



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

Carlos Alejandro Peña

Ingeniero en Bioinformática

Estudiante de Doctorado en Biotecnología Molecular, Facultad de Ciencias Biológicas,
Universidad de Concepción

carlosalepena@udec.cl

Asistente de Investigación, Ramírez Lab, Departamento de farmacología, Facultad de Ciencias
Biológicas, Universidad de Concepción

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

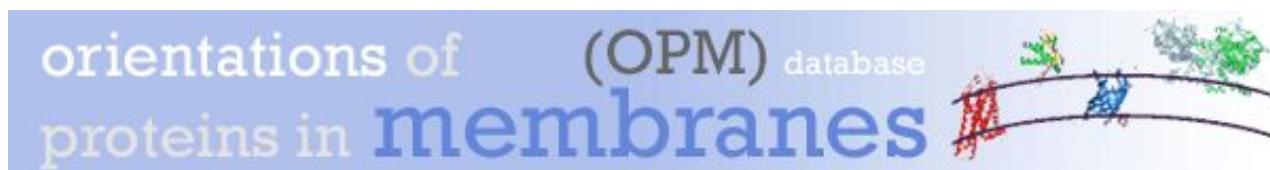
Julio 2024

Requisitos:

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



CHARMM-GUI
Effective Simulation Input Generator and More

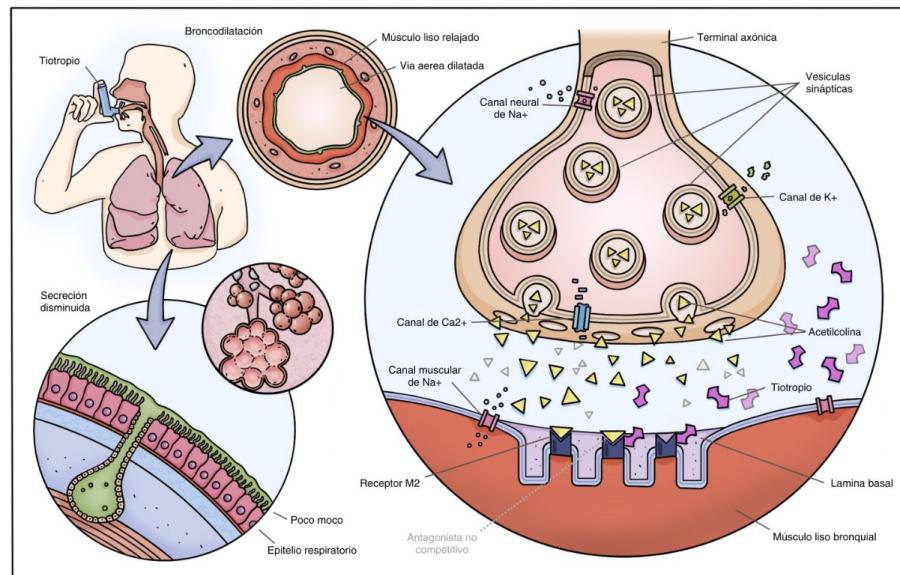


Caso de estudio

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

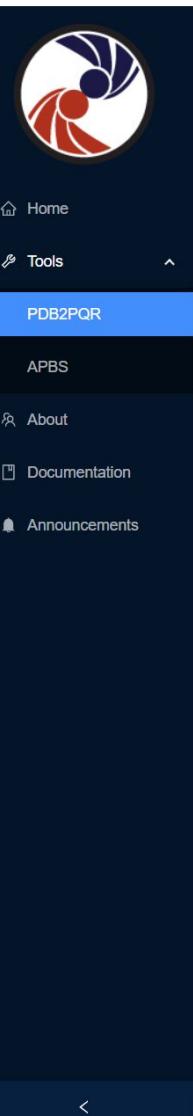
Method: X-RAY DIFFRACTION Resolution: 2.54 Å R-Value Free: 0.257 R-Value Work: 0.236 R-Value Observed: 0.237

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									
6804	ATOM	6804	HB2	CYS	A	512	4.460	22.908	-64.618
6805	ATOM	6805	HB3	CYS	A	512	4.981	21.361	-64.645
6806	ATOM	6806	HG	CYS	A	512	6.496	22.523	-62.456
6807	ATOM	6807	N	ARG	A	513	7.046	23.070	-67.569
6808	ATOM	6808	CA	ARG	A	513	6.896	23.039	-69.017
6809	ATOM	6809	C	ARG	A	513	7.303	21.679	-69.575
6810	ATOM	6810	O	ARG	A	513	7.970	21.594	-70.609
6811	ATOM	6811	CB	ARG	A	513	7.727	24.148	-69.666
6812	ATOM	6812	CG	ARG	A	513	7.368	25.569	-69.253
6813	ATOM	6813	CD	ARG	A	513	8.225	26.578	-69.931
6814	ATOM	6814	NE	ARG	A	513	7.885	27.938	-69.539
6815	ATOM	6815	CZ	ARG	A	513	8.510	29.012	-70.010
6816	ATOM	6816	NH1	ARG	A	513	9.622	28.907	-70.728
6817	ATOM	6817	NH2	ARG	A	513	8.040	30.222	-69.714
6818	ATOM	6818	OXT	ARG	A	513	7.067	20.547	-69.148
6819	ATOM	6819	H	ARG	A	513	7.916	23.388	-67.091
6820	ATOM	6820	HA	ARG	A	513	5.930	23.204	-69.249
6821	ATOM	6821	HB2	ARG	A	513	8.690	23.993	-69.433
6822	ATOM	6822	HB3	ARG	A	513	7.616	24.081	-70.660
6823	ATOM	6823	HG2	ARG	A	513	6.410	25.743	-69.489
6824	ATOM	6824	HG3	ARG	A	513	7.484	25.657	-68.262
6825	ATOM	6825	HD2	ARG	A	513	9.181	26.402	-69.694
6826	ATOM	6826	HD3	ARG	A	513	8.108	26.488	-70.921
6827	ATOM	6827	HE	ARG	A	513	7.143	28.072	-68.889
6828	ATOM	6828	HH11	ARG	A	513	9.998	28.006	-70.942
6829	ATOM	6829	HH12	ARG	A	513	10.085	29.731	-71.057
6830	ATOM	6830	HH21	ARG	A	513	7.214	30.315	-69.158
6831	ATOM	6831	HH22	ARG	A	513	8.513	31.037	-70.048
6832	TER								
6833	END								

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/6ol9>

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)acetoxy]-9,9-dimethyl-3-oxa-9-azoniatriacyclo[3.3.0-2,4-]nonane C ₁₉ H ₂₂ N O ₄ S ₂ LERNTVKEWCAPOY-DZZGSJMSA-N		
OLC Query on OLC	F [auth A]	(2R)-2,3-dihydroxypropyl (9Z)-octadec-9-enate C ₂₁ H ₄₀ O ₄ RZRNYUHWVFMP-GDCKJWNLSA-N		
P33 Query on P33	E [auth A]	3,6,9,12,15,18-HEXAOXACOSANE-1,20-DIOL C ₁₄ H ₃₀ O ₈ XP:JRQAZQMSCM-UHFFFAOYSA-N		
OLA Query on OLA	B [auth A], C [auth A]	OLEIC ACID C ₁₈ H ₃₄ O ₂ ZQPMPHVWECSIR-JKTRTIGZSA-N		

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

6OL9

Structure of the M5 muscarinic acetylcholine receptor

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

Classification: HYDROLASE/HYDROLASE INHIBITOR

Organism(s): Homo sapiens, *Tequatorivorus T4*

Expression System: *Spodoptera frugiperda*

Mutation(s): No

Membrane Protein: Yes

OPM PDBTM MemProMD

FASTA Sequence PDBx/mmCIF Format

PDB/mmCIF Format (gz) PDB/mmCIF Format (bz2)

BinaryCIF Format (gz) BinaryCIF Format (bz2)

PDB Format PDB Format (gz)

PDBML/XML Format (gz) PDBML/XML Format (bz2)

Structure Factors (CIF) Structure Factors (CIF - gz)

Structure Factors (CIF - bz2) Structure Factors (CIF - bz2)

3. Buscamos la molécula “OHK” 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

6OL9.pdb									
7009	HETATM	3199	C17	OLA	A		42.152	19.197	-46.306
7010	HETATM	3200	C18	OLA	A		42.959	19.736	-47.468
7011	HETATM	3201	C28	0A1203			36.420	25.486	-41.497
7012	HETATM	3202	029	OHK	A	1203	36.257	26.500	-42.114
7013	HETATM	3203	C3	OHK	A	1203	38.221	24.573	-39.917
7014	HETATM	3204	C32	OHK	A	1203	37.617	23.355	-42.023
7015	HETATM	3205	C34	OHK	A	1203	37.546	24.127	-38.773
7016	HETATM	3206	C35	OHK	A	1203	38.364	24.104	-37.632
7017	HETATM	3207	C36	OHK	A	1203	39.615	24.517	-37.897
7018	HETATM	3208	C41	OHK	A	1203	37.288	22.119	-41.468
7019	HETATM	3209	C42	OHK	A	1203	37.244	21.085	-42.440
7020	HETATM	3210	C43	OHK	A	1203	37.532	21.527	-43.682
7021	HETATM	3211	O10	OHK	A	1203	33.169	21.366	-41.480
7022	HETATM	3212	O11	OHK	A	1203	35.471	24.833	-40.820
7023	HETATM	3213	O33	OHK	A	1203	38.744	24.104	-42.089
7024	HETATM	3214	S37	OHK	A	1203	39.846	24.948	-39.520
7025	HETATM	3215	S44	OHK	A	1203	37.866	23.204	-43.723
7026	HETATM	3216	C1	OHK	A	1203	30.694	24.802	-40.971
7027	HETATM	3217	C3	OHK	A	1203	32.580	23.365	-39.987
7028	HETATM	3218	C4	OHK	A	1203	33.343	24.671	-39.575
7029	HETATM	3219	C5	OHK	A	1203	34.088	25.314	-40.840
7030	HETATM	3220	C6	OHK	A	1203	33.412	25.057	-42.185
7031	HETATM	3221	C7	OHK	A	1203	32.645	23.727	-42.312
7032	HETATM	3222	C8	OHK	A	1203	33.656	22.621	-42.001
7033	HETATM	3223	C9	OHK	A	1203	33.610	22.391	-40.563
7034	HETATM	3224	C12	OHK	A	1203	30.500	22.630	-41.356
7035	HETATM	3225	C30	OHK	A	1203	37.763	24.731	-41.372
7036	HETATM	3226	N2	OHK	A	1203	31.622	23.624	-41.160
7037	HETATM	3227	O22	F33	A1204		37.705	26.244	-25.441
7038	HETATM	3228	C21	F33	A1204		37.411	27.085	-26.563

Archivo.pqr (modificado)

6OL9.pqr									
ATOM	6830	HH21	ARG	A	513	7.214	30.315	-69.158	0.4493 0.6000
ATOM	6831	HH22	ARG	A	513	8.513	31.037	-70.048	0.4493 0.6000
TER									
6832	TER								
6833	ATOM	3201	C28	OHK	A	1203	36.420	25.486	-41.497
6834	ATOM	3202	O29	OHK	A	1203	36.257	26.500	-42.114
6835	ATOM	3203	C31	OHK	A	1203	38.221	24.573	-39.917
6836	ATOM	3204	C32	OHK	A	1203	37.617	23.355	-42.023
6837	ATOM	3205	C34	OHK	A	1203	37.546	24.127	-38.773
6838	ATOM	3206	C35	OHK	A	1203	38.364	24.104	-37.632
6839	ATOM	3207	C36	OHK	A	1203	39.615	24.517	-37.897
6840	ATOM	3208	C40	OHK	A	1203	37.288	22.119	-41.468
6841	ATOM	3209	C42	OHK	A	1203	37.244	21.085	-42.440
6842	ATOM	3210	C43	OHK	A	1203	37.532	21.527	-43.682
6843	ATOM	3211	O10	OHK	A	1203	33.169	21.366	-41.480
6844	ATOM	3212	O11	OHK	A	1203	35.471	24.833	-40.820
6845	ATOM	3213	O33	OHK	A	1203	38.744	24.464	-42.089
6846	ATOM	3214	S37	OHK	A	1203	39.846	24.948	-39.520
6847	ATOM	3215	S44	OHK	A	1203	37.866	23.204	-43.723
6848	ATOM	3216	C1	OHK	A	1203	30.694	24.802	-40.971
6849	ATOM	3217	C3	OHK	A	1203	32.580	23.365	-39.987
6850	ATOM	3218	C4	OHK	A	1203	33.343	24.671	-39.671
6851	ATOM	3219	C5	OHK	A	1203	34.088	25.314	-40.840
6852	ATOM	3220	C6	OHK	A	1203	33.412	25.057	-42.185
6853	ATOM	3221	C7	OHK	A	1203	32.645	23.727	-42.312
6854	ATOM	3222	C8	OHK	A	1203	33.656	22.621	-42.001
6855	ATOM	3223	C9	OHK	A	1203	33.610	22.391	-40.563
6856	ATOM	3224	C12	OHK	A	1203	30.500	22.630	-41.356
6857	ATOM	3225	C30	OHK	A	1203	37.763	24.731	-41.372
6858	ATOM	3226	N2	OHK	A	1203	31.622	23.624	-41.160
6859	END								

3. Construir sistema en CHARMM-GUI - Carga de archivos

1. Crear cuenta y acceder en CHARMM-GUI

<https://www.charmm-gui.org/?doc=input>

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

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.

Logout

Tutorial User Profile

Membrane Builder

Membrane Builder helps the user generate a series of CHARMM inputs necessary to build a protein/membrane complex for molecular dynamics simulations. A brief description of each step is given below. Among various other building schemes, either the "insertion" or the "replacement" method can be chosen by the user in step 3. (user can choose one of them in step 3, see below).

- Insertion method
A protein is inserted into a pre-equilibrated lipid bilayer with a hole whose size is comparable to the protein size (the libraries of lipid bilayers are available in [archive](#))
- Replacement method
A protein is first packed by lipid-like spheres whose positions are subsequently used to place randomly chosen lipid molecules from the library (the libraries of lipid molecules are available in [archive](#))

Please note that

- If you are not familiar with Membrane Builder, please first watch these [video demos](#) and also read the relevant references below.
- **NAMD inputs (v2.7b3 or after)** are provided for equilibration and production (see [STEP6](#)). Input files can be found in "namd" directory when you download tar archive ("charmm-gui.tgz") after all the input file generation.
- **GROMACS inputs (v5.0 or after)** are provided for minimization, equilibration, and production (see [STEP6](#)). Input files can be found in "gromacs" directory when you download tar archive ("charmm-gui.tgz") after all the input file generation. See [tar archive \("charmm-gui.tgz"\)](#) after all the input file generation. See [tar archive \("charmm-gui.tgz"\)](#) after all the input file generation. See [tar archive \("charmm-gui.tgz"\)](#) after all the input file generation.
- **OpenMM inputs (c39b1 or after)** are provided for equilibration and production (see [STEP6](#)). Input files can be found in "charmm_openmm" directory when you download tar archive ("charmm-gui.tgz") after all the input file generation.
- **Inputs (v8.10 or after)** are provided for minimization, equilibration, and production (see [STEP6](#)). Input files can be found in "tinker" directory when you download tar archive ("charmm-gui.tgz") after all the input file generation.
- The protein must be oriented with respect to a membrane bilayer whose normal is parallel to the Z-axis and whose center is located at Z=0
- DB structures are NOT pre-oriented, but can be oriented in STEP 2 (see below)
- If the OPM PDB does not contain "TER" between ATOM and HETATM, so that CHARMM-GUI often fails to recognize ligand molecules. In such case, the user should manually insert "TER" in appropriate places. In addition, all carbohydrate connection information is lost in OPM PDB files.
- Users can use Download Source RCSB and then use PPM web server (http://opm.phar.umich.edu/opm_server) in STEP 2 to obtain a oriented protein coordinate with respect to the membrane normal, so that users can have all molecular information (disulfide bonds / ligands / carbohydrates etc) in a RCSB PDB file
- a homogeneous lipid bilayer can be built with DMPC, DPPC, DOPC, POPC, DLPE, and POPE
- a heterogeneous lipid bilayer can be built with **434** different lipid molecules (see [lipid list](#))
- the heterogeneous Membrane Builder can be used for a homogeneous lipid bilayer (only using the replacement method)
- P21 crystal image is available in CHARMM input option
- rectangular and hexagonal geometries are available for a system shape in XY
- If you are not familiar with the first PDB reading step, please first watch these [video demos](#).

2. Seleccionar "Bilayer Builder" en Input Generator

Membrane Builder

Bilayer Builder

Membrane Builder

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

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

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

Lehigh University

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

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
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

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 HSD

Rename CYX to CYS

Upload CHARMM top & par for engineered residue

Topology: Seleccionar archivo Ningún archivo seleccionado

Parameter: Seleccionar archivo 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 Seleccionar archivo Ningún archivo seleccionado

The MOL2 file uploaded from Seleccionar archivo Ningún archivo seleccionado

force net charge 1

atom type gaff2

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
PROA NTER 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:

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

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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:

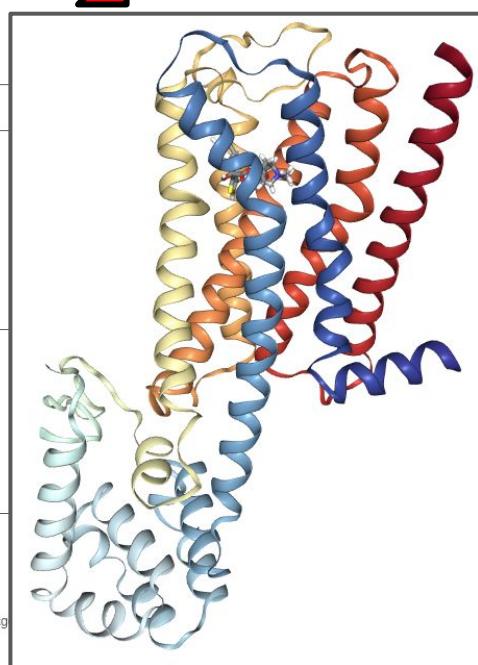
Generate Pore Water and Measure Pore Size

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

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

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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 Modeler
- PACE CG Builder
- Polymer Builder
- Drude Prepper
- Enhanced Sampler
- Free Energy Calculator
- LBS Finder & Refiner
- Ligand Designer
- High-Throughput Simulator
- QMMM Interface
- PBEO Solver
- Implicit Solvent Modeler
- UNICORN Builder
- MAP Utilizer
- DEER Facilitator
- NMR Structure Calculator
- Boundary Potential Utilizer
- GCMC/BD Ion Simulator

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

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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

Corresponde con la orientación y posición de OPM

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.

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

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

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

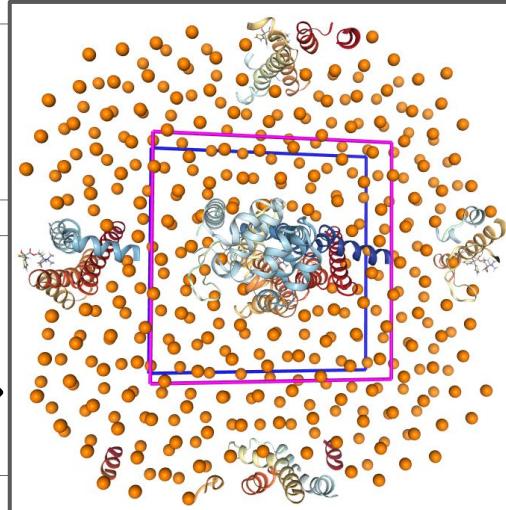
Please note that the ion count is an approximation based on geometry. The real number will be calculated in the next step.

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.

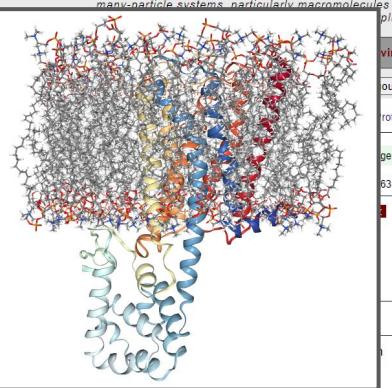
Building Ion and Waterbox
 Membrane components are generated. Due to time constraints, we first generate the lipid bilayer then generate ions and the water box. Click "Next Step" to generate ions and the water box.

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	

Membrane Builder

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20. Siguiente paso



Next Step: [Assemble Components](#)



Drude Prepper
Enhanced Sampler
Free Energy Calculator

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CHARMM-GUI

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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 Interface	
PBEQ Solver	
Implicit Solvent Modeler	
UNICORN Builder	
MAP Utilizer	
DEER Facilitator	
NMR Structure Calculator	
Boundary Potential Utilizer	
GCMC/BD Ion Simulator	

Membrane Builder

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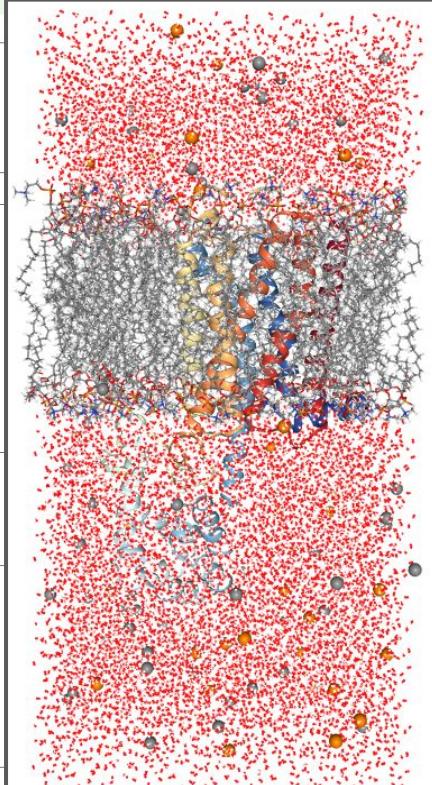
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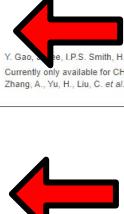
[download.tgz](#)

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](#)



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

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

Assembled PDB:	step5_assembly.pdb (view structure)
Input Generator Input:	step5_input.inp
Input Generator Output:	step5_input.out
CHARMM Minimization:	step5_input_minimization.str
Crystal Image:	crystal_image.str
FFT Calculation:	checkfft.py
Restraints:	membrane_restraint.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_positional_restraint2.str
Lipid Dihedral Restraint:	lipid_dihedral_restraint2.str
Equilibration Step 1:	equilibration_step1.inp
Equilibration Step 2:	equilibration_step2.inp
Equilibration Step 3:	equilibration_step3.inp
Equilibration Step 4:	equilibration_step4.inp
Equilibration Step 5:	equilibration_step5.inp
Equilibration Step 6:	equilibration_step6.inp
Production Input:	production.inp

Equilibration Input Notes:

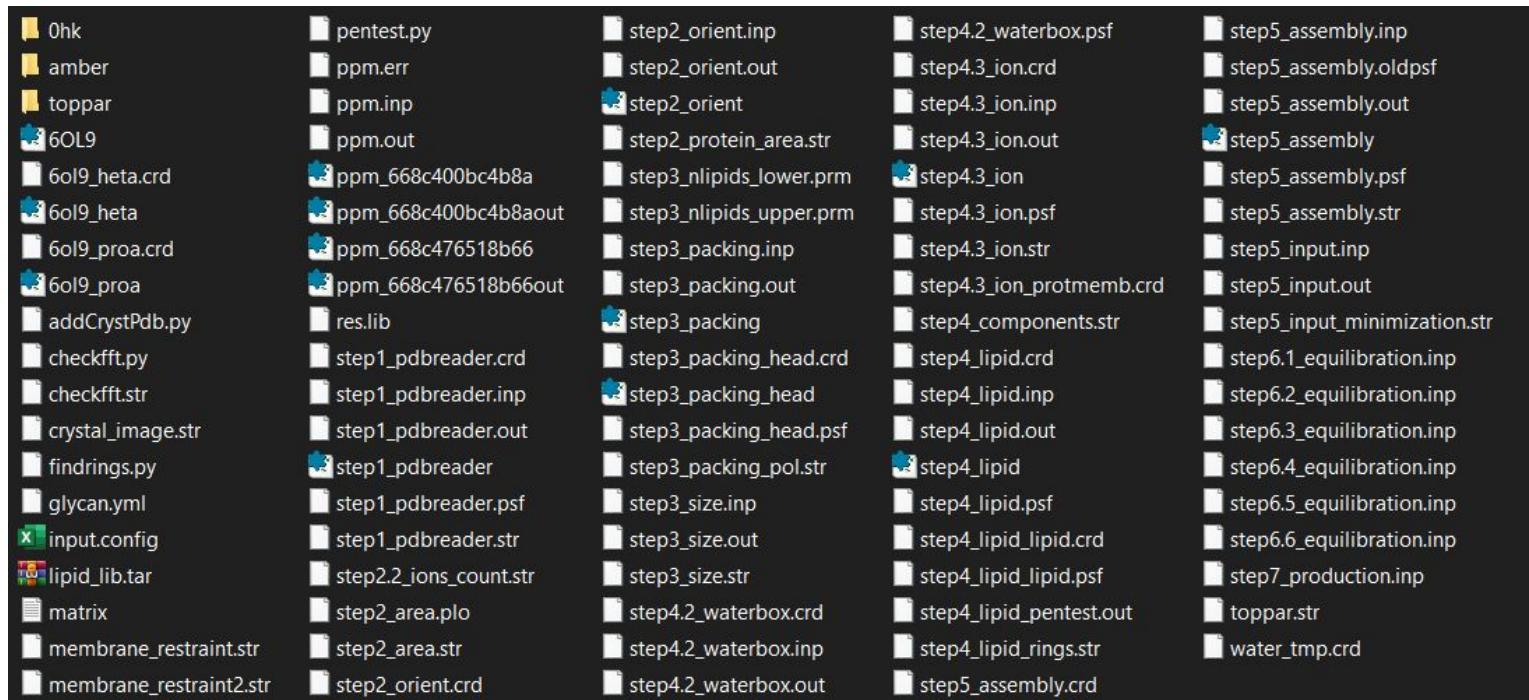
```

!----- Setup Restraints for Protein and Lipids (see @lipotype_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
!----- 10.0 5.0 2.5 1.0 0.5 0.1 0.0
!----- 10.0 2.5 2.5 1.0 0.5 0.1 0.0
!----- 2.5 2.5 2.5 1.0 0.5 0.1 0.0
!----- 2.5 2.5 2.5 1.0 0.5 0.1 0.0
!----- 2.5 2.5 2.5 1.0 0.5 0.1 0.0
!----- 10.0 0.0 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)

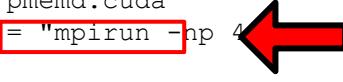
! Set water atom/residue names for SETTLE recognition
watnam='WAT',        ! Water residues are named WAT
owtnm='O',            ! Water oxygens are named O
/
&ewald
vdwmeth = 0,
/
```

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
```