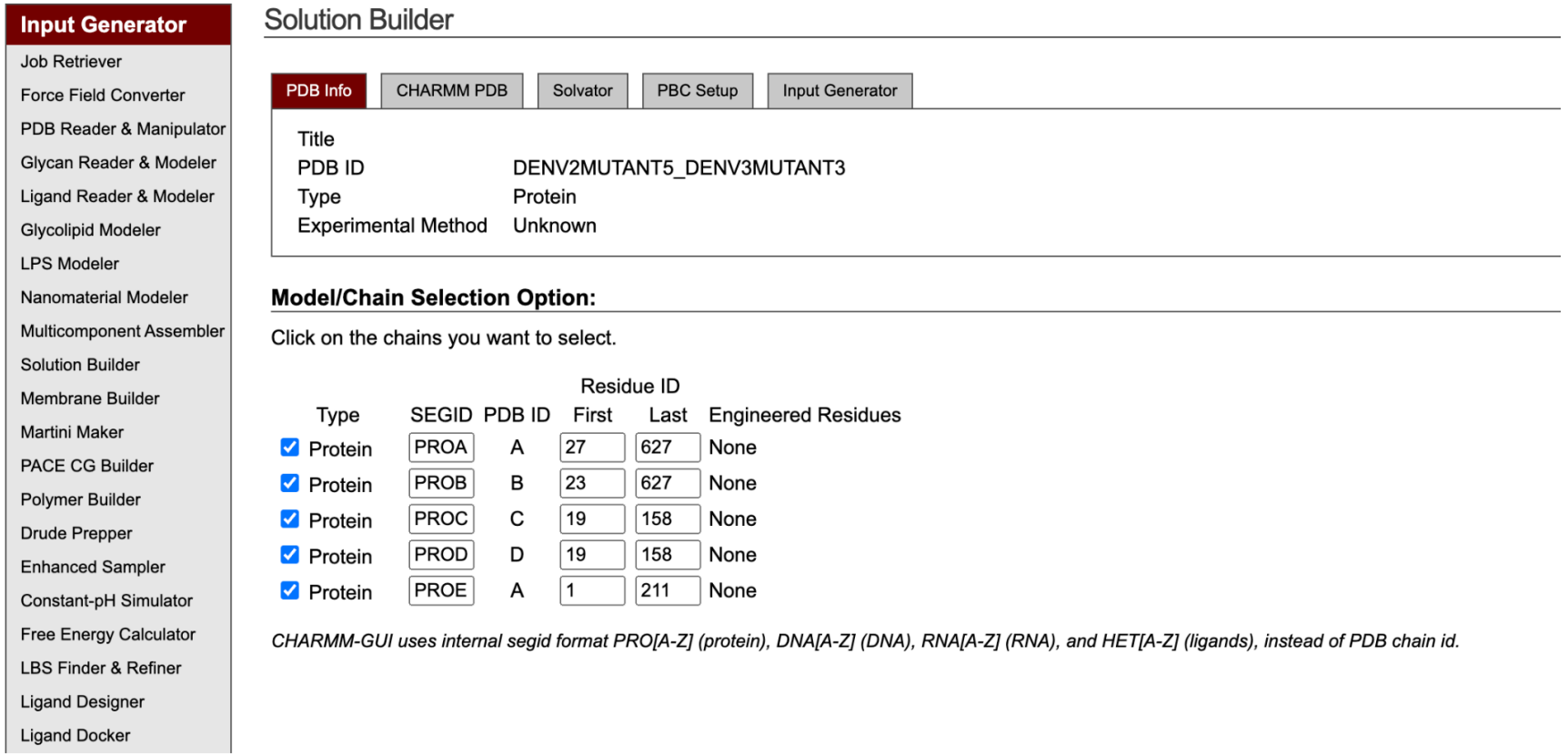
Parameter: template_mode to pdb100, msa_mode to mmseqs2_unirev_env, pair_mode to unpaired_unpaired, model_type to alphafold2_multimer_v3, num_recycles to 24, relax_max_iterations to 2000, and pairing_strategy to complete.

Molecular Dynamics

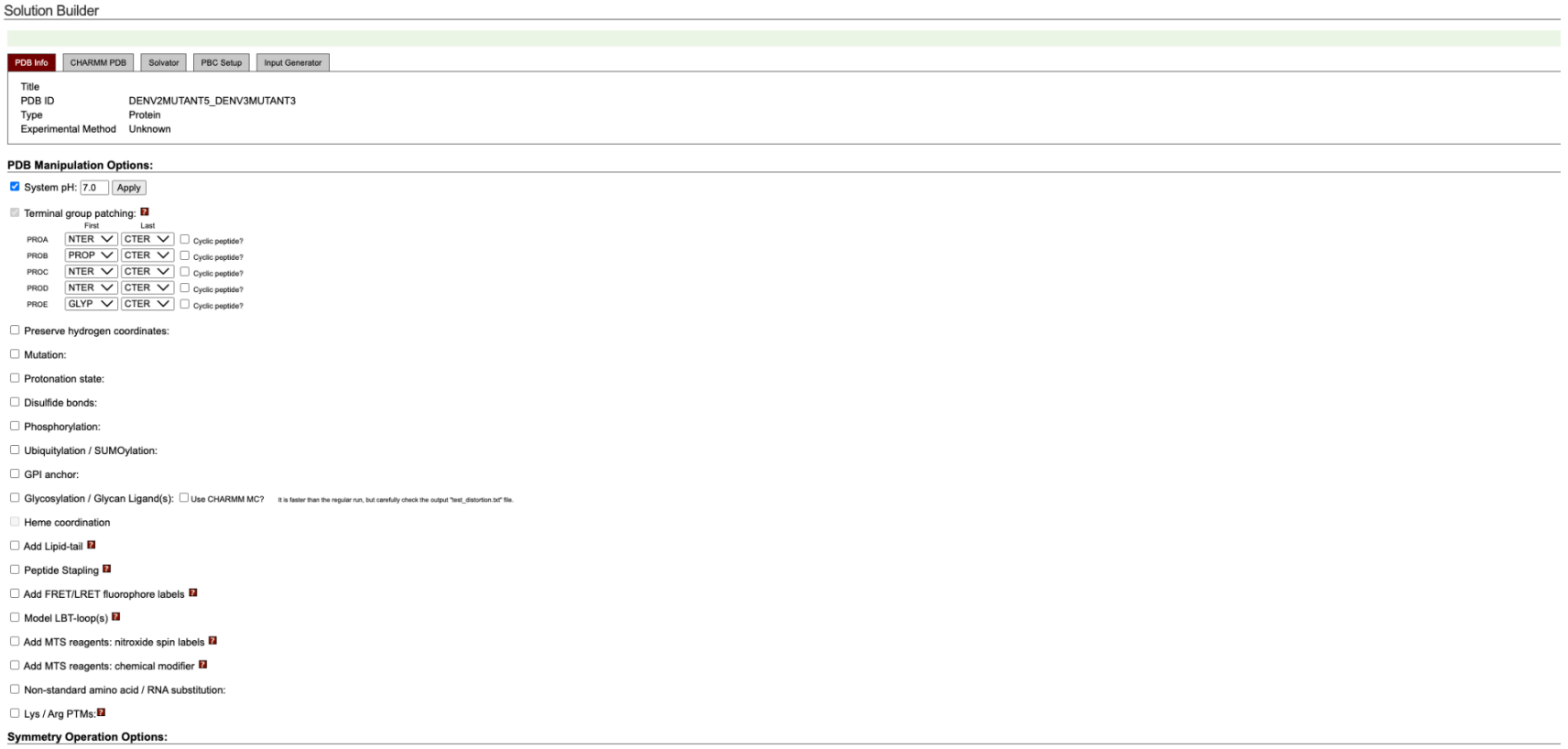
File preparation for molecular dynamics with CHARMM_GUI

Link : https://www.charmm-gui.org/
Login > Input generator > Solution builder

Step 1. Input the pdb file (PDB result by cluspro that contains protein-protein complex)
Step 2. Make sure all the chain are defined and PDB ID is correct



Step 3. Let the option exactly be the same as the default



Step 4. In this step, change the ions to NaCl, as the default setting is KCl. To do this, select NaCl in the ions option and add a simple ion type. Then, remove KCl by clicking the (-) button next to the option. Ensure that the formula contains only NaCl. Finally, press "Calculate Solvent Composition" to check the total ion concentration.

Solution Builder

PDB Info | CHARMM PDB | Solvator | PBC Setup | Input Generator

Original PDB File: DENV2MUTANT5_DENV3MUTANT3.pdb (view structure)
Individual Chains: denv2mutant5_denv3mutant3_proa.pdb
denv2mutant5_denv3mutant3_prob.pdb
denv2mutant5_denv3mutant3_proc.pdb
denv2mutant5_denv3mutant3_prod.pdb
denv2mutant5_denv3mutant3_proe.pdb
CHARMM Input: step1_pdbreader.inp
CHARMM Output: step1_pdbreader.out
CHARMM PDB: step1_pdbreader.pdb (view structure)
CHARMM CRD: step1_pdbreader.crd
CHARMM PSF: step1_pdbreader.psf

Computed Energy:

Please beware of that the computed energy is CHARMM single-point energy and is displayed to make sure all the coordinates are defined.

```
ENER ENR:  Eval#     ENERgy      Delta-E         GRMS
ENER INTERN:          BONDs       ANGLes       UREY-b    DIHEdrals    IMPRopers
ENER CROSS:           CMAPs        PMF1D        PMF2D        PRIMO
ENER EXTERN:        VDWaals         ELEC       HBONds          ASP         USER
 ----------      ---------    ---------    ---------    ---------    ---------
ENER>        0  25722.50758     0.00000   173.39056
ENER INTERN>     1890.41349  4463.77576   481.49143  17781.66048    61.43508
ENER CROSS>       -754.03733     0.00000     0.00000      0.00000
ENER EXTERN>    26385.17844 -24587.40977     0.00000      0.00000     0.00000
 ----------      ---------    ---------    ---------    ---------    ---------
```

Waterbox Size Options:

- ○ Specify Waterbox Size
- ◉ Fit Waterbox Size to Protein Size

Waterbox type: Rectangular (Currently, the octahedral box is supported only for CHARMM and NAMD)
Enter Edge Distance: 10.0

Add Ions:

☑ Include Ions

Ion Placing Method: Monte-Carlo

☑ Basic Ion Types

NaCl | Add Simple Ion Type

☐ More Ion Types

Formula	Cation	Anion	Concentration	Neutralizing	
NaCl	Na^+	Cl^-	0.15	◉	-

Calculate Solvent Composition

Ion	Count
Na^+	442
Cl^-	414

Please note that the ion count is an approximation based on geometry. The real number will be calculated in the next step.

Step 5.

PDB Info | CHARMM PDB | **Solvator** | PBC Setup | Input Generator

CHARMM PDB:	step1_pdbreader.pdb (view structure)	
Solvator Input:	step2_solvator.inp	
Solvator Output:	step2_solvator.out	
Solvator PDB:	step2_solvator.pdb (view structure)	
Solvator CRD:	step2_solvator.crd	
Solvator Stream:	step2_solvator.str	
Waterbox Info:	step2.1_waterbox.prm	
Water Box:	step2.1_waterbox.inp	Input file for water box
	step2.1_waterbox.out	Output file for water box
	step2.1_waterbox.str	Stream file for water box reading
	step2.1_waterbox.pdb (view structure)	water box PDB file
	step2.1_waterbox.crd	water box CRD file
	step2.1_waterbox.prm	
Ions:	step2.2_ions.inp	Input file for ions
	step2.2_ions.out	Output file for ions
	step2.2_ions.str	Ion Information
	step2.2_ions.pdb	ions PDB file

System Size:

Box Type	Rectangle		
Crystal Type	CUBIC		
System Size	A	170	Dimension along the A (X) axis
	B	170	Dimension along the B (Y) axis
	C	170	Dimension along the C (Z) axis
Crystal Angle	Alpha	90.0	Angle between the axis B and C
	Beta	90.0	Angle between the axis A and C
	Gamma	90.0	Angle between the axis A and B

Periodic Boundary Condition Options:

- (•) Generate grid information for PME FFT automatically
- ( ) Explicit grid information for PME FFT

X Y Z

[ ] [ ] [ ]

Step 6. Change the temperature to 310K.

Force Field Options:

AMBER

AMBER Force Fields

Protein	DNA	RNA	Glycan	Lipid	Water	Ligand
FF14SB	OL15	OL3	GLYCAM_06j	Lipid21	TIP3P	GAFF2

- [x] Hydrogen mass repartitioning
- [ ] 12-6-4 ion

Glycolipids and lipoglycans are not supported in current CHARMM-GUI Amber FF implementation.

Input Generation Options:

- [ ] AMBER
- [x] GROMACS
- [ ] OpenMM
- [ ] NAMD
- [ ] GENESIS
- [ ] Tinker

Equilibration Input Generation Options:

- (•) NVT Ensemble

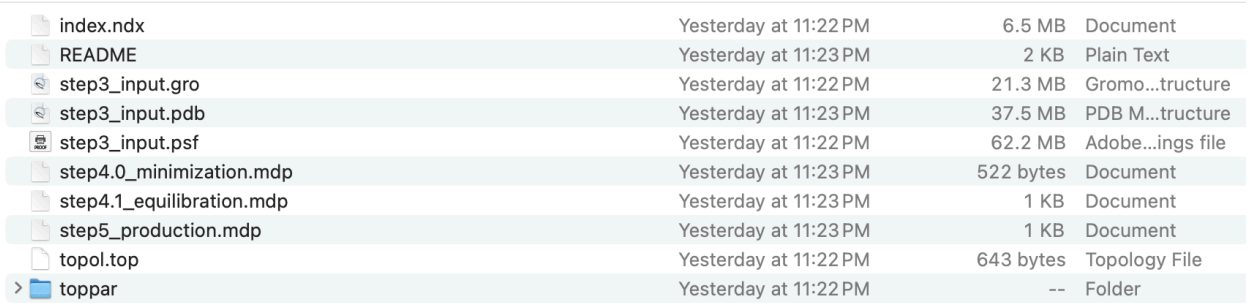
Dynamics Input Generation Options:

- (•) NPT Ensemble
- ( ) NVT Ensemble

Temperature: 310 K

Step 7. Download the file and make sure the gromacs folder contains exactly same as in the picture below

Input from CHARMM_GUI



index.ndx	Yesterday at 11:22 PM	6.5 MB	Document
README	Yesterday at 11:23 PM	2 KB	Plain Text
step3_input.gro	Yesterday at 11:22 PM	21.3 MB	Gromo...tructure
step3_input.pdb	Yesterday at 11:23 PM	37.5 MB	PDB M...tructure
step3_input.psf	Yesterday at 11:22 PM	62.2 MB	Adobe...ings file
step4.0_minimization.mdp	Yesterday at 11:23 PM	522 bytes	Document
step4.1_equilibration.mdp	Yesterday at 11:23 PM	1 KB	Document
step5_production.mdp	Yesterday at 11:23 PM	1 KB	Document
topol.top	Yesterday at 11:22 PM	643 bytes	Topology File
toppar	Yesterday at 11:22 PM	--	Folder

Running MD

1. gmx grompp -f step4.0_minimization.mdp -o step4.0_minimization.tpr -c step3_input.gro -r step3_input.gro -p topol.top -n index.ndx -maxwarn 1
2. gmx mdrun -v -deffnm step4.0_minimization
3. gmx grompp -f step4.1_equilibration.mdp -o step4.1_equilibration.tpr -c step4.0_minimization.gro -r step3_input.gro -p topol.top -n index.ndx -maxwarn 1
4. gmx mdrun -v -deffnm step4.1_equilibration
5. export GMX_MAXCONSTRWARN=-1
6. gmx grompp -f step5_production.mdp -o step5_1.tpr -c step4.1_equilibration.gro -p topol.top -n index.ndx -maxwarn 1
7. gmx mdrun -v -deffnm step5_1

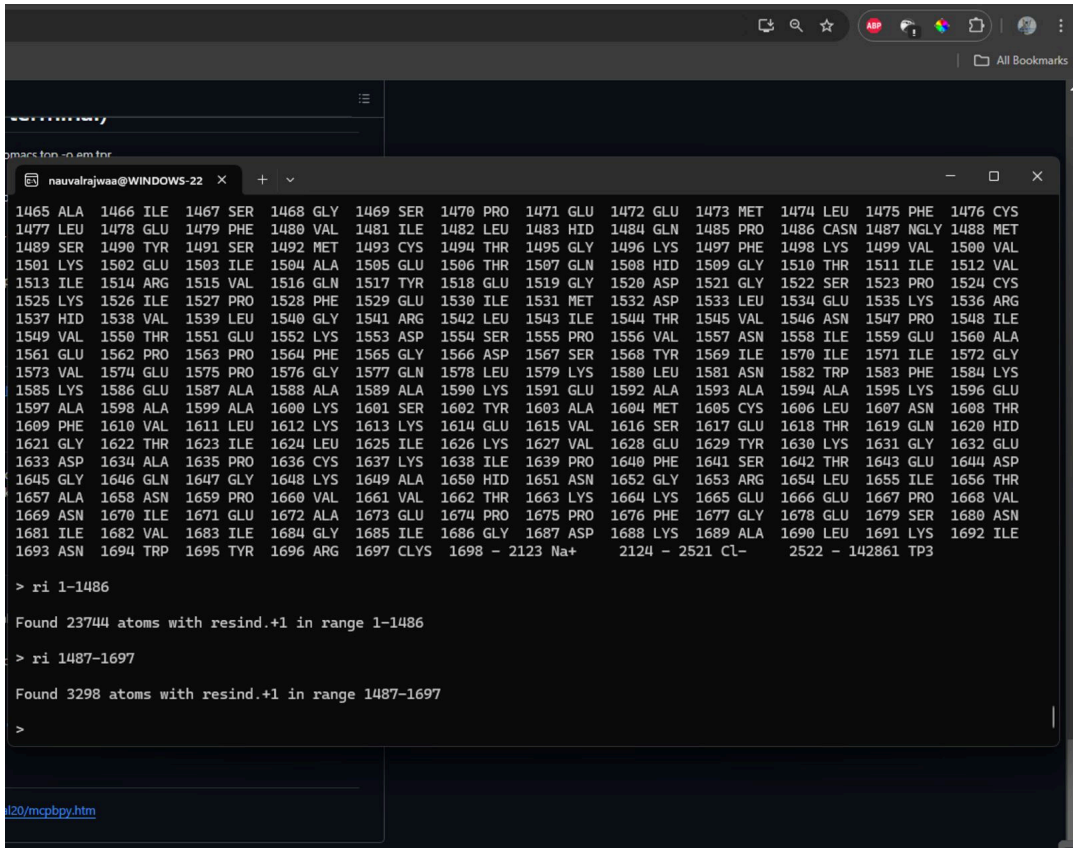
MMPBSA

Installation tool

https://valdes-tresanco-ms.github.io/gmx_MMPBSA/dev/installation/

Preparation

1. gmx make_ndx -f step5_1.gro -o new_index.ndx
2. Make a group index for the ligand and receptor proteins. To do this, give each protein a unique index so that they may be easily distinguished. Verify that the system appropriately labels the ligand and receptor proteins. Accurate identification and study of the two proteins' interactions depend on this phase.
   - ri 1-1486 (reseptor)
   - ri 1487-1697
   - q
3. gmx trjconv -f step5_1.xtc -o md_1_noPBC.xtc -s step5_1.tpr -pbc mol -center -n new_index.ndx -ur compact
4. Select "Protein" and "System" by choosing values 1 and 0, respectively. Ensure that the correct options are assigned to accurately define the system's components. This step is crucial for proper configuration and analysis.



Running MMPBSA

5. conda activate gmxMMPBSA
6. gmx_MMPBSA -O -i mmpbsa.in -cs step5_1.tpr -cp topol.top -ci new_index.ndx -cg 16 17 -ct md_1_noPBC.xtc