



Tutorial de construcción de sistemas con membrana bilipídica usando CHARMM-GUI para AMBER u otros programas de simulación molecular compatibles

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<https://ramirezlab.github.io/>

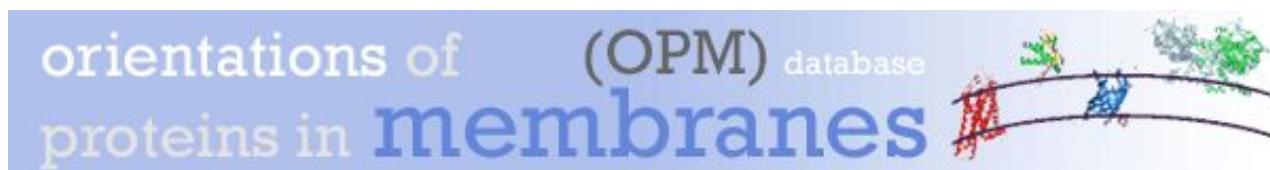
Julio 2024

Requisitos:

- Acceso a internet
- AMBER <https://ambermd.org/index.php>



CHARMM-GUI
Effective Simulation Input Generator and More

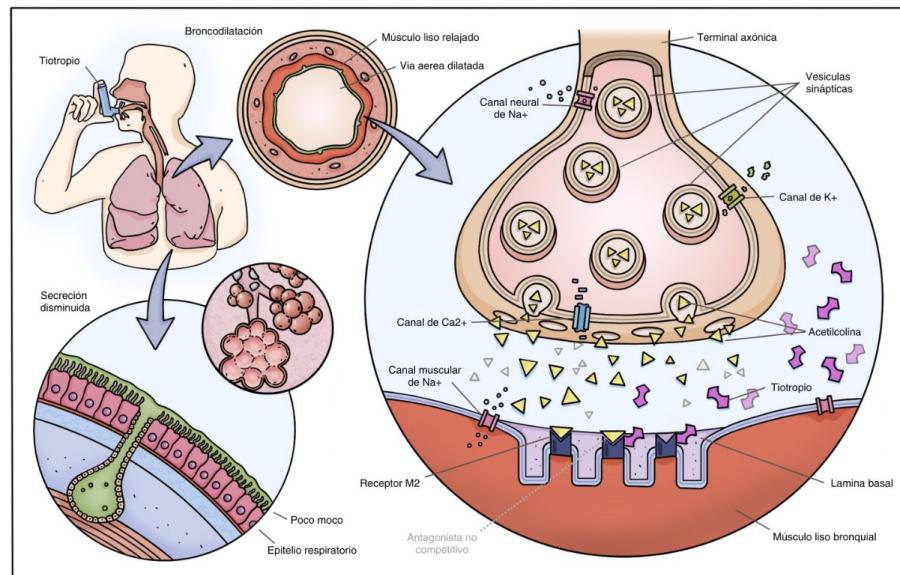


Caso de estudio

Para este tutorial vamos a estudiar mediante simulación de dinámica molecular (MD) la interacción entre el receptor muscarínico de acetilcolina M5 y el fármaco Tiotropio, un antagonista específico para los receptores muscarínicos M1 a M5 presentes en el tejido liso de los pulmones, usado como un broncodilatador para tratar la obstrucción pulmonar crónica y el asma. El Tiotropio es un inhibidor competitivo con la acetilcolina evitando los efectos colinérgicos en el músculo liso, relajándose y reduciendo la secreción de moco.

A continuación construiremos un sistema completo con proteína-ligando, agua, iones y membrana usando la plataforma CHARMM-GUI para luego calcular una breve simulación de dinámica molecular usando AMBER.

Mecanismo de acción y efectos del Tiotropio



<https://es.wikipedia.org/wiki/Tiotropio>

Estructura cristalográfica del receptor muscarínico de acetilcolina M5 unido a Tiotropio obtenida por difracción de rayos X:

<https://www.rcsb.org/structure/6OL9>

PDB PROTEIN DATA BANK 222,036 Structures from the PDB 1,068,577 Computed Structure Models (CSM)

3D Structures Enter search term(s), Entry ID(s), or sequence Include CSM Advanced Search | Browse Annotations Help

PDB-101 wwPDB EMDDataResource NAKB wwPDB Foundation PDB-Dev

Structure Summary Structure Annotations Experiment Sequence Genome Versions

Biological Assembly 1 6OL9 Display Files Download Files Data API

Explore in 3D: Structure | Sequence Annotations | Electron Density | Validation Report | Ligand Interaction (OHK) | Predict Membrane

Global Symmetry: Asymmetric - C1 Global Stoichiometry: Monomer - A1

Structure of the M5 muscarinic acetylcholine receptor (M5-T4L) bound to tiotropium

PDB DOI: <https://doi.org/10.2210/pdb6OL9/pdb>

Classification: HYDROLASE/HYDROLASE INHIBITOR

Organism(s): Homo sapiens, Tequattrovirus T4

Expression System: Spodoptera frugiperda

Mutation(s): No

Membrane Protein: Yes OPM PDBTM MemProtMD mpstruc

Deposited: 2019-04-16 Released: 2019-12-11

Deposition Author(s): Vuckovic, Z., Christopoulos, A., Thal, D.M.

Funding Organization(s): Wellcome Trust, National Health and Medical Research Council (NHMRC, Australia)

Experimental Data Snapshot

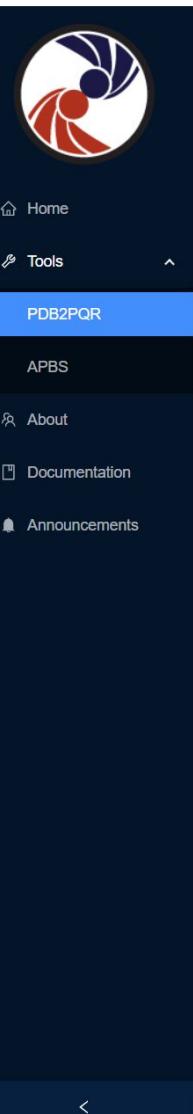
Method: X-RAY DIFFRACTION Resolution: 2.54 Å R-Value Free: 0.257 R-Value Work: 0.236 R-Value Observed: 0.237

wwPDB Validation 3D Report Full Report

Currently 6OL9 does not have a validation slider image.

1. Calcular estados de protonación con PBD2PQR

<https://server.poissonboltzmann.org/pdb2pqr>



Tools / PDB2PQR Job Configuration

1. Ingresar código PDB o subir archivo PDB

PDB Selection
* PDB Source
PDB ID Upload a PDB file
* Please enter a PDB ID
6OL9

For continued support of this server, please register your use of this software:
 Register Here

pKa Options
pH: 7.4
 No pKa calculation
 Use PROPKA to assign protonation states at provided pH

Forcefield Options
Please choose a forcefield to use
AMBER CHARMM PEOEPB PARSE SWANSON TYL06 User-defined Forcefield

Please choose an output naming scheme to use
Internal naming scheme AMBER CHARMM PARSE PEOEPB SWANSON TYL06

Additional Options
 Ensure that new atoms are not rebuilt too close to each other
 Optimize the hydrogen bonding network
 Assign charges to the ligand specified in a MOL2 file
 Create an APBS input file
 Add/keep chain IDs in the PQR file
 Insert whitespaces between atom name and residue name, between x and y, and between y and z
 Make the protein's N-terminus neutral (requires PARSE forcefield)
 Make the protein's C-terminus neutral (requires PARSE forcefield)
 Remove the waters from the output file

2. Ajustar pH, en este caso 7.4 fisiológico y seleccionar PROPKA para asignar los estados de protonación

3. Seleccionar AMBER como campo de fuerza y para sintaxis de nombres

4. Seleccionar "mantener ID de cadenas en el archivo PQR"

5. Iniciar cálculo

Start Job

Tools / Job Status / 43ftbzbd_20240708

To return to your results after leaving, [save this page](#).

Job ID: 43ftbzbd_20240708 Job Type: PDB2PQR Time Elapsed: 00:00:10

Submitted Pending Job Start Running Complete

PDB2PQR Input Files
6OL9.pdb 569.61 KB [Download](#)

PDB2PQR Output Files
43ftbzbd.pqr 466.97 KB [Download](#)
43ftbzbd.log 133.33 KB [Download](#)
pdb2pqr-metrics.json 620 Bytes [Download](#)
pdb2pqr.stdout.txt 0 Bytes [Download](#)
pdb2pqr.stderr.txt 86 KB [Download](#)
43ftbzbd.in 435 Bytes [Download](#)

6. Descargar archivo *.pqr

Use results with APBS >

2. Agregar ligando al archivo pqr Archivo.pqr (original)

6OL9.pqr x

1. El archivo “*.pqr” descargado de PDB2PQR contiene solo la proteína y ha perdido todas las moléculas co-cristalizadas con ella. Para analizar la interacción con Tiotropio mediante simulación molecular se lo debe reincorporar

<https://www.rcsb.org/structure/6o19>

Small Molecules				
Ligands 4 Unique				
ID	Chains 	Name / Formula / InChI Key	2D Diagram	3D Interactions 
OHK (Subject of Investigation/LOI) Query on OHK	D [auth A]	(1R,2R,4S,5S,7S)-7-[{hydroxy(dithiophen-2-yl)acetyl}oxy]-9,9-dimethyl-3-oxa-9-azoniaracyclo[3.3.1.0^{2,4}]nonane C ₁₉ H ₂₂ N O ₄ S ₂ LERNTVKEWCAPOY-DZZGGSBJMSA-N		 Interactions  Interactions & Density 
OLC Query on OLC	F [auth A]	(2R)-2,3-dihydroxypropyl (9Z)-octadec-9-enate C ₂₁ H ₄₀ O ₄ RZRNNAYUHW/FMIP/GDCKJWNLSA-N		 Interactions  Interactions & Density 
P33 Query on P33	E [auth A]	3,6,9,12,15,18-HEXAOXAICO SANE-1,20-DIOL C ₁₄ H ₃₀ O ₈ XPJRQAIZZQMSCM-UHFFFAOYSA-N		 Interactions  Interactions & Density 
OLA Query on OLA	B [auth A], C [auth A]	OLEIC ACID C ₁₈ H ₃₄ O ₂ ZQPPMHVWECSIRJ-KTKRTIGZSA-N		 Interactions  Interactions & Density 

2. En la sección de ligandos del PDB 6OL9 identificamos el Tiotropio con el ID "0HK" y descargamos 6OL9 en formato pdb

3. Buscamos la molécula “0HK” en 6OL9.pdb y copiamos todas sus líneas en el archivo pqr después de la proteína y la cabecera “TER”.

4. Finalmente guardamos los cambios en el archivo pqr

Archivo.pdb

7009	HETATM	3119	C17	OLA	A		42.152	19.197	-46.306	1.00	93.31	C
7010	HETATM	3200	C18	OLA	A		42.959	19.736	-47.468	1.00	92.89	C
7011	HETATM	3201	C28	OKH	A1203		36.420	25.486	-41.497	1.00	56.58	C
7012	HETATM	3202	C29	OKH	A1203		36.257	26.500	-42.114	1.00	56.97	O
7013	HETATM	3203	C31	OKH	A1203		38.221	24.573	-39.917	1.00	65.21	C
7014	HETATM	3204	C32	OKH	A1203		37.617	23.355	-42.023	1.00	58.31	C
7015	HETATM	3205	C34	OKH	A1203		37.546	24.127	-38.773	1.00	49.04	C
7016	HETATM	3206	C35	OKH	A1203		38.364	24.104	-37.632	1.00	57.73	C
7017	HETATM	3207	C36	OKH	A1203		39.615	24.517	-37.897	1.00	70.66	C
7018	HETATM	3208	C41	OKH	A1203		37.288	22.119	-41.468	1.00	37.65	C
7019	HETATM	3209	C42	OKH	A1203		37.244	21.085	-42.440	1.00	51.01	C
7020	HETATM	3210	C43	OKH	A1203		37.532	21.527	-43.462	1.00	60.94	C
7021	HETATM	3211	O10	OKH	A1203		33.169	21.366	-41.480	1.00	61.96	O
7022	HETATM	3212	O11	OKH	A1203		35.471	24.833	-40.820	1.00	58.30	O
7023	HETATM	3213	O33	OKH	A1203		38.744	25.461	-42.089	1.00	51.36	C
7024	HETATM	3214	S37	OKH	A1203		39.846	24.948	-39.520	1.00	75.99	S
7025	HETATM	3215	S44	OKH	A1203		37.866	23.204	-43.723	1.00	71.91	S
7026	HETATM	3216	C1	OKH	A1203		30.694	24.802	-40.971	1.00	55.05	C
7027	HETATM	3217	C9	OKH	A1203		32.580	23.365	-39.987	1.00	58.10	C
7028	HETATM	3218	C9	OKH	A1203		33.343	24.671	-39.371	1.00	57.95	C
7029	HETATM	3219	C5	OKH	A1203		34.088	25.314	-40.840	1.00	60.44	C
7030	HETATM	3220	C6	OKH	A1203		33.412	25.057	-42.185	1.00	60.10	C
7031	HETATM	3221	C7	OKH	A1203		32.645	23.727	-42.312	1.00	58.23	C
7032	HETATM	3222	C8	OKH	A1203		33.656	22.621	-42.001	1.00	60.73	C
7033	HETATM	3223	C9	OKH	A1203		33.610	22.391	-40.563	1.00	61.01	C
7034	HETATM	3224	C12	OKH	A1203		30.500	22.630	-41.356	1.00	40.48	C
7035	HETATM	3225	C30	OKH	A1203		37.763	24.731	-41.372	1.00	59.33	C
7036	HETATM	3226	NE1	OKH	A1203		31.622	23.624	-41.160	1.00	56.55	N
7037	HETATM	3227	O22	P33	A1204		37.705	26.244	-25.441	1.00	111.91	O
7038	HETATM	3228	C21	P33	A1204		37.411	27.085	-26.563	1.00	111.98	C

Archivo.pqr (modificado)

3. Construir sistema en CHARMM-GUI - Selección y modificación moléculas iniciales (Proteína y/o ligando)

CHARMM-GUI

Effective Simulation Input Generator and More

CHARMM is a versatile program for atomic-level simulation of many-particle systems, particularly macromolecules of biological interest. - M. Karplus

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Bilayer Builder

PDB Info STEP 1 STEP 2 STEP 3 STEP 4 STEP 5 STEP 6

Title
PDB ID 6OL9
Type Protein
Experimental Method Unknown

Model/Chain Selection Option:

Click on the chains you want to select.

Type	SEGID	PDB ID	First	Last	Engineered Residues
<input checked="" type="checkbox"/> Protein	PROA	A	26	1160	CYX, HID
<input checked="" type="checkbox"/> Hetero	HETA	A			0HK

CHARMM-GUI uses internal segid format PRO[A-Z] (protein), DNA[A-Z] (DNA), RNA[A-Z] (RNA), and HET[A-Z] (ligands), instead of PDB chain id.

5. Seleccionar las moléculas que se quieren incluir en el sistema, en este caso la proteína y la heteromolecula (Tiotropio)

6. Siguiente Paso

Next Step: Manipulate PDB



CHARMM-GUI

Effective Simulation Input Generator and More

Lehigh University / Department of Biological Sciences / Department of Chemistry / Department of Bioengineering / Im Lab
Problems, Questions, & Comments? / Contact / Forum / Copyright(c) 2006-2024 by the Im Lab

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User Profile

Bilayer Builder

PDB Info STEP 1 STEP 2 STEP 3 STEP 4 STEP 5 STEP 6

Title
PDB ID 6OL9
Type Protein
Experimental Method Unknown

PDB Manipulation Options:

System pH: 7.4 [Apply]

Renaming Engineered Residues:

Rename HID to

Rename CYX to

Upload CHARMM top & par for engineered residue

Topology: Ningún archivo seleccionado

Parameter: Ningún archivo seleccionado

Reading Hetero Chain Residues:

0HK Rename to 0HK CSML Search Click this if you want to generate your ligand FF using the PDB coordinate.

Use CHARMM General Force Field to generate CHARMM top & par files (using ParamChem service)

Use Antechamber to generate CHARMM top & par files

The SDF file from RCSB

The SDF file uploaded from Ningún archivo seleccionado

The MOL2 file uploaded from Ningún archivo seleccionado

force net charge 1

atom type

charge method AM1-BCC

Use OpenFF to generate CHARMM top & par files

Upload CHARMM top & par for hetero chain

Protonate/Deprotonate based on selected pH

Terminal group patching:

First Last CTER Cyclic peptide?

Preserve hydrogen coordinates:

Mutation:

Protonation state:

Disulfide bonds:

Phosphorylation:

Ubiquitylation / SUMOylation:

GPI anchor:

Glycosylation / Glycan Ligand(s): Use CHARMM MC? It is faster than the regular run, but carefully check the output "test_dissociation.txt" file

Heme coordination:

Add Lipid-tail:

Peptide Stapling:

Add FRET/LRET fluorophore labels:

Model LBT-loop(s):

Add MTS reagents: nitroxide spin labels:

Add MTS reagents: chemical modifier:

Non-standard amino acid / RNA substitution:

Lys / Arg PTMs:

Symmetry Operation Options:

7. Seleccionar pH, en este caso vamos a trabajar con pH neutro fisiológico 7,4.

8. Para parametrizar el Tiotropio (0HK) seleccionamos antechamber a partir del archivo SDF de RCSB y agregamos una carga neta de 1. La carga depende de la molécula y del pH en el que se esté trabajando, en este caso vamos a trabajar con la carga formal disponible en la tarjeta de PubChem del Tiotropio

<https://pubchem.ncbi.nlm.nih.gov/compound/5487427#section=Chemical-and-Physical-Properties>

PubChem Tiotropium (Compound)

3 Chemical and Physical Properties

3.1 Computed Properties

Property Name	Property Value	Reference
Molecular Weight	392.5 g/mol	Computed by PubChem 2.2 (PubChem release 2021.10.14)
XLogP3-AA	2.3	Computed by XLogP3 3.0 (PubChem release 2021.10.14)
Hydrogen Bond Donor Count	1	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Hydrogen Bond Acceptor Count	6	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Rotatable Bond Count	5	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Exact Mass	392.09902553 g/mol	Computed by PubChem 2.2 (PubChem release 2021.10.14)
Monoisotopic Mass	392.09902553 g/mol	Computed by PubChem 2.2 (PubChem release 2021.10.14)
Topological Polar Surface Area	116 Å ²	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Heavy Atom Count	26	Computed by PubChem
Formal Charge	1	Computed by PubChem
Complexity	564	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)

9. Siguiente Paso

Next Step: Generate PDB and Orient Molecule

3. Construir sistema en CHARMM-GUI - Orientación y posición inicial

CHARMM-GUI

Effective Simulation Input Generator and More

CHARMM is a versatile program for atomic-level simulation of many-particle systems, particularly macromolecules of biological interest. - M. Karplus

Bilayer Builder

STEP 1

Original PDB File: [6OL9.pdb \(view structure\)](#)

Individual Chains: [6OL9_proa.pdb](#) [6OL9_heta.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

ENER_EUR:	EVAL#	ENERGY	DELTAE	GROTS
ENER INTERN:		BBENDS	ANGLES	UREY-B
ENER CROSS:		CHAPS	PWFLD	PWFD
ENER EXTERN:		VDWPAIRS	ELEC	DIHEDR
-----	-----	-----	-----	-----
ENER>	0	38488.74653	0.00000	283.55698
ENER INTERN>	35105.08390	968.78866	96.40315	3603.23
ENER CROSS>	-14.66275	0.00000	0.00000	0.00
ENER EXTERN>	5867.19582	-7283.92453	0.00000	0.00
-----	-----	-----	-----	-----

Topology and Parameter Files:

Below is the topology and parameter files that are

0HK
 Topology: [0hk/0hk.rtf](#)
 Topology: [0hk/0hk_g.rtf](#)
 Parameter: [0hk/0hk.prm](#)

Orientation Options:

Use PDB Orientation
 Align the First Principal Axis Along Z
 Align a Vector (Two Atoms) Along Z
 Run PPM 2.0

Please select the chains sending to PPM server.
 PROA

Positioning Options:

Rotate Molecule respect to the X axis [] Degree
 Rotate Molecule respect to the Y axis [] Degree
 Translate Molecule along Z axis [] Angstrom
 Flip Molecule along the Z axis

Area Calculation Options:

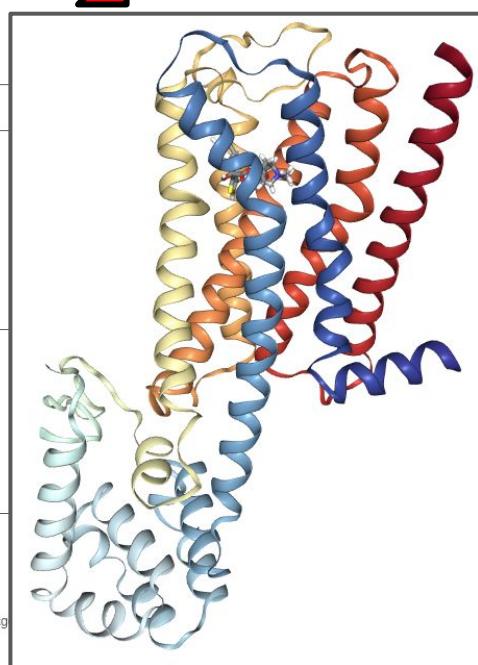
Generate Pore Water and Measure Pore Size

Apart from the text, there are several red arrows pointing to specific sections: one arrow points to the "CHARMM PDB" link in the "CHARMM Output" section; another arrow points to the "Topology and Parameter Files" section; a third arrow points to the "Orientation Options" section; and a fourth arrow points to the "Positioning Options" section.

A partir de éste paso (y los siguientes) ya podemos previsualizar nuestro sistema y sus cambios haciendo click en "view structure"

10. En el caso de proteínas transmembranales se puede recurrir a OPM para obtener la ubicación de la membrana corriendo PPM. Si no funcionara o si no se está satisfecho con el resultado se puede orientar manualmente usando las opciones de orientación y posición de este paso

11. Siguiente Paso



(OPM) database

orientations of proteins in membranes

6OL9 >> Muscarinic acetylcholine receptor M5

Type: Transmembrane (3 classes)
 Class: Alpha-helical polytopic (156 superfamilies)
 Superfamily: Rhodopsin-like receptors and pumps (12 families) CL192
 Family: G-protein coupled receptors, family A (664 proteins) 9.A.14 (TCDB) IPR00001 IPR000276 PDBsum
 Species: Homo sapiens (2659 proteins)
 Localization: Eukaryotic plasma membrane (3316 proteins)

Hydrophobic Thickness or Depth: 31.8 Å
 Tilt Angle: 3°
 ΔG_{transf} : -72.4 kcal/mol
 PDB Sum: [6OL9](#), MSD: [6OL9](#), MMDB: [6OL9](#), Encompass: [6OL9](#)

Links to 6OL9:
 subunit A (N terminus extracellular side)
 Resolution: 2.54
 Primary PDB representation: 6OL9
 Other PDB entries representing this structure: none

Download OPM File: [6OL9.pdb](#), PDB Sum: [6OL9](#), PDB: [6OL9](#)

Ubicación y orientación de la membrana para 6OL9 en OPM

11. Siguiente Paso

Next Step: Calculate Cross-Sectional Area

<https://opm.phar.umich.edu/proteins/4797>

3. Construir sistema en CHARMM-GUI - Tamaño de la caja de “agua”, composición y tamaño del parche de membrana

CHARMM-GUI

Effective Simulation Input Generator and More

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Some lectures, job postings, and FAQ are now available. See update log for update history and giving for donation. Contact info is given below.

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User Profile

JOB ID: 2021450263

download.tgz

Membrane Builder

PDB Info STEP 1 STEP 2 STEP 3 STEP 4 STEP 5 STEP 6

CHARMM PDB: step1_pdbreader.pdb (view structure)

Orientation Input: step2_orient.inp

Orientation Output: step2_orient.out

Oriented PDB: step2_orient.pdb (view structure) (please view this structure before you move to the next step)

Area Calculation: step2_area.str step2_area.plo step2_protein_area.str

Calculated cross sectional area of the protein

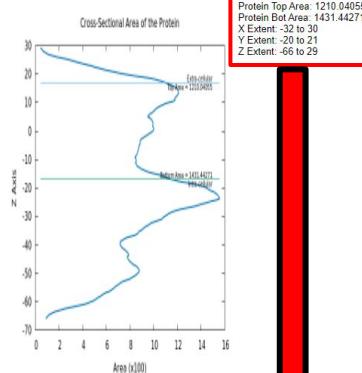
Calculated cross sectional area along Z axis

Calculated cross sectional area of the protein

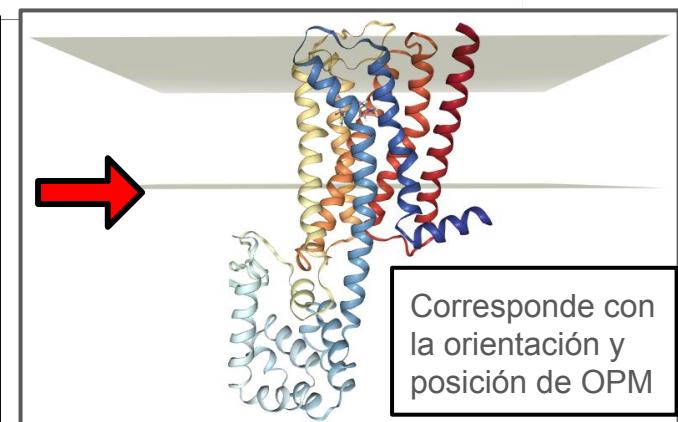
Bookmarked this link. If you want to comeback to this page

Download tgz

Calculated Cross Sectional Area:



12. SIEMPRE
comprobar la posición
de la membrana, en
caso de estar mal
colocada corregir
manualmente en el
paso anterior,
haciendo click en
atrás y recargar en el
explorador de internet



Protein Projection onto XY:

Projection of protein portions above and below the membrane surface onto the membrane surface can be performed only to exclude pseudo lipid spheres underneath the protein portion during the lipid sphere packing.

- Turn on protein projection onto XY of upper leaflet
 - Turn on protein projection onto XY of lower leaflet
- This option is not recommended to use unless it is absolutely necessary.

System Size Determination Options:

- Homogeneous Lipid "Homogeneous Lipid" option is no longer supported. You can use "Heterogeneous Lipid" option even for homogeneous lipid bilayer building.
- Heterogeneous Lipid

1. Box Type: Rectangular (Currently, only CHARMM, NAMD, and GROMACS support the hexagonal box)

2. Length of Z based on:
 Water thickness 22.5 (Minimum water height on top and bottom of the system)
 Hydration number 50 (Number of water molecules per one lipid molecule)

3. Length of XY based on:
 Ratios of lipid components
 Numbers of lipid components

Length of X and Y: 67 (initial guess)
(The system size along the X and Y must be the same)

13. Colocar lípidos basado en una razón y definir el tamaño del parche cuadrado de membrana, calculable como la mayor diferencia entre X o Y de la sección transversal + 5 o más, en función de los lípidos en la membrana.

Show the system info click this once you fill the following table:

Lipid Type	Charge [e]	Tail Info. [sn1/sn2]	Images	Upperleaflet Ratio (Integer)	Lowerleaflet Ratio (Integer)	Surface Area
------------	------------	----------------------	--------	------------------------------	------------------------------	--------------

Sterols

PA (phosphatidic acid) Lipids

PC (phosphatidylcholine) Lipids

DDPC	0	10.0 / 10.0	[Image]	0	0	64.1
DCPC	0	11.0 / 11.0	[Image]	0	0	64.1
DLPC	0	12.0 / 12.0	[Image]	0	0	64.1
DMPC	0	14.0 / 14.0	[Image]	0	0	64.1
DPPC	0	16.0 / 16.0	[Image]	0	0	63.0
DSPC	0	18.0 / 18.0	[Image]	0	0	65.6
PSPC	0	16.0 / 18.0	[Image]	0	0	68.3
PYPC	0	16.0 / 16.1	[Image]	0	0	64.1
POPC	0	16.0 / 18.1	[Image]	1	1	68.3
PLPC	0	16.0 / 18.2	[Image]	0	0	65.2
PILPC	0	16.0 / 18.2	[Image]	0	0	60.0
PEPC	0	16.0 / 22.1	[Image]	0	0	66.5
SOPC	0	18.0 / 18.1	[Image]	0	0	66.2
SLPC	0	18.0 / 18.2	[Image]	0	0	65.2
DRPC	0	14.1 / 14.1	[Image]	0	0	67.0
DYPC	0	16.1 / 16.1	[Image]	0	0	67.0
YOPC	0	16.1 / 18.1	[Image]	0	0	67.0
DOPC	0	18.1 / 18.1	[Image]	0	0	69.7
OEPC	0	18.1 / 22.1	[Image]	0	0	66.5
DUPC	0	18.2 / 18.2	[Image]	0	0	70.0
DGPC	0	20.1 / 20.1	[Image]	0	0	66.7
DEPC	0	22.1 / 22.1	[Image]	0	0	66.5
DNPC	0	24.1 / 24.1	[Image]	0	0	62.3
DLIPC	0	18.2 / 18.2	[Image]	0	0	65.2

15. Click en “show the system info” para mostrar la previsualización de lípidos de la membrana y poder continuar

14. Colocar lípidos en razón de 1 es a 1 (1:1) de POPC en la capa superior e inferior de la membrana

Calculated Number of Lipids:		
Lipid Type	Upperleaflet Number	Lowerleaflet Number
POPC	48	52
Calculated XY System Size:		
Protein Area	1483.76767	1215.59053
Lipid Area	3278.4	3551.6
# of Lipids	48	52
Total Area	4762.16767	4767.19053
Protein X Extent	33.73	
Protein Y Extent	31.42	
Average Area	4764.68	
A	69.03	
B	69.03	

16. Siguiente paso

Next Step: Determine the System Size

3. Construir sistema en CHARMM-GUI - Seleccionar iones y sus concentraciones

CHARMM-GUI
Effective Simulation Input Generator and More

CHARMM is a versatile program for atomic-level simulation of many-particle systems, particularly macromolecules of biological interest. - M. Karplus

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Membrane Builder

PDB Info STEP 1 STEP 2 **STEP 3** STEP 4 STEP 5 STEP 6

Oriented PDB: [step2_orient.pdb \(view structure\)](#)
 System Size Input: [step3_size.inp](#)
 System Size Output: [step3_size.out](#)
 System Size: [step3_size.str](#)
 Packing Simulation: [step3_packing.inp](#)
[step3_packing.out](#)
[crystal_image.str](#)
[step3_packing_top.str](#)
[step3_packing.pdb \(view structure\)](#)

Packing Simulation Input
 Packing Simulation Output
 Crystal Image
 Topology File of Pseudo Lipid Spheres
 Generated Packed System (*please view this structure before you move to the next step*)

Determined System Size:
 Box Type Rectangle
 Crystal Type TETRAGONAL
 System Size A 69.0643762 Dimension along the A (X) axis
 B 69.0643762 Dimension along the B (Y) axis
 C 139.387 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
 # of Lipids on Top 52
 on Bottom 49
 Z Center -18.6635 Center of the system along the Z axis

System Building Options:
 Insertion method Build system using insertion method
 Replacement method Build system using replacement method
 Check lipid ring (and protein surface) penetration

For this system, insertion method can not be used. Replacement method will be used instead.

Component Building Options:
 Include Ions
 Ion Placing Method: Distance
 Basic Ion Types
[KCl](#) Add Simple Ion Type
 More Ion Types
 Formula Cation Anion Concentration Neutralizing
 KCl K+ Cl- 0.15
 Calculate Solvent Composition
 Ion Count
 K+ 38
 Cl- 60

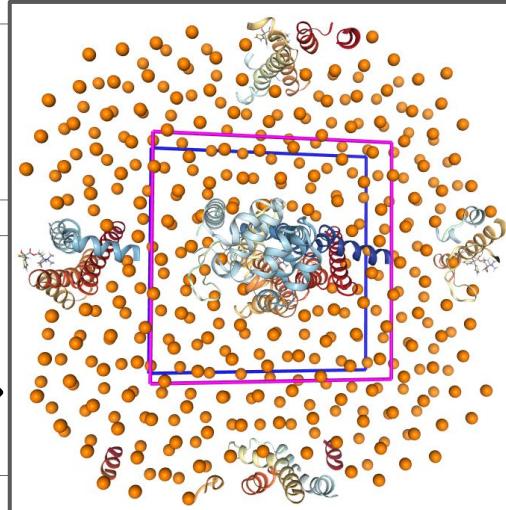
Please note that the ion count is an approximation based on geometry. The real number will be calculated in the next step.

Verificar que la proteína no esté demasiado cerca de sí misma por las condiciones periódicas de X e Y

17. En este caso vamos a usar KCl en una concentración de 0.15 M, como viene por defecto, pero se puede cambiar a lo que requiera el sistema

18. Siguiente paso

Next Step: Build Components




CHARMM-GUI
Effective Simulation Input Generator and More

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Membrane Builder

PDB Info STEP 1 STEP 2 STEP 3 **STEP 4** STEP 5 STEP 6

Oriented PDB: [step2_orient.pdb \(view structure\)](#)
 Component Input: [step4_lipid.inp](#)
 Component Output: [step4_lipid.out](#)
 Component Number: [step4_components.str](#)
 Component PDB: [step4_lipid.pdb \(view structure\)](#)

Check lipid penetration
 The protein surface penetration check finds the lipid tails that go beyond the protein surface, and the lipid ring penetration check detects the lipid tails that pass through the cyclic groups (e.g. resolve many of these bad contacts, but one might need to visually check the following lipid molecules to ensure the following contacts are resolved. The user can regenerate the lipid bilayer).

Protein surface penetration:
 No protein surface penetration is found.
 Lipid ring penetration:
 No lipid ring penetration is found.

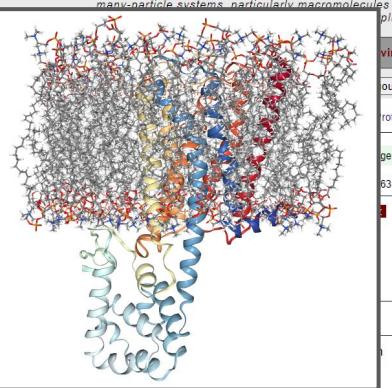
Building Ion and Waterbox
 Membrane components are generated. Due to time constraints, we first generate the lipid bilayer then generate ions and the water box. Click "Next Step" to generate ions and the water box.

Verificación de la colocación de fosfolípidos en el sistema

19. Siguiente paso

Next Step: Assemble Components

9




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3. Construir sistema en CHARMM-GUI - Seleccionar Campo de fuerza (Force Field), programas, ensamble y temperatura para la MD

CHARMM-GUI

Effective Simulation Input Generator and More

CHARMM is a versatile program for atomic-level simulation of many-particle systems, particularly macromolecules of biological interest. - M. Karplus

Input Generator
Job Retriever
Force Field Converter
PDB Reader & Manipulator
Glycan Reader & Modeler
Ligand Reader & Modeler
Glycolipid Modeler
LPS Modeler
Nanomaterial Modeler
Multicomponent Assembler
Solution Builder



Drude Prepper
Enhanced Sampler
Free Energy Calculator

Membrane Builder

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20. Siguiente paso



Next Step: [Assemble Components](#)

CHARMM-GUI

Effective Simulation Input Generator and More

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Input Generator
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Nanomaterial Modeler
Multicomponent Assembler
Solution Builder
Membrane Builder
Martini Maker
PACE CG Builder
Polymer Builder
Drude Prepper
Enhanced Sampler
Free Energy Calculator
LBS Finder & Refiner
Ligand Designer
High-Throughput Simulator
QM/MM Interface
PBEQ Solver
Implicit Solvent Modeler
UNICORN Builder
MAP Utilizer
DEER Facilitator
NMR Structure Calculator
Boundary Potential Utilizer
GCMC/BD Ion Simulator

CHARMM36m

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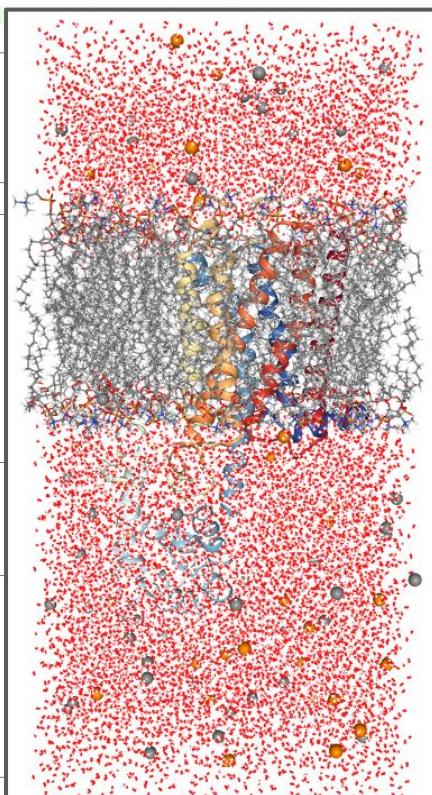
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Sistema ensamblado

Dimensiones finales del sistema



21. Campo de fuerza y opciones adicionales, seleccionamos CHARMM36m, pues está optimizado para sistemas con membranas lipídicas



22. Programa a usar para la simulación molecular, en este caso AMBER



Equilibration Options:

- P21 image transformation (only available for CHARMM)
- CHARMM DOMDEC (only available for CHARMM)
- Generate grid information for PME FFT automatically
- Explicit grid information for PME FFT

x y z

- NVT ensemble
- NPT ensemble
- NPAT ensemble
- NPQt ensemble

Surface Tension [0] (dyn/cm)

Temperature [310] K

23. Ensamble y temperatura de las simulaciones, en este caso producción NPT y temperatura 310 K fisiológica

24. Último paso



Next Step: [Generate Equilibration and Dynamics Inputs](#)

3. Construir sistema en CHARMM-GUI - Descargar archivos para la simulación

CHARMM-GUI
Effective Simulation Input Generator and More

CHARMM is a versatile program for atomic-level simulation of many-particle systems, particularly macromolecules of biological interest. - M. Karpus

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Input Generator

Job Retriever
Force Field Converter
PDB Reader & Manipulator
Glycan Reader & Modeler
Ligand Reader & Modeler
Glycolipid Modeler
LPS Modeler
Nanomaterial Modeler
Multicomponent Assembler
Solution Builder
Membrane Builder
Martini Maker
PACE CG Builder
Polymer Builder
Drude Prepper
Enhanced Sampler
Free Energy Calculator
LBS Finder & Refiner
Ligand Designer
High-Throughput Simulator
QM/MM Interfacer
PBEQ Solver
Implicit Solvent Modeler
UNICORN Builder
MAP Utilizer
DEER Facilitator
NMR Structure Calculator
Boundary Potential Utilizer
GCMC/BD Ion Simulator

PDB Info | STEP 1 | STEP 2 | STEP 3 | STEP 4 | STEP 5 | **STEP 6**

Assembled PDB: [step5_assembly.pdb](#) (view structure)
Input Generator Input: [step5_input.inp](#)
Input Generator Output:
CHARMM Minimization:
Crystal Image: [crystal_image.str](#)
FFT Calculation:
Restraints:
[membrane_restraint.str](#)
[membrane_restraint2.str](#)
Equilibration Inputs:
[step6_1_equilibration.inp](#)
[step6_2_equilibration.inp](#)
[step6_3_equilibration.inp](#)
[step6_4_equilibration.inp](#)
[step6_5_equilibration.inp](#)
[step6_6_equilibration.inp](#)
Production Inputs: [step7_production.inp](#)
Lipid Positional Restraint
Lipid Dihedral Restraint

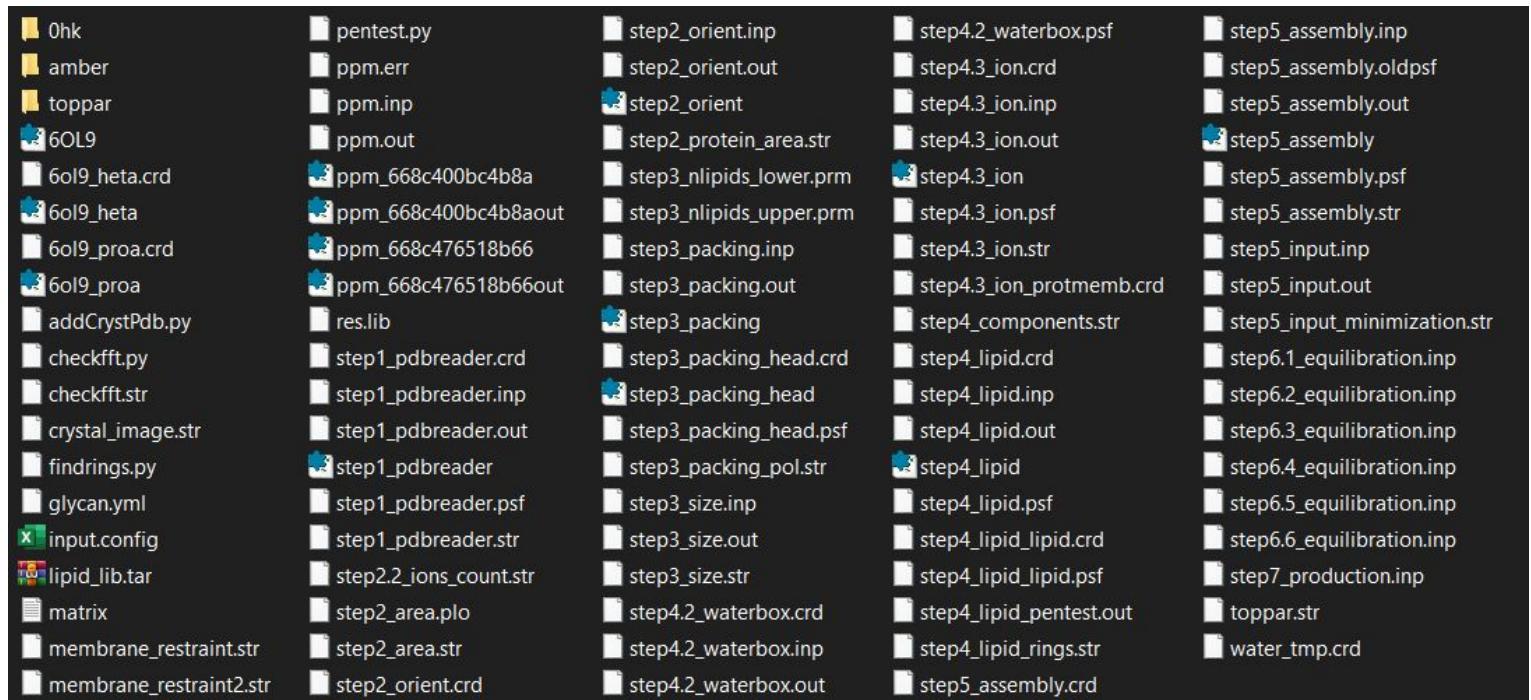
Equilibration Input Notes:

```
! Setup Restraints for Protein and Lipids (see @liptype_restraint.str)
!
! Suggested Equilibration Scheme [Reducing Force Constants]
!(5 Cycles, 1 cycle = 50 - 100 ps.)
!
!-----.
! 1 cycle   2 cycle   3 cycle   4 cycle   5 cycle   6 cycle
!-----.
! BB      10.0      5.0      2.5      1.0      0.5      0.1
! SC      5.0       2.5      1.0      0.5      0.1      0.0
! vforce   2.5       2.5      1.0      0.5      0.1      0.0
! tforce   2.5       2.5      1.0      0.5      0.1      0.0
! ion     10.0      0.0      0.0      0.0      0.0      0.0
!-----.
!
! Equilibration
!
! To reduce the possible problem with the numerical integration with
! the uncorrelated system, 1 fs time-step is used only for the first-step of
! equilibration.
! It is still possible that you may need to use 1 fs for the next equilibration
! steps if your system is initially very very unstable (rare cases).
!
! ** Note: change "nstep" to reduce the number of dynamics steps
```

25. Finalmente descargar archivos para la simulación molecular: minimización, equilibrado, y producción

Protocolo propuesto por CHARMM-GUI para equilibrar la simulación molecular y sus restricciones en kcal/molÅ²

Los archivos descargados incluyen todas las configuraciones y scripts usados en cada paso, archivos de topología y parámetros, más los archivos de configuración de simulación para AMBER



4. Simulación de dinámica molecular - Configuración del archivo de producción para AMBER

Archivo de producción, step7_production.mdin

```
A NPT simulation for common production-level simulations
&cntrl
imin=0,           ! No minimization
irest=1,          ! This IS a restart of an old MD simulation
ntx=5,            ! So our inpcrd file has velocities

! Temperature control
ntt=3,            ! Langevin dynamics
gamma_ln=1.0,      ! Friction coefficient (ps^-1)
temp0=310,         ! Target temperature

! Potential energy control
cut=12.0,          ! nonbonded cutoff, in Angstroms
fswitch=10.0,       ! Force-based switching

! MD settings
nstlim=5000000,   ! 10 ns total
at=0.002,          ! time step (ps) ← Red arrow pointing to the text

! SHAKE
ntc=2,             ! Constrain bonds containing hydrogen
ntf=2,             ! Do not calculate forces of bonds containing hydrogen

! Control how often information is printed
ntpr=1000,          ! Print energies every 1000 steps
ntwx=10000,         ! Print coordinates every 10000 steps to the trajectory
ntwr=10000,         ! Print a restart file every 10K steps (can be less frequent)
! ntwv=-1,           ! Uncomment to also print velocities to trajectory
! ntwf=-1,           ! Uncomment to also print forces to trajectory
ntxo=2,             ! Write NetCDF format
ioutfm=1,           ! Write NetCDF format (always do this!)

! Wrap coordinates when printing them to the same unit cell
iwrap=1,

! Constant pressure control.
barostat=2,         ! MC barostat... change to 1 for Berendsen
ntp=3,               ! 1=isotropic, 2=anisotropic, 3=semi-isotropic w/ surften
pres0=1.0,            ! Target external pressure, in bar

! Constant surface tension (needed for semi-isotropic scaling). Uncomment
! for this feature. csurften must be nonzero if ntp=3 above
csurften=3,          ! Interfaces in 1=yz plane, 2=xz plane, 3=xy plane
gamma_ten=0.0,        ! Surface tension (dyne/cm). 0 gives pure semi-iso scaling
ninterface=2,         ! Number of interfaces (2 for bilayer)

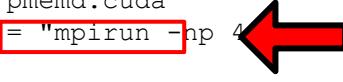
! Set water atom/residue names for SETTLE recognition
watnam='WAT',        ! Water residues are named WAT
owtnm='O',            ! Water oxygens are named O
/
&ewald
vdwmeth = 0,
/
```

Tiempo de simulación, calculado como $nstlim * dt$, en este caso $5.000.000 * 0.002 = 10.000$ ps o 10 ns

Cantidad de pasos para escribir coordenadas de la trayectoria, en este caso se escribirían 500 frames

4. Simulación de dinámica molecular - Automatizar cálculos con el archivo README de CHARMM-GUI

```
#!/bin/csh
#
# Generated by CHARMM-GUI
(http://www.charmm-gui.org) v3.7
#
# All input files were optimized for
AMBER16 or above, so lower version of
AMBER can cause some errors.
# In this script, the parallel (MPI)
version is commented out. Use this
line for parallel execution instead
# (adjust for your MPI and the number
of CPUs you want to use).
Alternatively, if you have access to
# pmemd.cuda or are willing to use
sander, you can replace "pmemd" with
pmemd.cuda or sander and "pmemd.MPI"
# with pmemd.cuda.MPI or sander.MPI
#
# There is a known issue in current
CHARMM-GUI AMBER inputs with "sander".
# If you are willing to use "sander"
for your simulation, please remove
"&end" line in all minimization /
equilibration
# inputs.
```

```
set amber = pmemd.cuda
# set amber = "mpirun -np 4" 
set init = step5_input
set mini_prefix = step6.0_minimization
set equi_prefix =
step6.%d_equilibration
set prod_prefix = step7_production
set prod_step = step7

# Minimization
# In the case that there is a problem
during minimization using a
pmemd.cuda, please try to use pmemd
only for
# the minimization step.
if (-e dihe.restraint) sed -e
"s/FC/250.0/g" dihe.restraint >
${mini_prefix}.rest
pmemd -O -i ${mini_prefix}.mdin -p
${init}.parm7 -c ${init}.rst7 -o
${mini_prefix}.mdout -r
${mini_prefix}.rst7 -inf
${mini_prefix}.mdinfo -ref
${init}.rst7
```

```
# Equilibration
set cnt = 1
set cntmax = 6
set fc =
{'250.0','100.0','50.0','50.0','25.0'}

while ( ${cnt} <= ${cntmax} )
@ pcnt = ${cnt} - 1
    set istep = `printf ${equi_prefix}
${cnt}`
    set pstep = `printf ${equi_prefix}
${pcnt}`
    if ( ${cnt} == 1 ) set pstep =
${mini_prefix}

        if (-e dihe.restraint && ${cnt} <
${cntmax}) then
            sed -e "s/FC/${fc[$cnt]}/g"
dihe.restraint > ${istep}.rest
        endif
        ${amber} -O -i ${istep}.mdin -p
${init}.parm7 -c ${pstep}.rst7 -o
${istep}.mdout -r ${istep}.rst7 -inf
${istep}.mdinfo -ref ${init}.rst7 -x
${istep}.nc
        @ cnt += 1
end
```

```
# Production
set cnt      = 1
set cntmax = 10

while ( ${cnt} <= ${cntmax} )
@ pcnt = ${cnt} - 1
    set istep = ${prod_step}_${cnt}
    set pstep = ${prod_step}_${pcnt}
    if ( ${cnt} == 1 ) set pstep =
`printf ${equi_prefix} 6`

        ${amber} -O -i ${prod_prefix}.mdin
-p ${init}.parm7 -c ${pstep}.rst7 -o
${istep}.mdout -r ${istep}.rst7 -inf
${istep}.mdinfo -x ${istep}.nc
        @ cnt += 1
end
```

2. Dar permisos de ejecución y ejecutar README.sh

```
$ chmod 774 README.sh
$ ./README.sh
```