



UNIVERSIDAD NACIONAL DE INGENIERÍA

FACULTAD DE CIENCIAS

LABORATORIO DE INVESTIGACIÓN EN BIOPOLÍMEROS Y METALOFÁRMACOS



Uso de herramientas *in silico* para el estudio del *Efecto Caballo de Troya* como estrategia contra la resistencia a los antibióticos

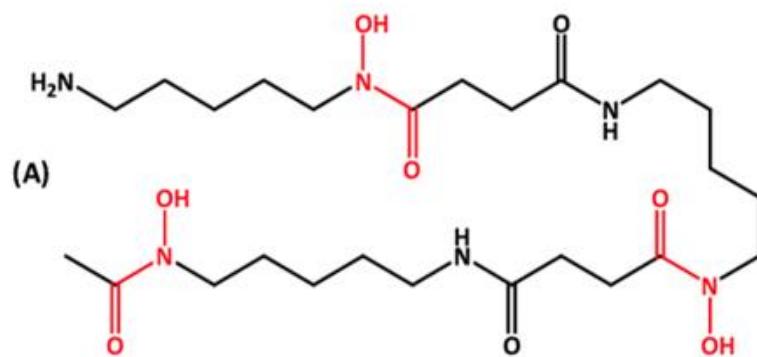
Jesus Antonio Alvarado Huayhuaz

Orientadora: Ana Cecilia Valderrama Negrón

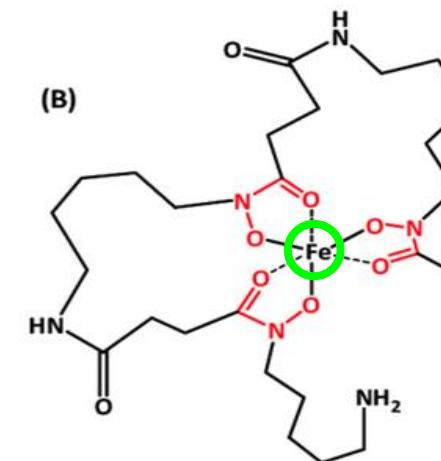
Sideróforo Desferrioxamina

✓ Desferrioxamina (DFO): Sideróforo - *Streptomices pilosus*

Formula: C₂₅H₄₈N₆O₈
MW: 561.69 g/mol



[Fe³⁺] ~ 10⁻¹⁸ M
 ↓ Biodisponibilidad



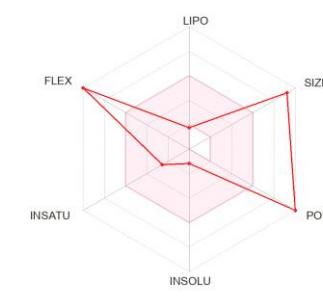
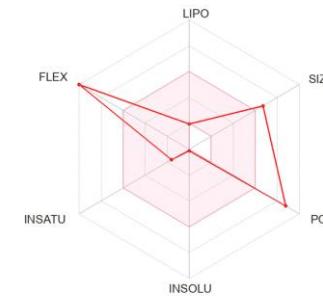
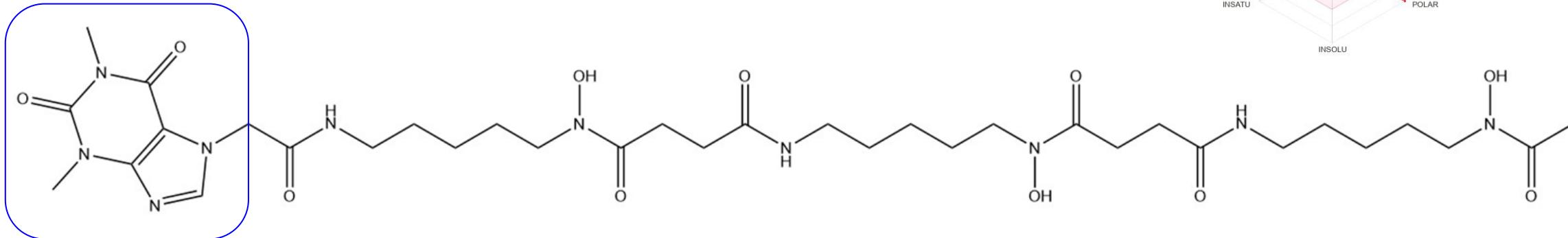
↑ Estabilidad
 (logβ = 42,33)



Enfermedades por
 sobrecarga de hierro

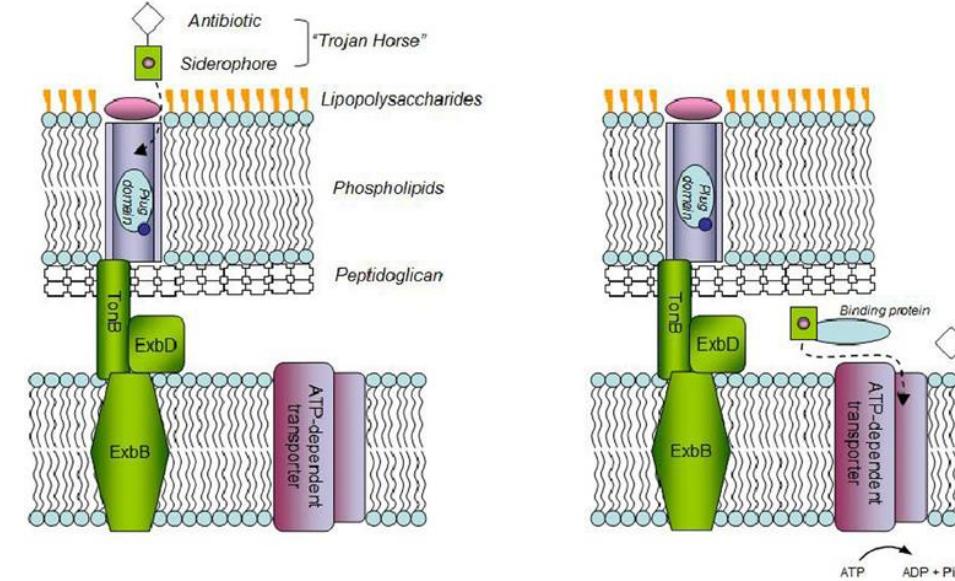
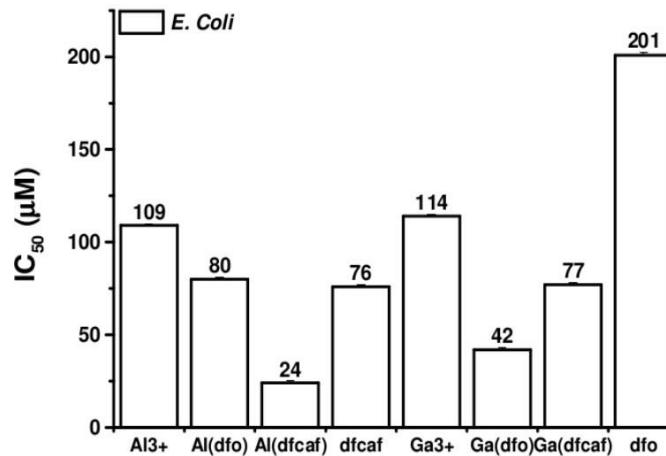
Desferrioxamina + cafeína (DFCAF)

- ✓ DFO: ↓ Permeabilidad celular,
Druglikeness 😞: Violaciones: Lipinski: 3,
Ghose: 3, Veber: 2, Egan: 1, Muegge: 4
 - ✓ DFO+cafeína (DFCAF): ↑ permeabilidad celular
 - ✓ Terapia de quelación vs Cáncer.
 - ✓ Efecto Caballo de Troya - patógenos AMR.



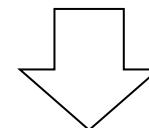
Sideróforos y THA

- ✓ Promiscuidad: Expresión de múltiples proteínas de absorción de hierro, incluídas para sideróforos exógenos (no nativos de la bacteria)
- ✓ Algunas bacterias como *Neisseria spp.* y *Haemophilus influenzae*, **no sintetizan sideróforos**, pero producen receptores para proteínas humanas que contienen hierro, como las transferrinas.

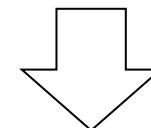


Planteamiento y formulación del problema

- ✓ THA es un mecanismo prometedor para la administración de fármacos
- ✓ Modificaciones en Fe-DFO (intercambio de metal por Al, Ga, Cd o síntesis de derivados de DFO) han mostrado eficacia en actividad antibacterial ($\downarrow IC_{50}$)



- ✓ *Acinetobacter baumannii* es un patógeno prioritario (Nivel 1) por la OMS.
- ✓ Estudios de aplicación de THA son cajas negras a nivel molecular*.

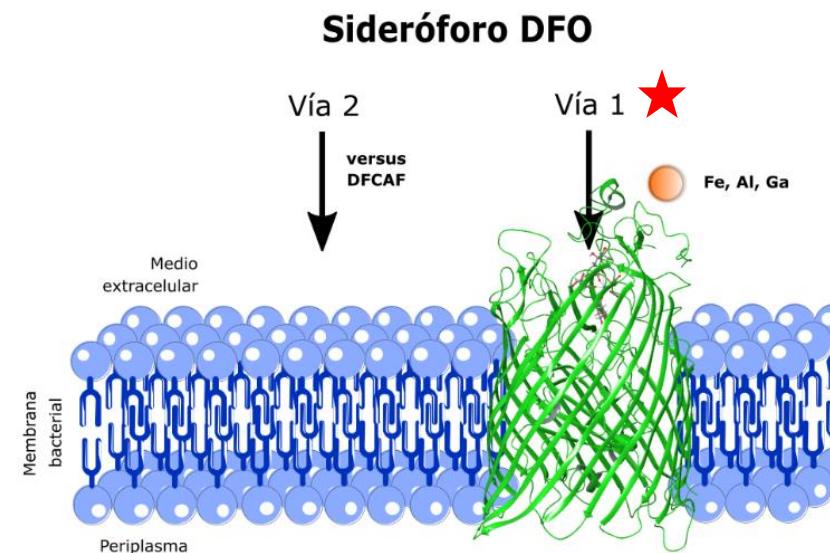


- ✓ ¿Podemos usar las herramientas *in silico* para estudiar el efecto Caballo de Troya contra *Acinetobacter baumannii*?

Objetivos

General

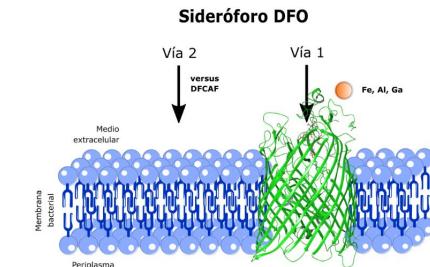
- ✓ Identificar las interacciones en el reconocimiento molecular de Fe-DFO, Al-DFO y Ga-DFO, en *Acinetobacter baumannii*, por medio de herramientas *in silico* en el receptor FhuE y, fuera de este, empleando el sideróforo DFO y su derivado con cafeína, DFCAF, para evaluar su potencial efecto “Caballo de Troya”.



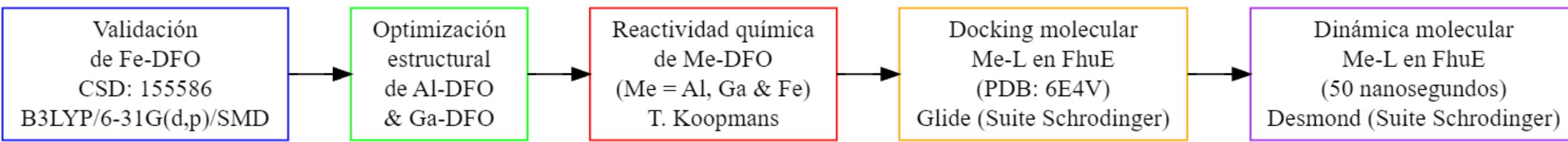
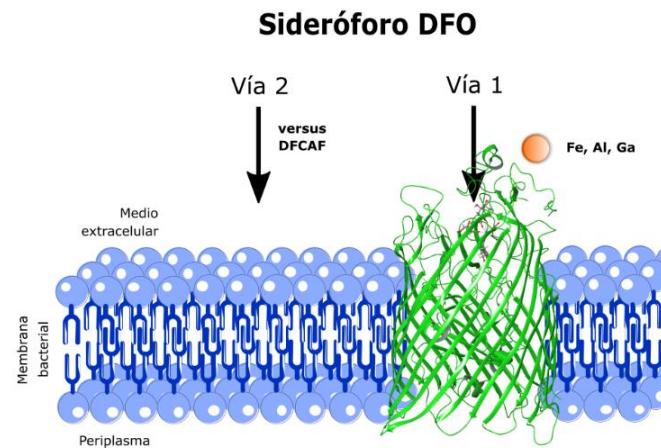
Objetivos

Específicos

- ✓ 1. Analizar la reactividad química de los complejos Me-DFO y sus interacciones intermoleculares con la proteína receptora.
- ✓ 2. Evaluar la actividad de DFCAF a través del receptor y de la membrana.
- ✓ 3. Diseñar un modelo predictor de permeabilidad a la membrana celular.
- ✓ 4. Analizar la influencia de la isomería de Fe-DFO en el reconocimiento molecular.
- ✓ 5. Analizar las interacciones intermoleculares de DFO versus DFCAF en una membrana basada en POPE por 50 nanosegundos de simulaciones de dinámica molecular.
- ✓ 6. Realizar estudios de DM *in vitro* en *A. baumannii* con administración de DFO y DFCAF*.
- ✓ 7. Analizar la toxicidad en *A. baumannii* y biofilmes cuando se administra DFO y DFCAF cargados en liposomas de asolecitina de soja.



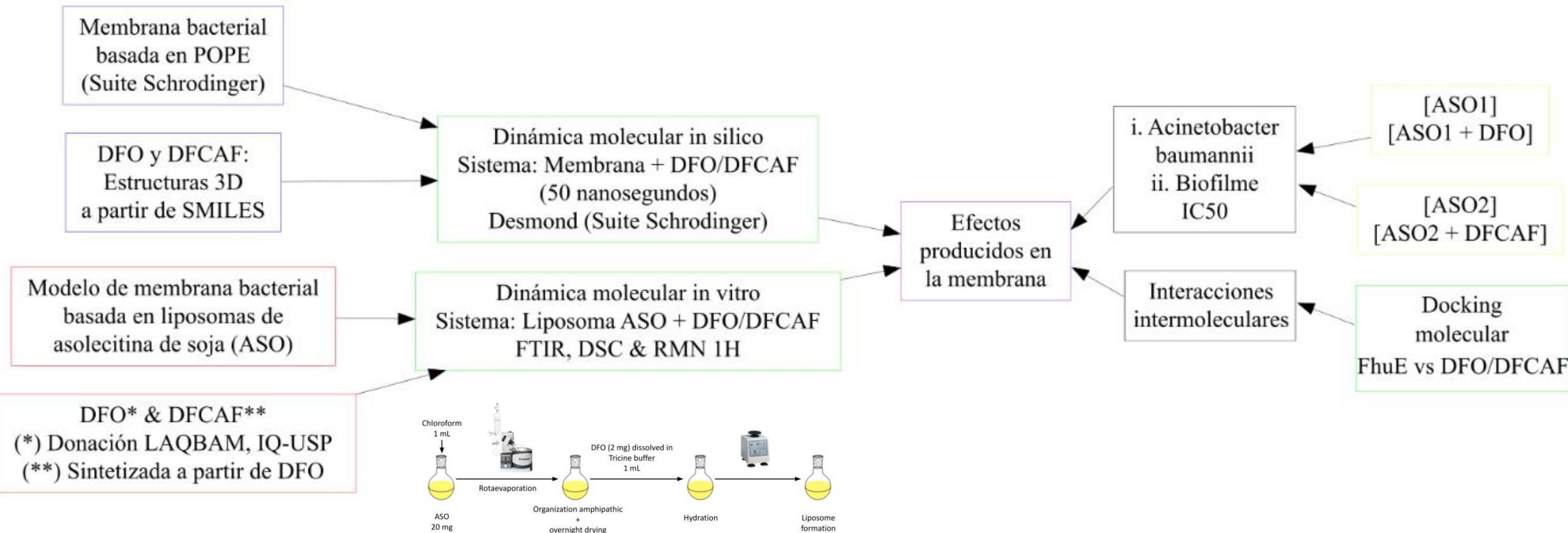
Vía 1



Metodología empleada para evaluar THA debido al reconocimiento molecular en FhuE.

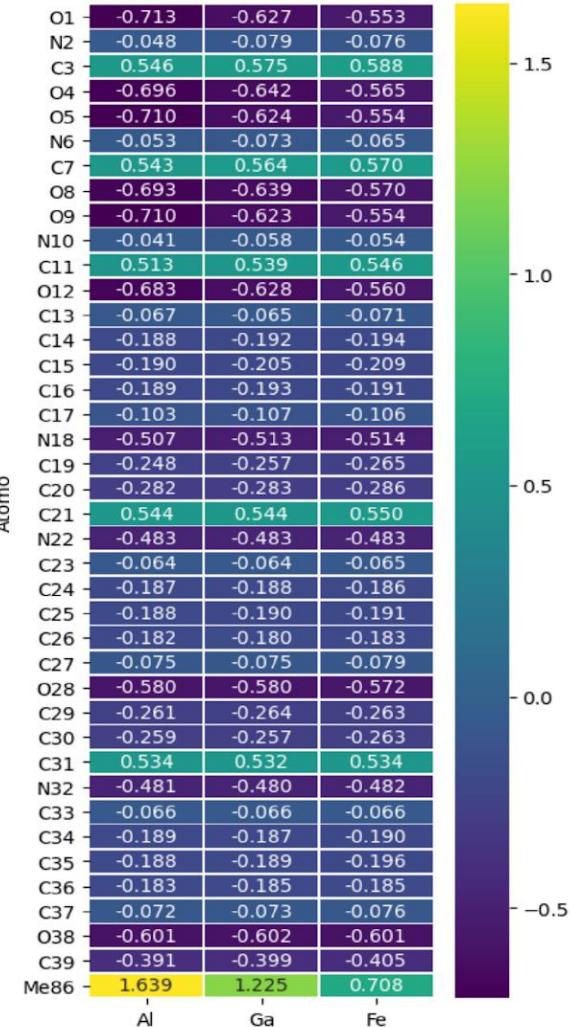
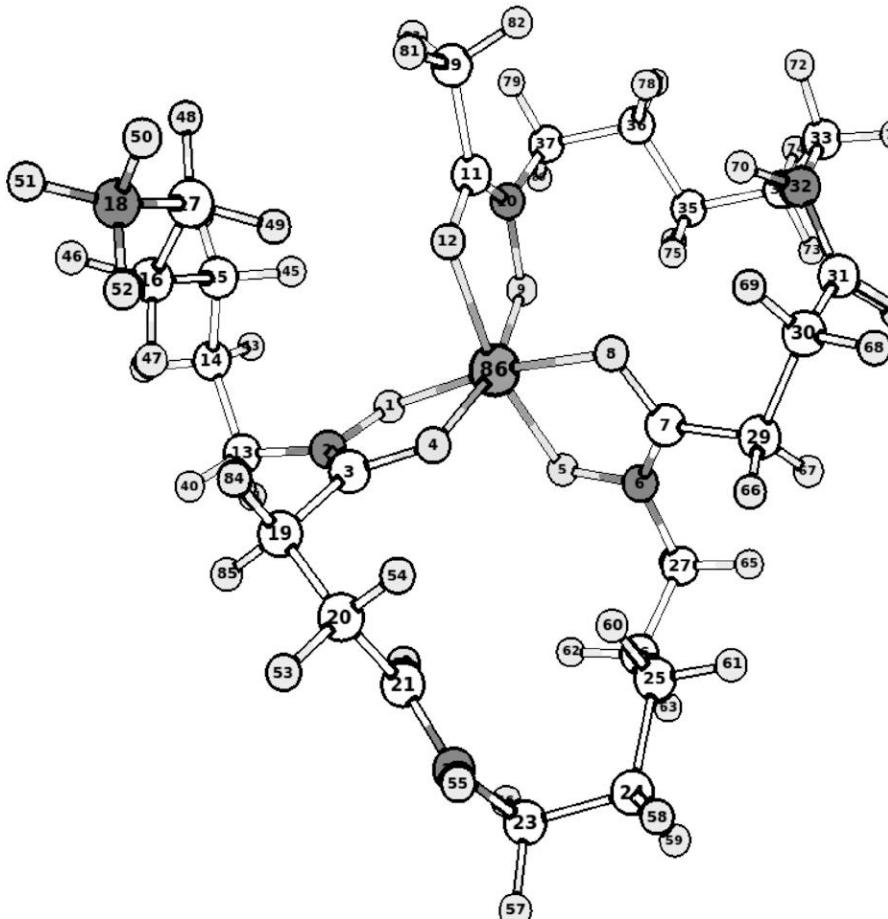
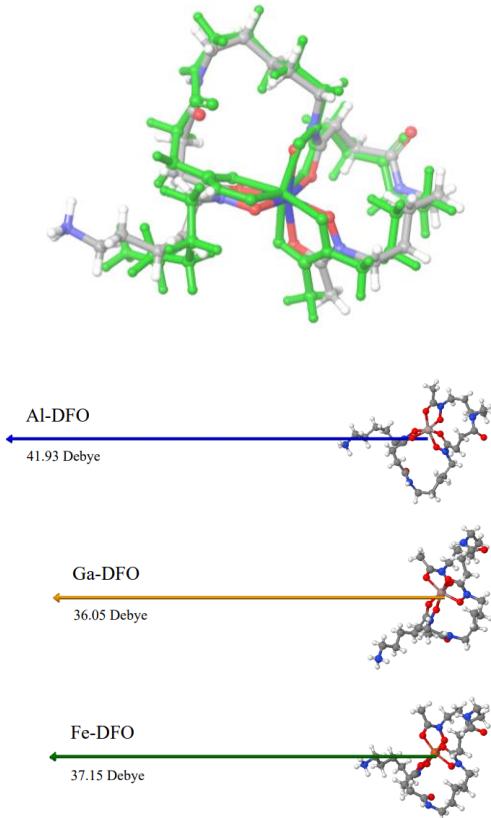
Vía 2

Metodología empleada para evaluar THA sin optar por el reconocimiento en FhuE.



Estructura y reactividad

- ✓ RMSD (Fe-DFO): 0.8 Å
- ✓ Átomo 86: Al, Ga, Fe



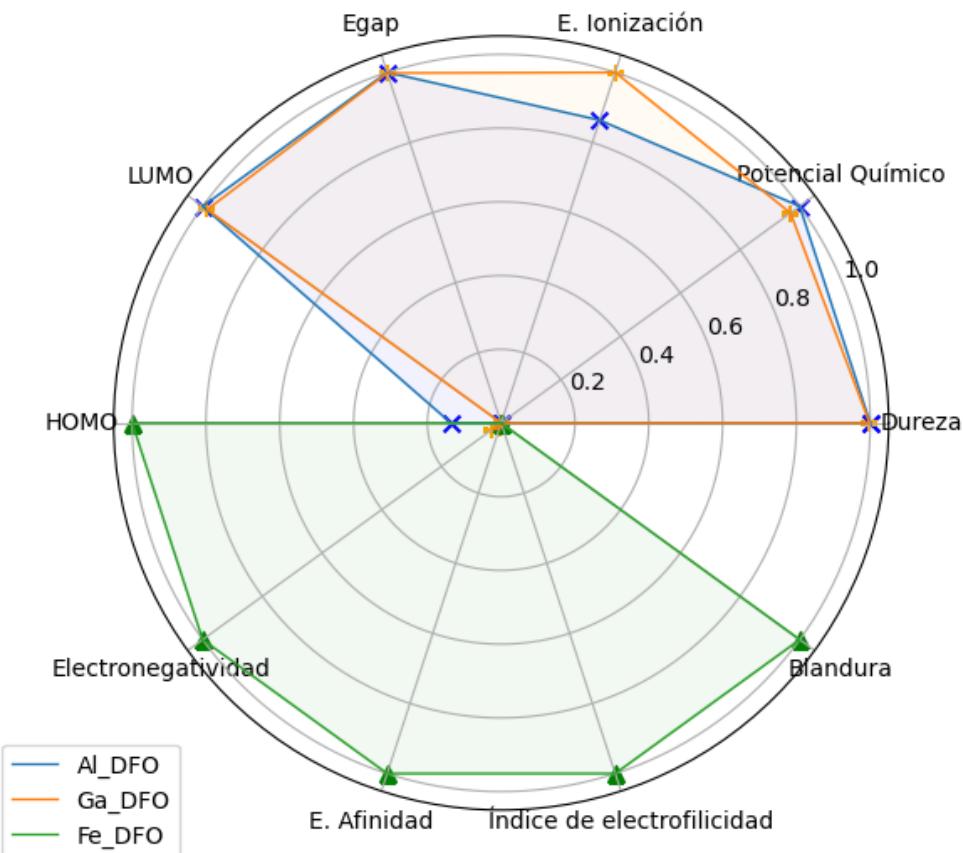
Estructura y reactividad

Reactividad global de los complejos Me-DFO.

Compuesto	HOMO / eV	LUMO / eV	Energía gap / eV	Energía de ionización / eV	Afinidad Electrónica / eV
Al-DFO	-5.83	0.13	5.96	5.83	-0.13
Ga-DFO	-5.87	0.09	5.96	5.87	-0.09
Fe-DFO	-5.62	-2.47	3.15	5.62	2.47

Continuación de la Tabla 2.

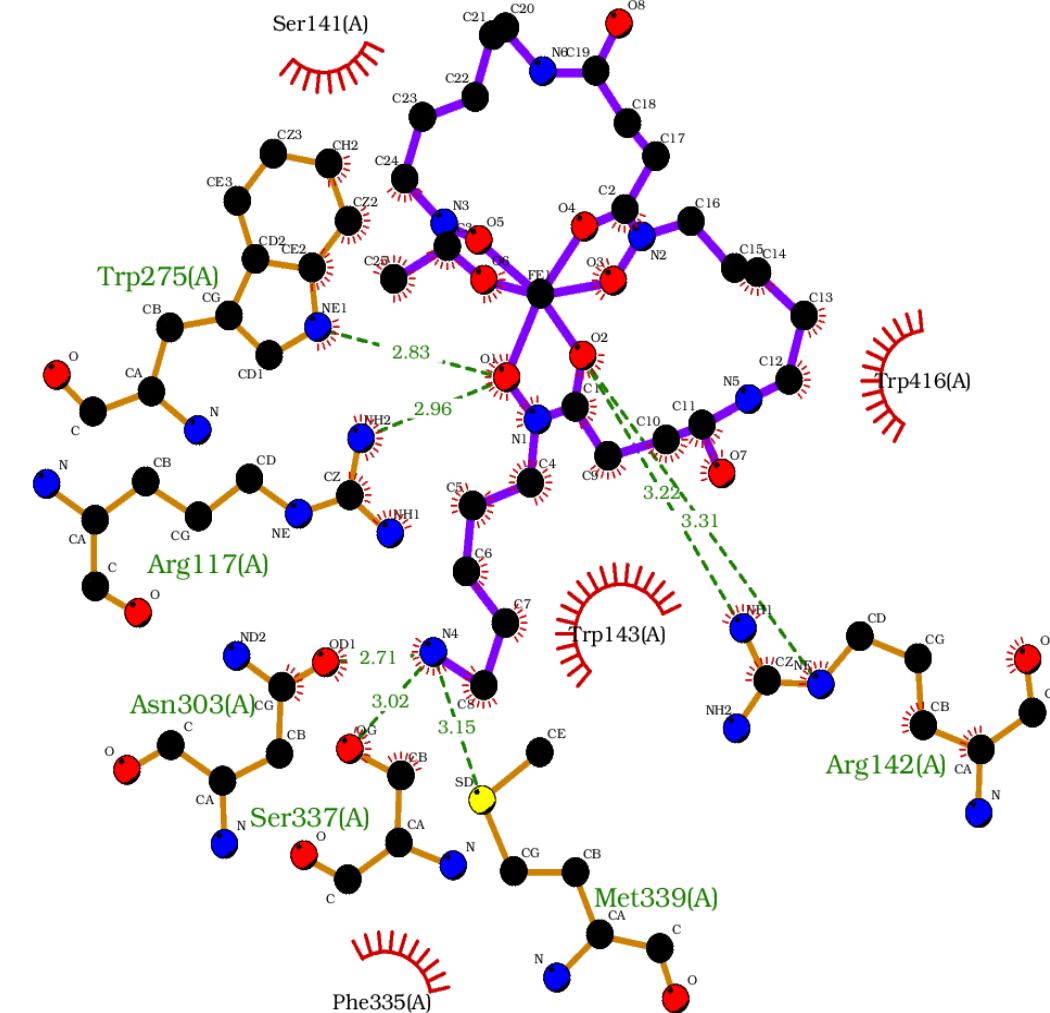
Compuesto	Potencial químico	Electronegatividad	Dureza	Blandura	Índice de electrofilicidad
Al-DFO	-2.85	2.85	2.98	0.17	1.37
Ga-DFO	-2.89	2.89	2.98	0.17	1.4
Fe-DFO	-4.04	4.04	1.57	0.32	5.19



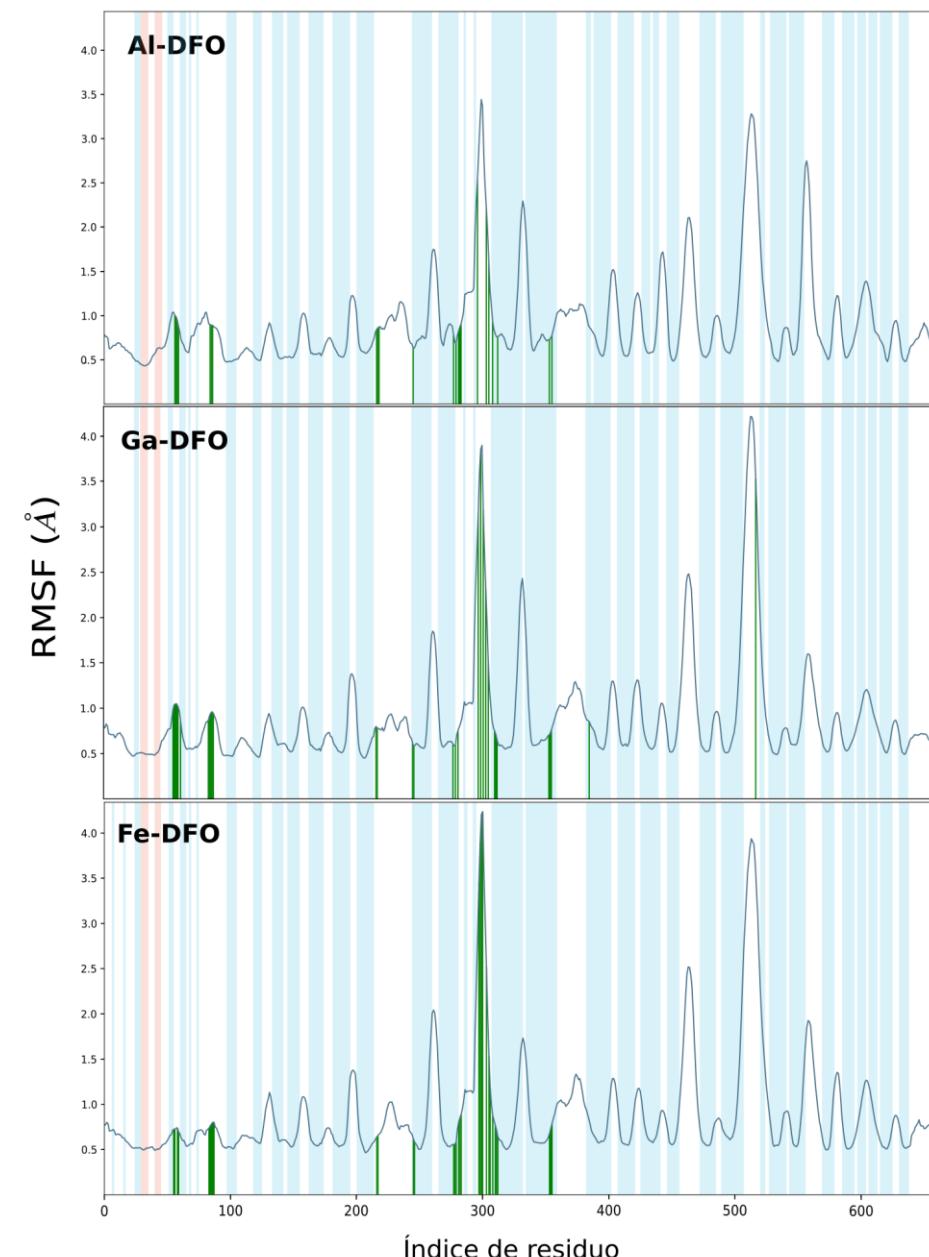
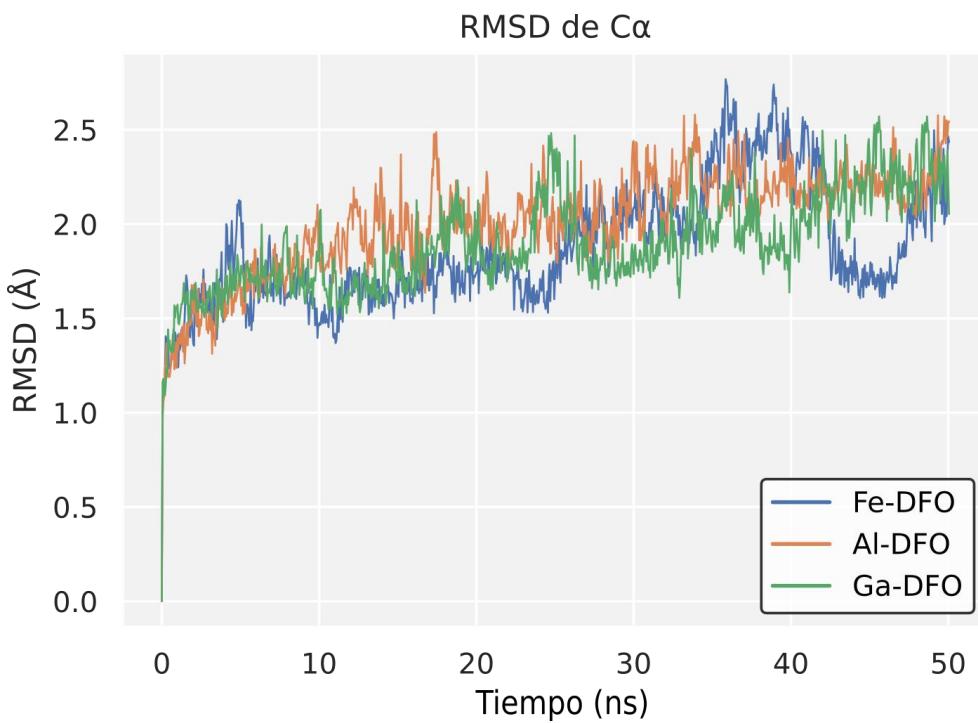
Docking molecular

Docking score e interacciones intermoleculares de Me-DFO con FhuE.

Compuesto	Docking score / kcal.mol ⁻¹	Enlace de hidrógeno	Lipofílico
HWS	-5.814	Arg117, Arg142, Trp275, Asn373	Ser141, Trp143, Ser337, Met339, Gly371, Trp416
Al-DFO	-5.772	Arg117, Arg142, Trp275, Asn303, Ser337	Trp143, Phe335, Met339, Trp416
Ga-DFO	-5.926	Arg117, Arg142, Trp275, Asn303, Ser337, Met339,	Ser141, Trp143, Phe335, Trp416
Fe-DFO	-5.880	Arg117, Arg142, Trp275, Asn303, Ser337, Met339	Ser141, Trp143, Phe335, Trp416



Simulaciones de Dinámica Molecular



$$RMSD_x = \sqrt{\frac{1}{N} \sum_{i=1}^N (r'_i(t_x)) - r_i(t_{ref}))^2}$$

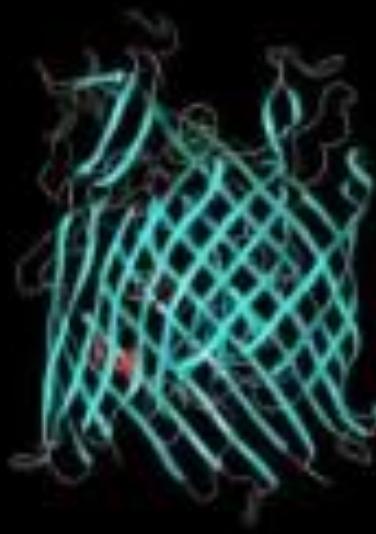
$$RMSF_i = \sqrt{\frac{1}{T} \sum_{t=1}^T \langle (r'_i(t)) - r_i(t_{ref}))^2 \rangle}$$

Simulaciones de Dinámica Molecular



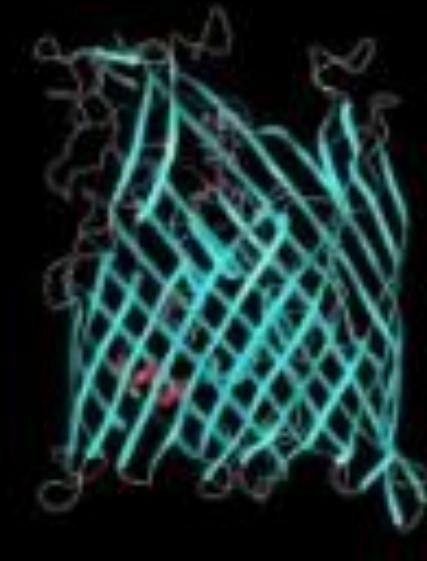
✓ Fe-DFO

<https://youtu.be/Wr3V32W4PZg>



✓ Al-DFO

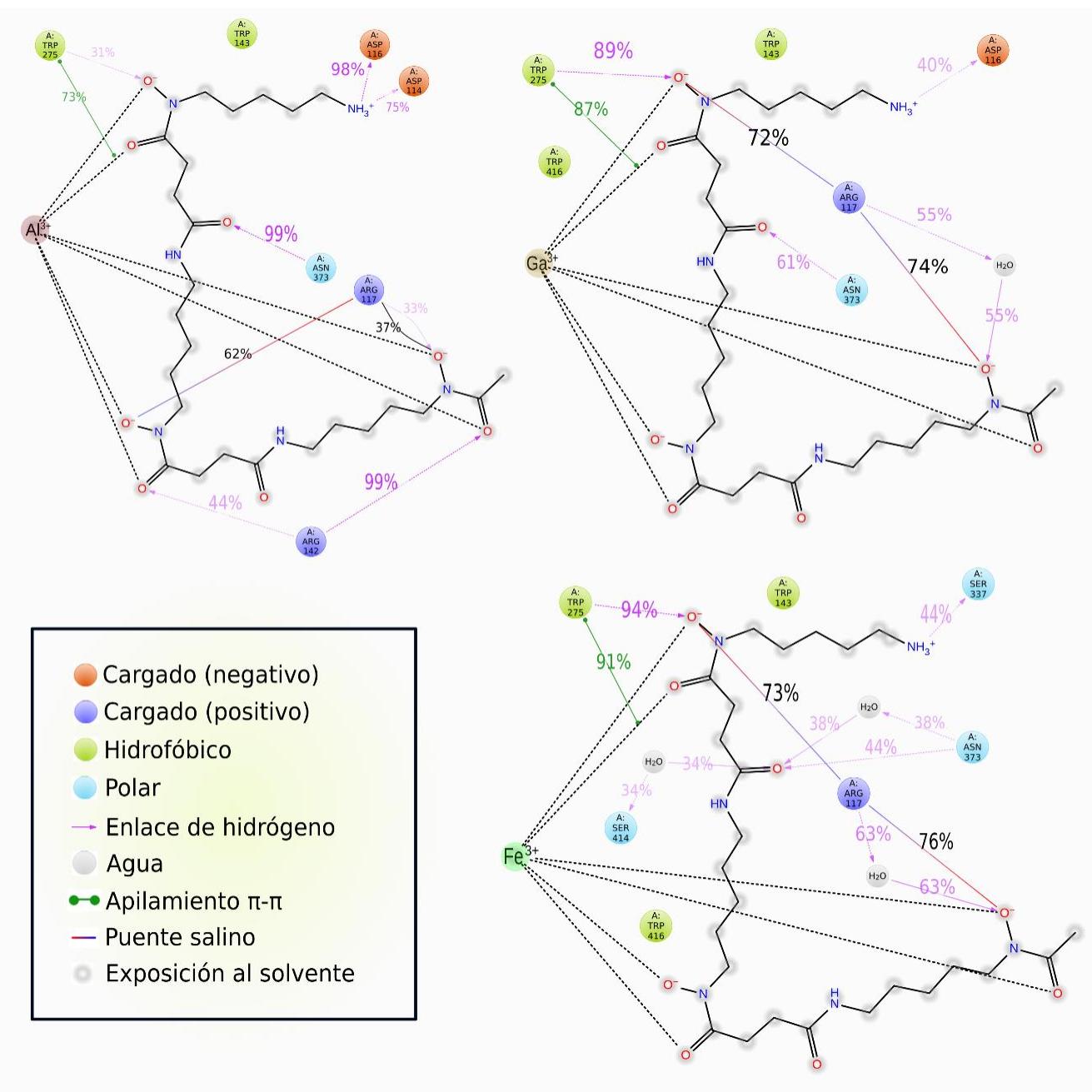
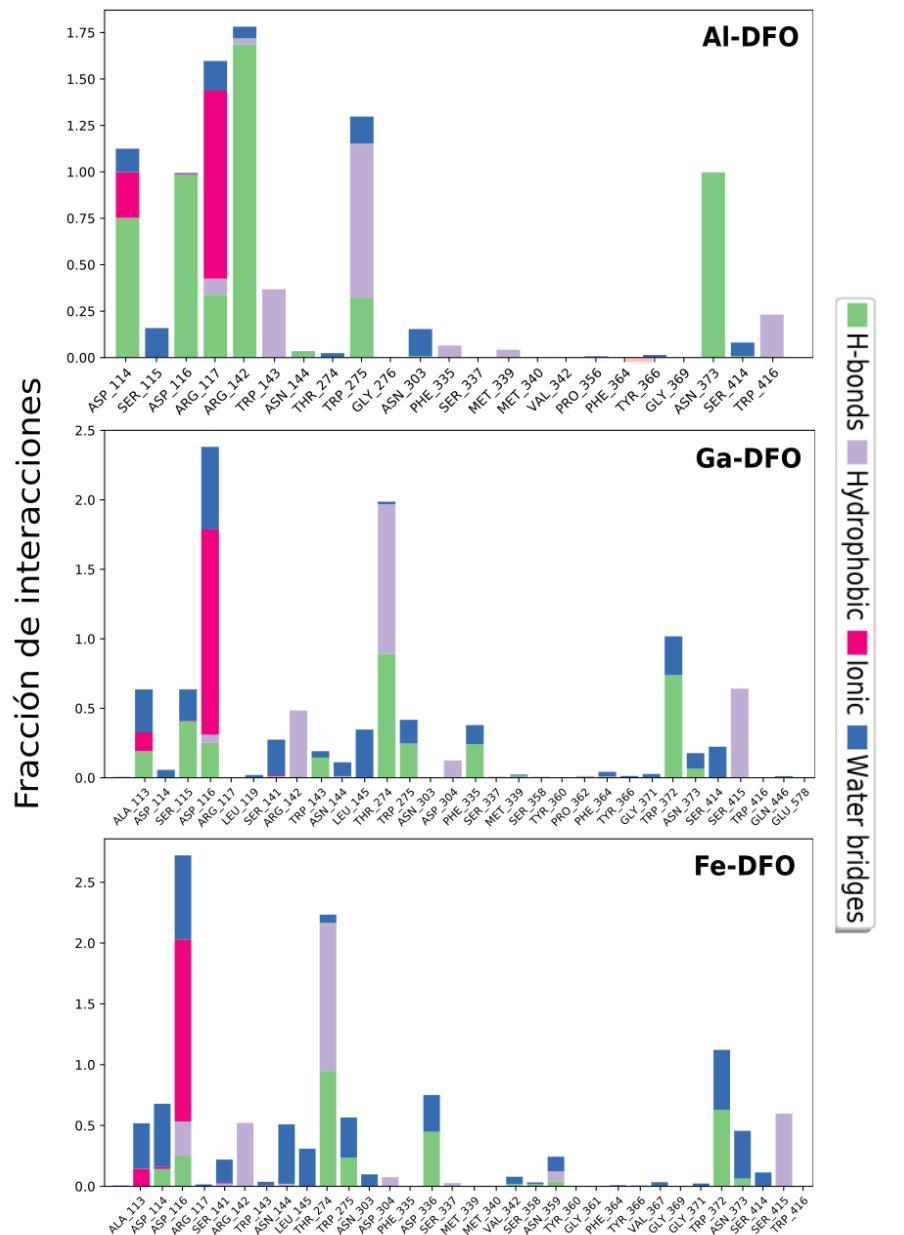
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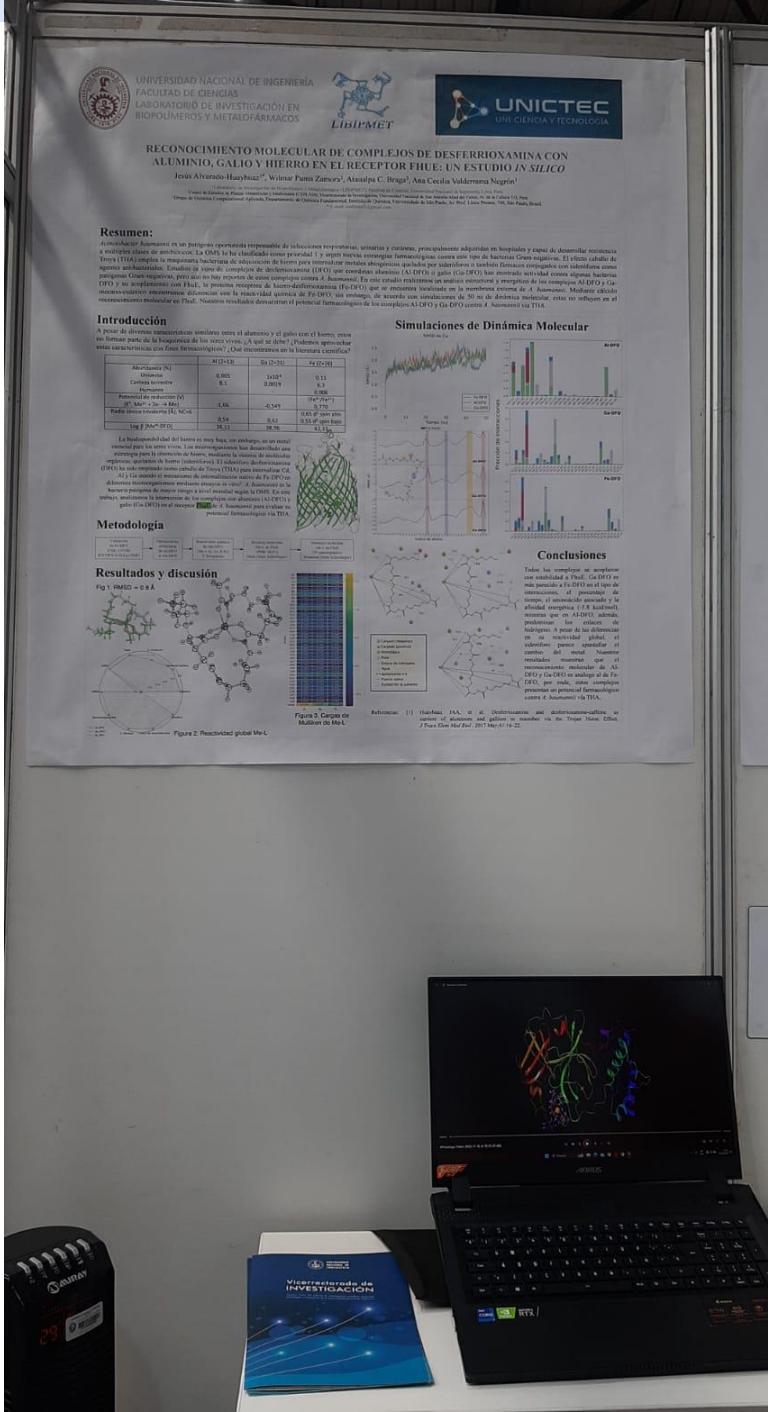


✓ Ga-DFO

<https://youtu.be/WKwiqy9Q58w>

Diagrama de barras de la fracción de interacciones







Prediction of Siderophores Partition Coefficient using Artificial Neural Networks

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² Laboratorio de Ingeniería Biomédica, Universidad Peruana Cayetano Heredia, Lima, Peru

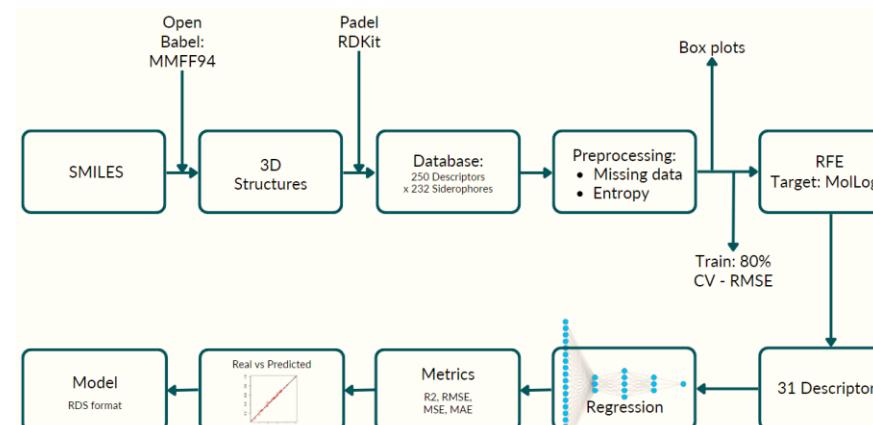
³ Centro de Ciências Computacionais (C3), Universidade Federal do Rio Grande, Av. Itália km 08, Campus Carreiros, Rio Grande 96201-900, Brazil

Abstract. The octanol-water partition coefficient ($\log P$) is a recurring molecular descriptor in the empirical rules that estimate the druglikeness of a molecule. $\log P$ is used as a measure of lipophilicity and cell permeability. Its prediction saves chemical reagents, effort and time. Quantum mechanical methods are the most efficient but have a high computational cost and other methods are limited by molecular weight, number and type of atoms. Siderophores are molecules synthesized by microorganisms for the acquisition of iron, with a molecular weight generally greater than 500 Daltons. In recent years, they have been used as a Trojan Horse (THA) to internalize toxic agents in microorganisms. In this work, we have collected 232 siderophores and used 250 molecular descriptors to efficiently predict $\log P$ using artificial neural networks. These results are useful for the selection of molecules with pharmacological potential via THA.

Keywords: $\log P$ · siderophore · artificial neural networks

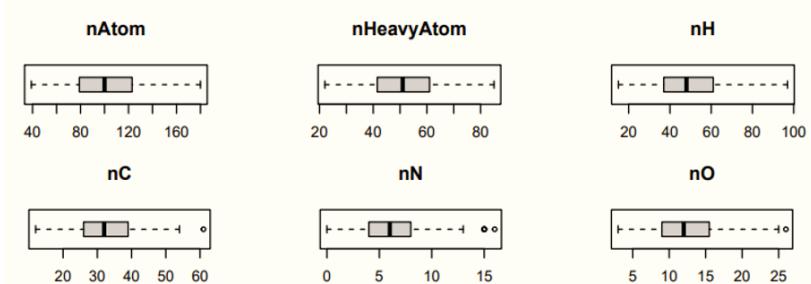
Factor: Permeabilidad

Model	Druglikeness	Ref.
Lipinsky	MW<500, $\log P < 4.15$, N ou O <10, NH ou OH <5	[10]
Ghose	160<MW<480, $-0.4 < \log P < 5.6$, $40 < MR < 130$, $20 < \text{atoms} < 70$	[7]
Veber	Rotatable bonds<10, TPSA <140	[19]
Egan	$\log P < 5.88$, TPSA<131.6	[5]
Muegge	$200 < MW < 600$, $-2 < \log P < 5$, TPSA<150, Num. rings<7, NumC>4, heteroatoms>1, rotatable bonds< 15, H-bond acc<10, H-bond don<5	[14]

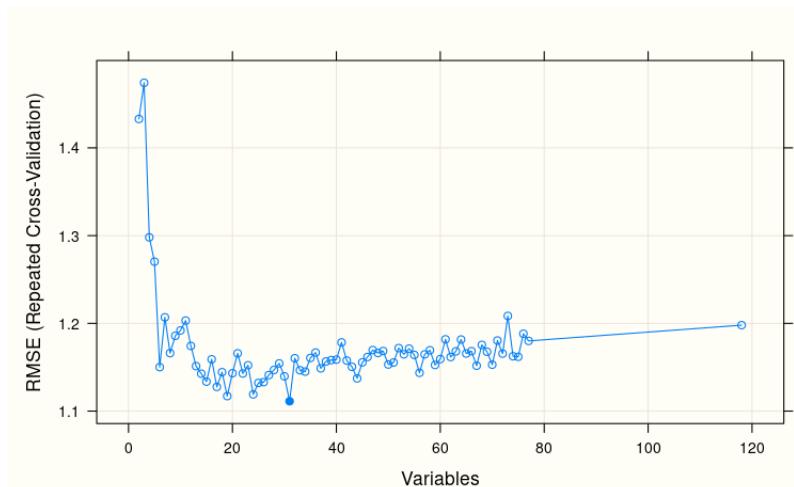


EDA, Preproc, Entrenamiento, Validación

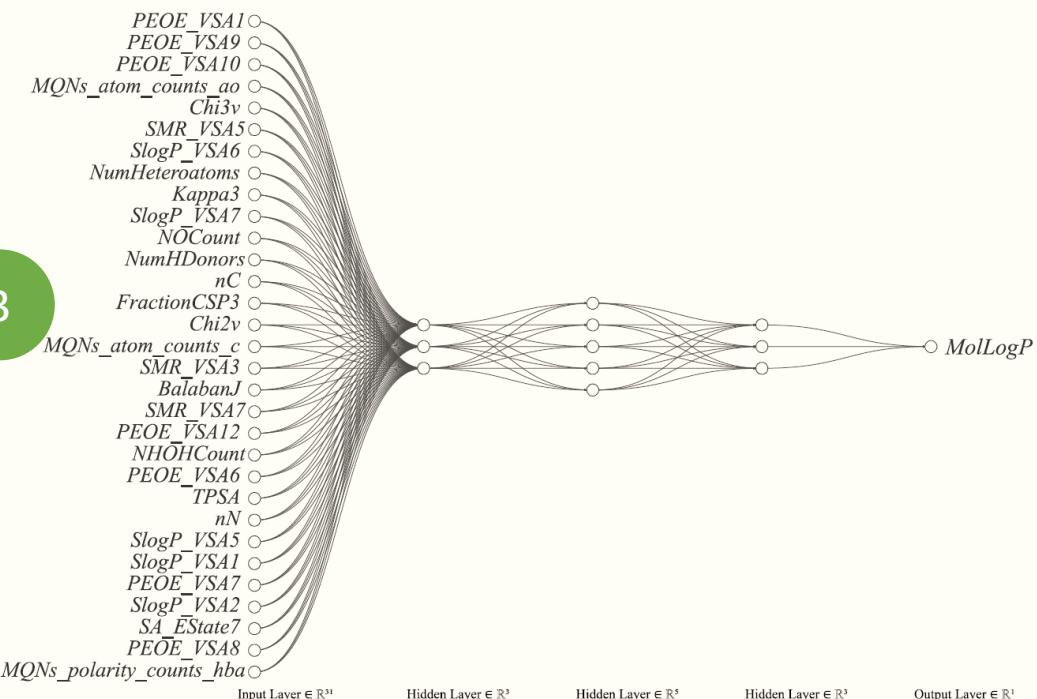
1



2



3



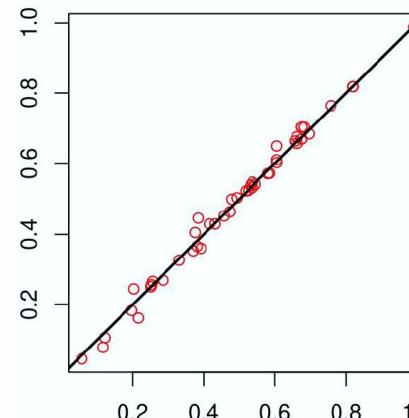
Real vs Predicted

$$R^2 = 1 - \frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{\sum_{i=1}^n (y_i - \bar{y})^2}$$

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^n (y_i - \hat{y}_i)^2}$$

$$MSE = \frac{1}{n} \sum_{i=1}^n (y_i - \hat{y}_i)^2$$

$$MAE = \frac{1}{n} \sum_{i=1}^n |y_i - \hat{y}_i|$$



4



Stereochemistry may not influence the molecular recognition of Ferrioxamine

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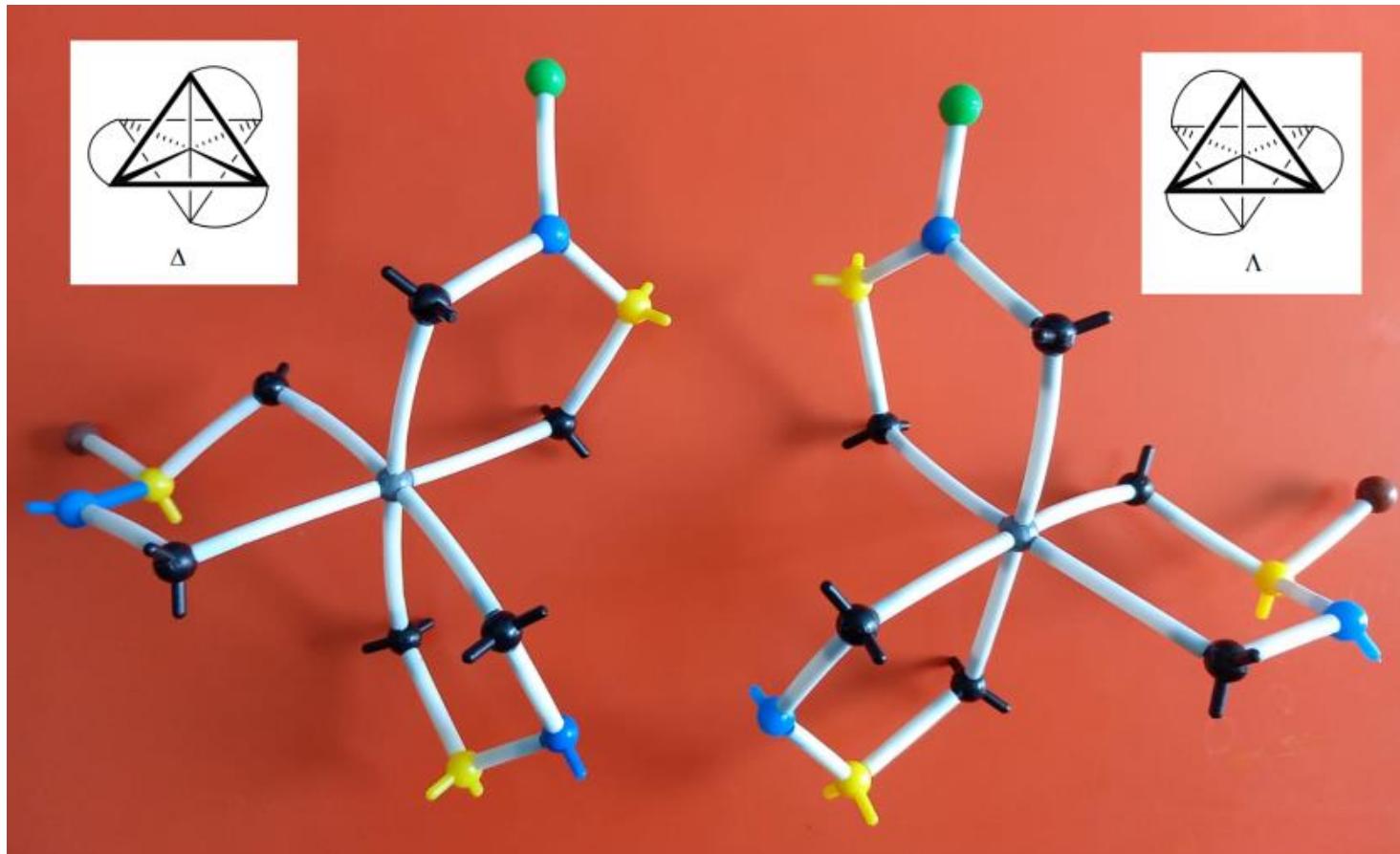
² Centro de Ciências Computacionais (C3), Universidade Federal do Rio Grande, Av. Itália km 08, Campus Carreiros, Rio Grande 96201-900, Brazil

³ Department of Fundamental Chemistry, Institute of Chemistry, University of São Paulo, Lineu Prestes Av., 748, São Paulo, 05508-900, São Paulo, Brazil

Abstract. The bioavailability of iron is extremely low despite being an essential element for life. The molecular mechanism of iron acquisition in bacteria depends on the chemistry of siderophores. Since microorganisms exhibit promiscuity in the use of exogenous siderophores, the imitation and chemical modification of these compounds have been employed in drug development. Hydroxamate iron-siderophore complexes, such as ferrioxamine, feature a chiral metal center, thus a single structure can generate a series of stereoisomers with different molecular recognition. In this work, we developed a database with ferrioxamine stereoisomers to address this question. Using computational tools, we constructed the three-dimensional molecules of ferrioxamine from its SMILES representation and docked them to the FoxA receptor of *Pseudomonas aeruginosa*. On average, the free binding energy was -9.3 kcal/mol, and the most frequent intermolecular interactions were: GLY657 86%, ASN662 81%, PRO659 87%, coincidentally with the control. Unsupervised machine learning methods were employed to search for patterns through PCA, KMeans, and DBSCAN, using molecular descriptors, and a better clustering was found for k=2. Finally, through multivariate hypothesis testing, we found no significant difference between the groups with higher and lower FEB. These results shed light on the utilization of iron despite its stereochemistry in the molecular recognition of ferrioxamine.

Keywords: ferrioxamine · artificial intelligence · stereochemistry

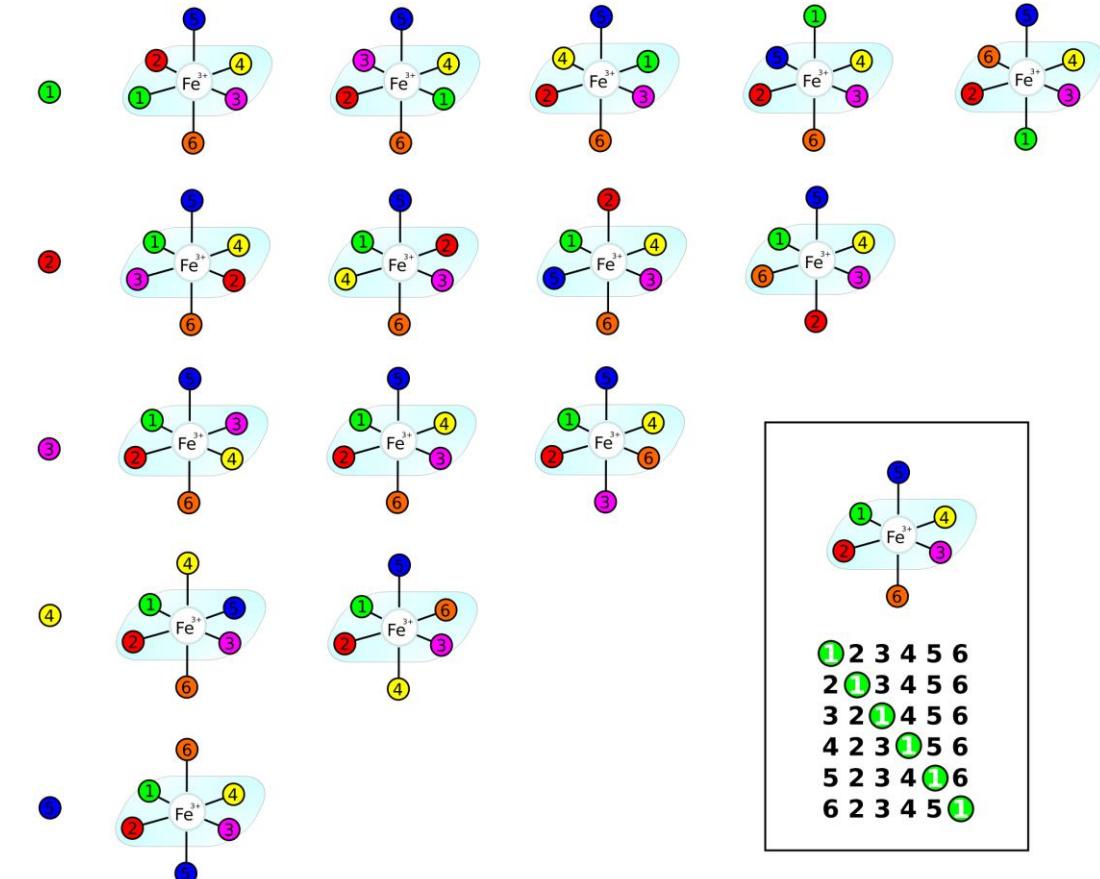
Factor: Isomería



Además de los isómeros ópticos (enantiómeros delta y lambda), están los isómeros geométricos (fac y mer)

Base de datos

PDB ID	Released	R (Å)	MW (kDa)	Ligand	Organism	Reference
8B14	2023-02-08	2.60	150.86	APO	EC/EC	[4]
6Z8A	2020-11-25	2.95	86.61	6L0 (mer,Δ)	PA/EC	[17]
6E4V	2019-08-28	2.00	79.76	HWS (fac,Δ)	AB/EC	[9]
6I98	2019-08-28	2.80	79.01	APO	PA/EC	[12]
6I96	2019-08-28	1.85	81.19	0UE (fac,Δ)	PA/EC	[12]
4CU4	2014-04-09	2.30	88.63	MJ25	EC/EC	[15]
2O5P	2007-10-30	2.77	174.41	APO	PA/PA	[3]
2GRX	2006-06-13	3.30	216.87	FCI (fac,Δ)	EC/EC	[18]
1IFI1	2001-08-29	2.90	83.47	RIF	EC/EC	[8]
1QFG	2000-07-26	2.50	85.03	APO	EC/EC	[6]
1QFF	2000-07-26	2.70	85.24	FCI (fac,Δ)	EC/EC	[6]
1QKC	2000-06-05	3.10	85.15	ALB (fac,Δ)	EC/EC	[8]
1QJQ	2000-06-05	2.95	84.92	PFC (fac,Δ)	EC/EC	[8]
2FCP	1999-01-13	2.50	83.37	APO	EC/EC	[7]
1FCP	1999-01-13	2.70	82.79	FCI (fac,Δ)	EC/EC	[7]
1BY5	1999-01-13	2.60	81.34	Ferrichrome (fac,Δ)	EC/EC	[14]
1BY3	1999-01-13	2.74	80.37	APO	EC/EC	[14]



molsimplify -lig "(ON3CCCCNC(=O)C5)(ON1CCCC[NH+])(OC2C5)(OC3C)(OC1C4)(ON2CCCCNC(=O)C4)"
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Combinatoria

```

[] x = '123456'

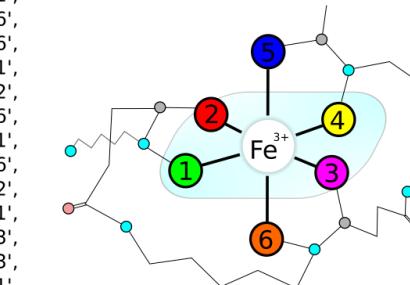
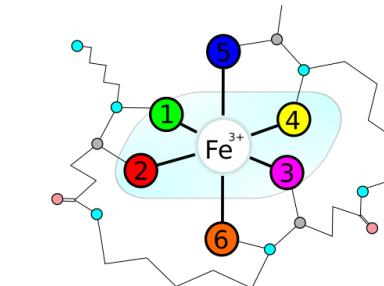
[] def permutacion(palabra):
    if len(palabra) == 1:
        return [palabra]
    perms = permutacion(palabra[1:])
    char = palabra[0]
    result = []
    for perm in perms:
        for i in range(len(perm) + 1):
            result.append(perm[:i] + char + perm[i:])
    return result

[] x = '123456'
grupo = permutacion(x)
grupo

[] len(grupo)
720

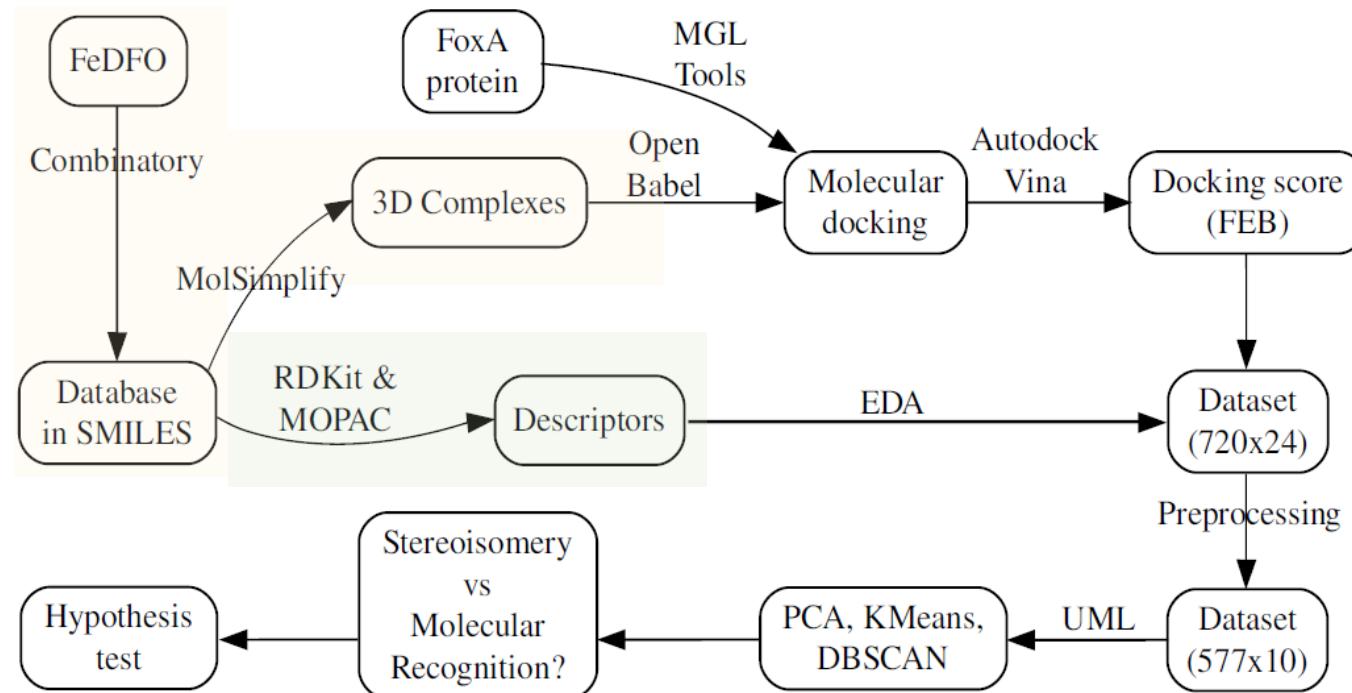
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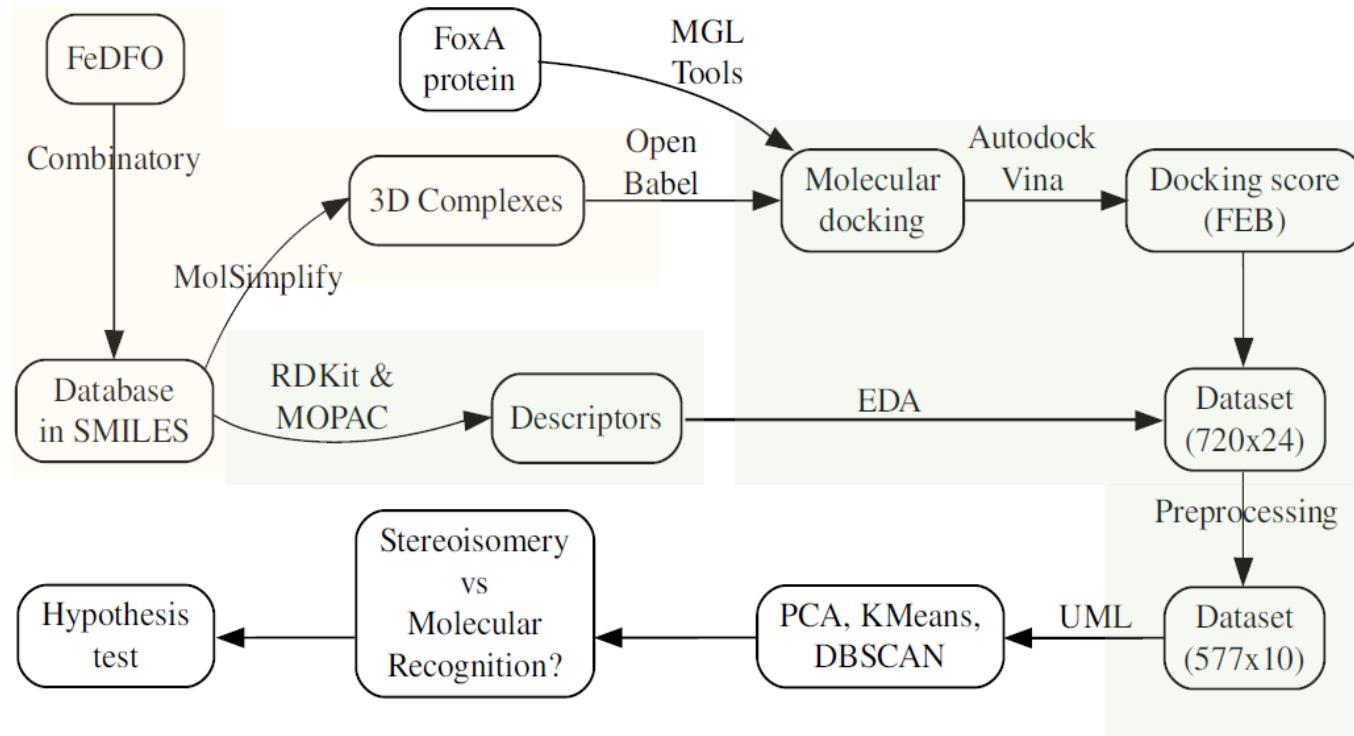
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Combinatoria

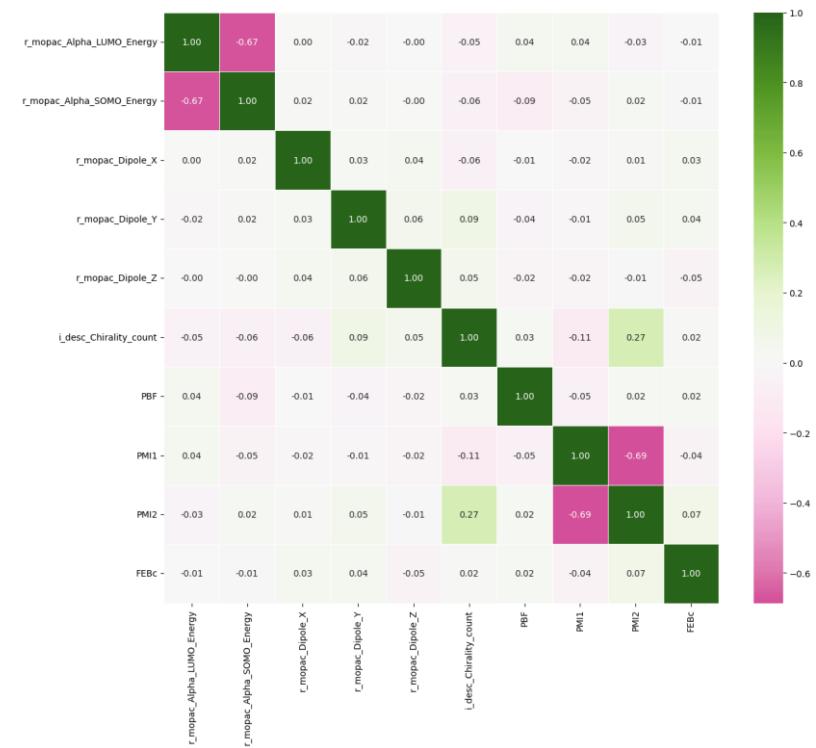


Number	RDKit	Mopac
1	PBF	r_mopac_Alpha_LUMO_Energy,
2	PMI1	r_mopac_Alpha_SOMO_Energy,
3	PMI2	r_mopac_Beta_LUMO_Energy
4	PMI3	r_mopac_Beta_SOMO_Energy
5	NPR1	r_mopac_Dipole
6	NPR2	r_mopac_Dipole_X
7	RadiusOfGyration	r_mopac_Dipole_Y
8	InertialShapeFactor	r_mopac_Dipole_Z
9	Eccentricity	r_mopac_Ionization_Energy
10	Asphericity	r_mopac_MOPAC_Heat_of_Formation
11	SpherocityIndex	r_mopac_MOPAC_Total_Energy_EV
12		i_desc_Chirality_count

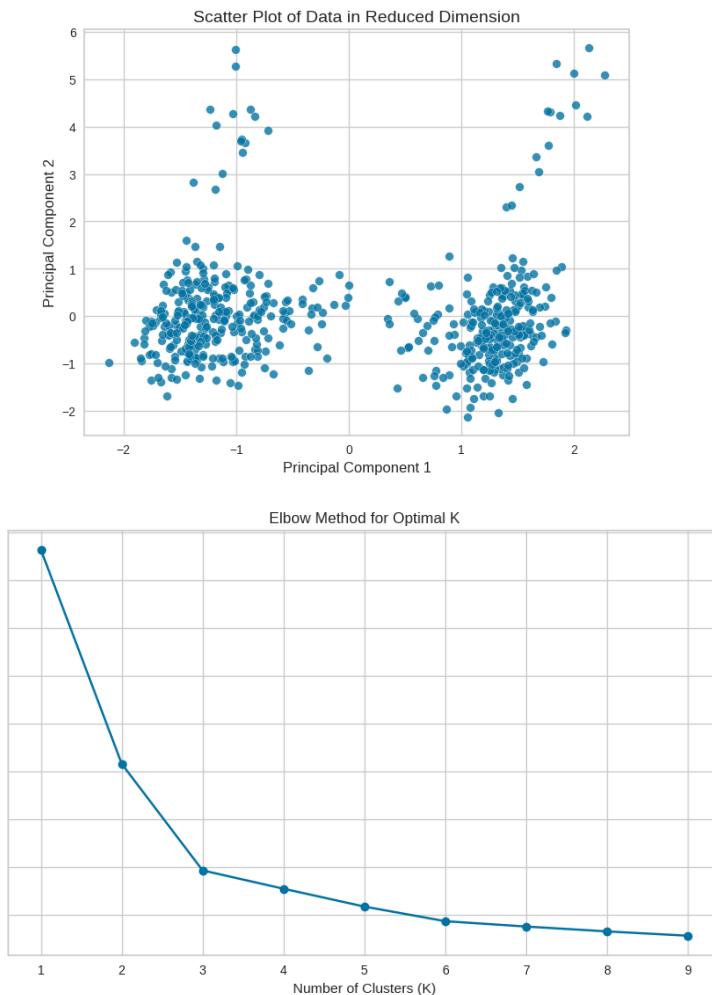
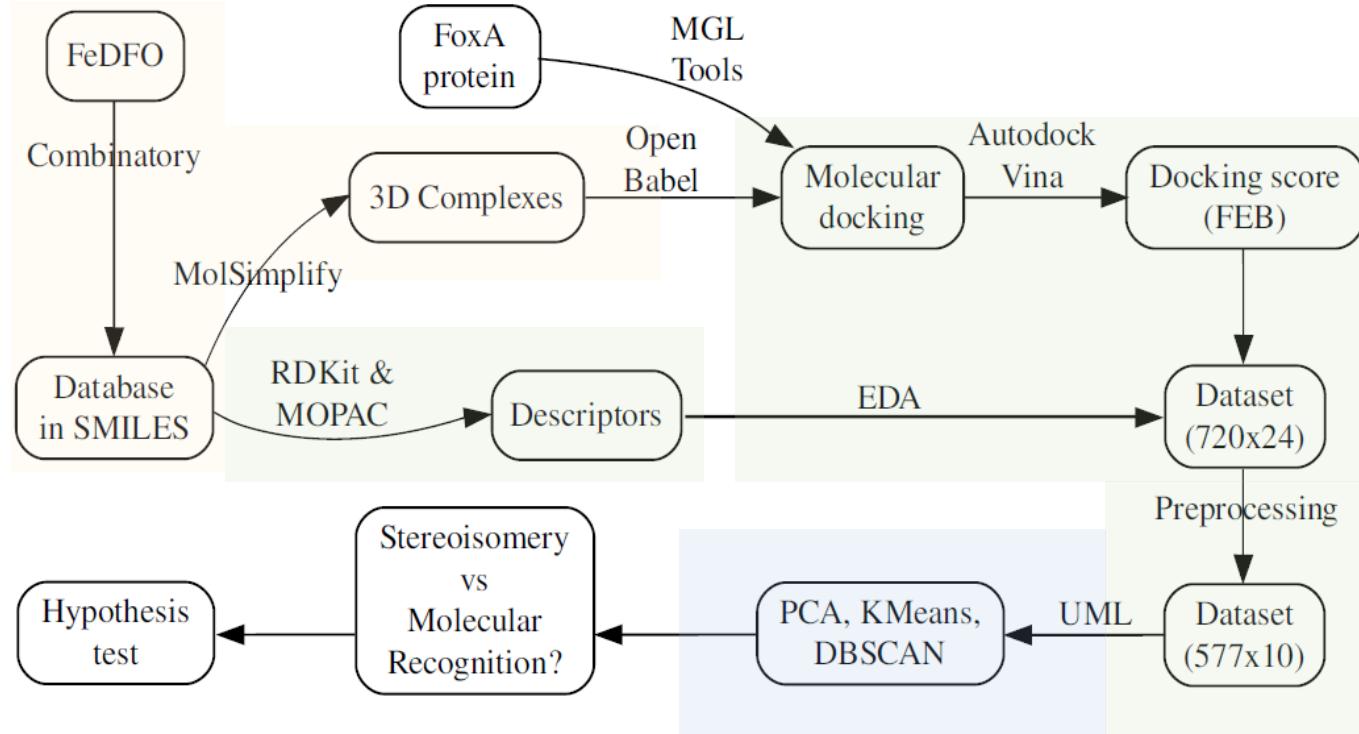
Docking molecular



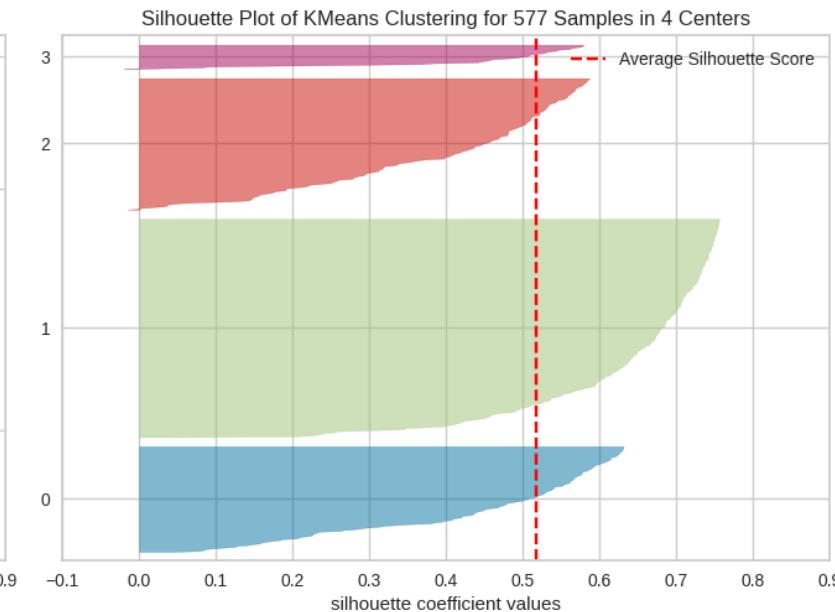
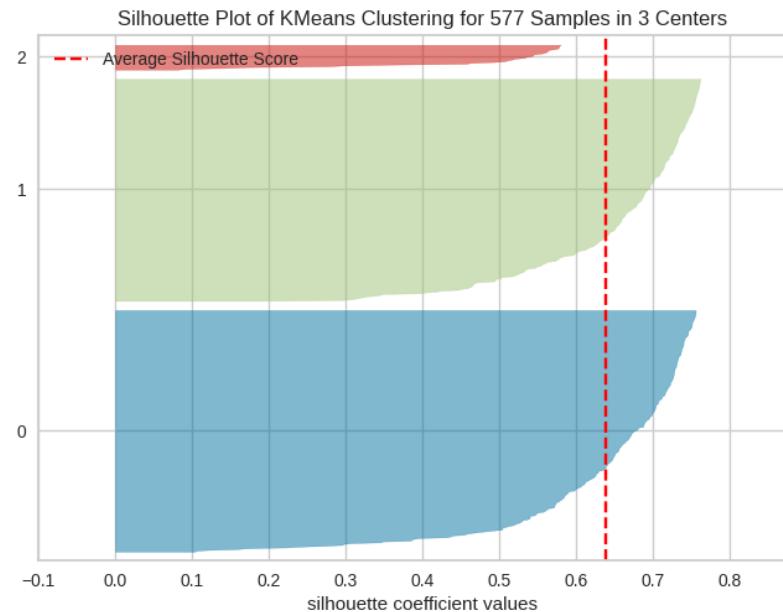
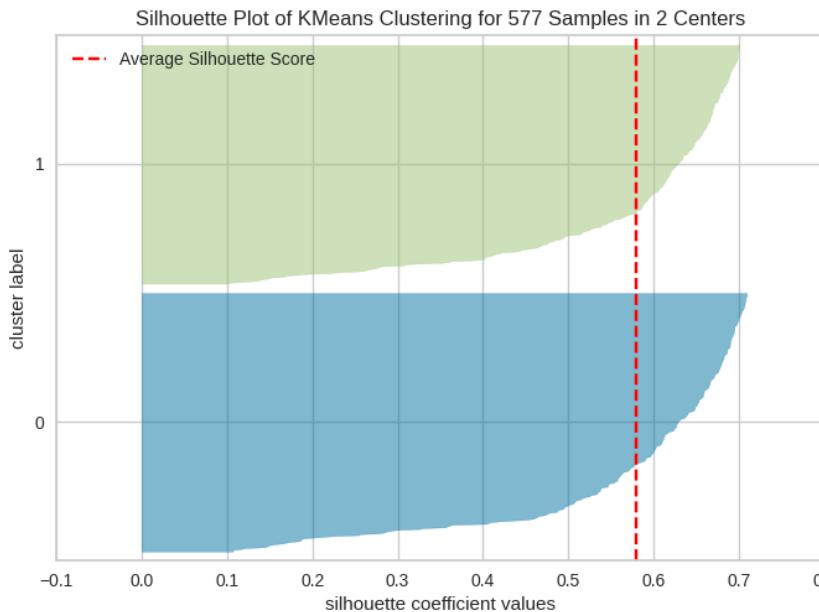
GLY657 86%, ASN662 81% y PRO659 87%.



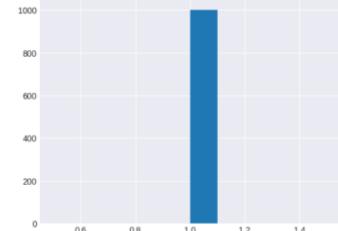
ML no supervisado



Agrupamiento



Test de Hipótesis

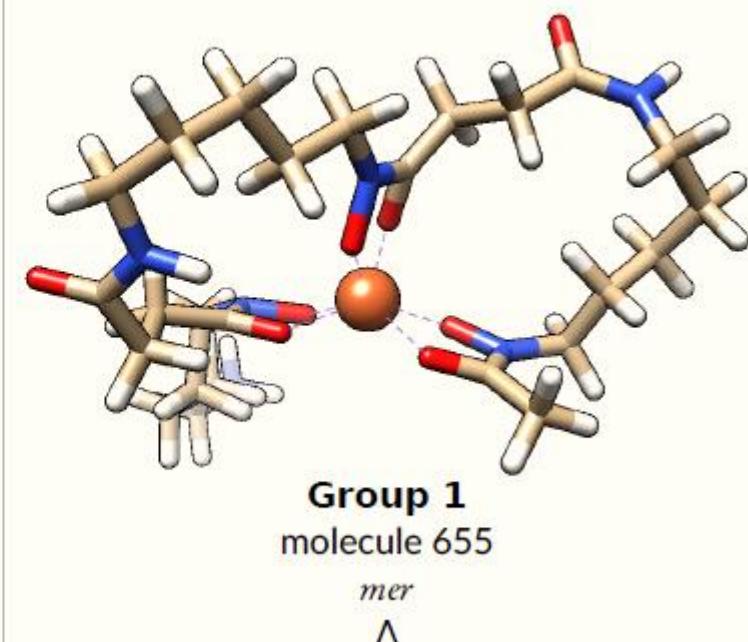
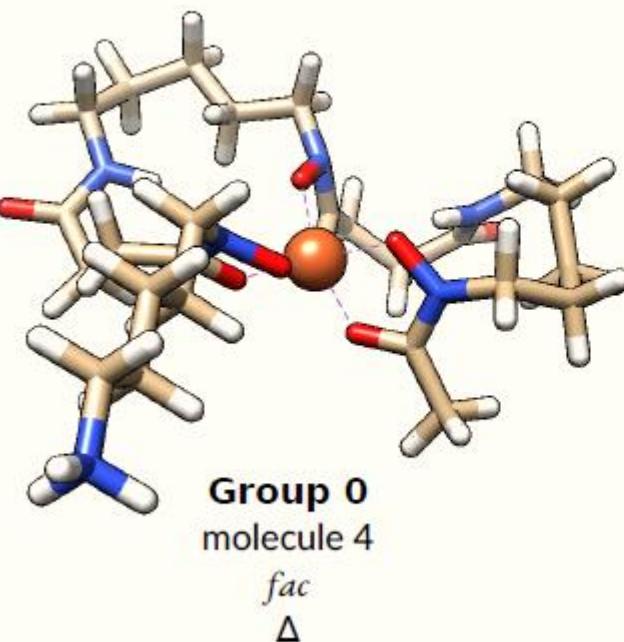


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r_mopac_Beta_LUMO_Energy	0.832039	7.84e-27	False
r_mopac_Beta_SOMO_Energy	0.911851	5.18e-20	False
r_mopac_Dipole	0.894959	9.24e-22	False
r_mopac_Dipole_X	0.994242	0.00775	False
r_mopac_Dipole_Y	0.950629	8.66e-15	False
r_mopac_Dipole_Z	0.971505	1.25e-10	False
r_mopac_Ionization_Energy	0.903782	7.09e-21	False
r_mopac_MOPAC_Heat_of_Formation	0.929750	7.28e-18	False
r_mopac_MOPAC_Total_Energy_EV	0.947942	3.13e-15	False
i_desc_Chirality_count	0.887456	1.80e-22	False
PBF	0.842910	4.49e-26	False
PMI1	0.791204	2.25e-29	False
PMI2	0.711190	2.16e-33	False
PMI3	0.761613	5.53e-31	False
NPR1	0.884148	8.99e-23	False
NPR2	0.750946	1.59e-31	False
RadiusOfGyration	0.730013	1.55e-32	False
InertialShapeFactor	0.722078	6.67e-33	False
Eccentricity	0.395425	1.50e-43	False
Asphericity	0.799134	6.50e-29	False
SpherocityIndex	0.961910	9.93e-13	False
FEBC	0.975398	1.20e-09	False

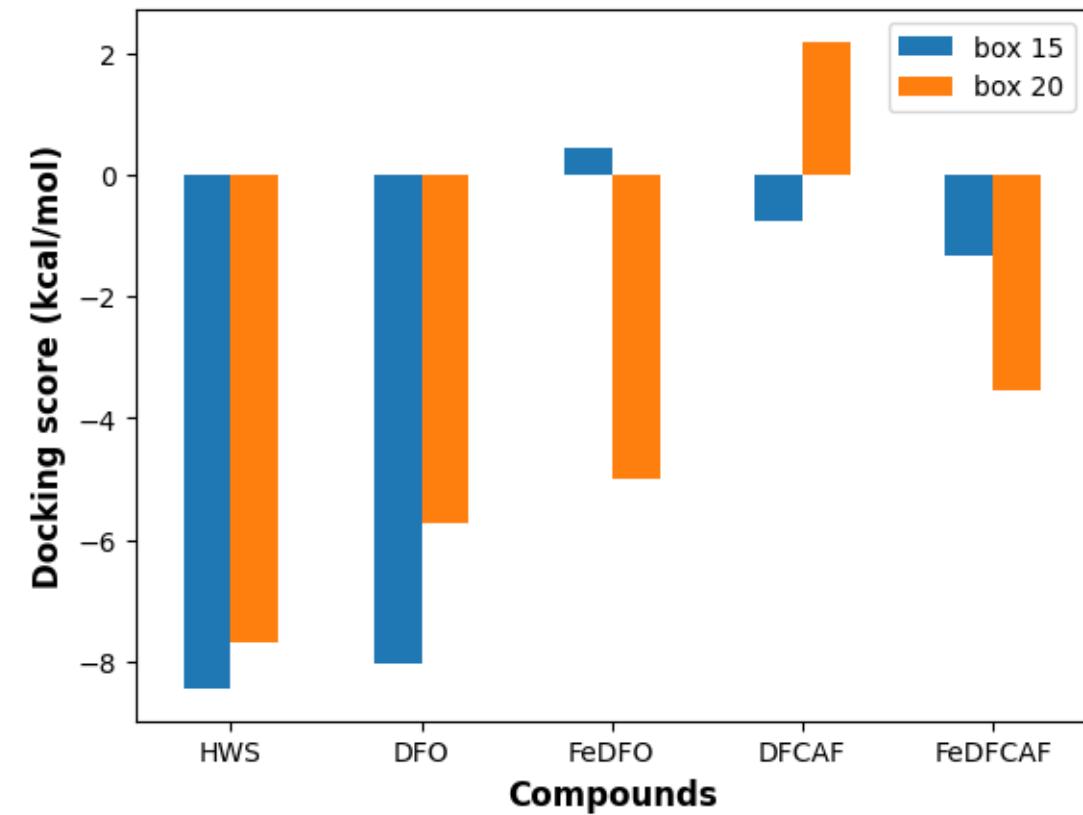
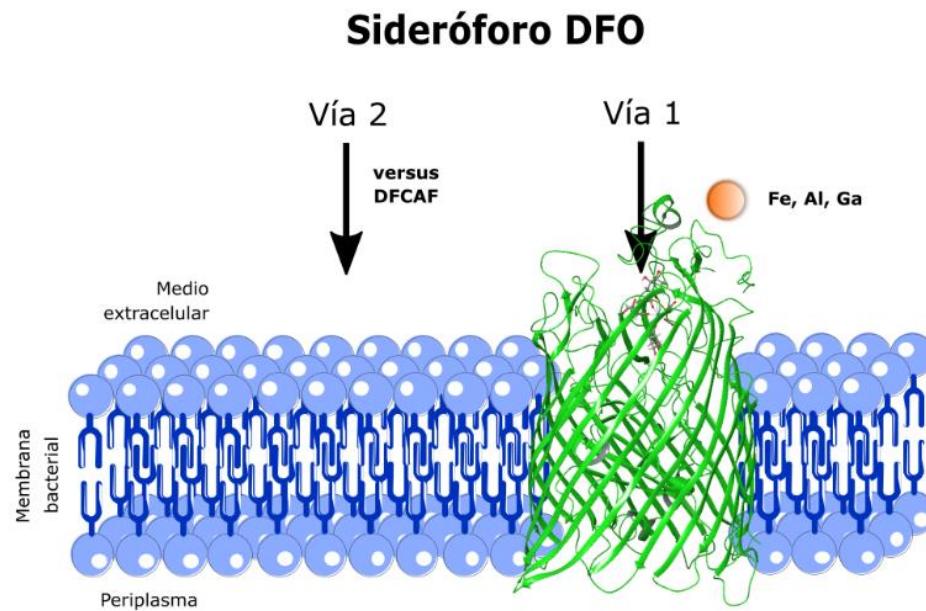
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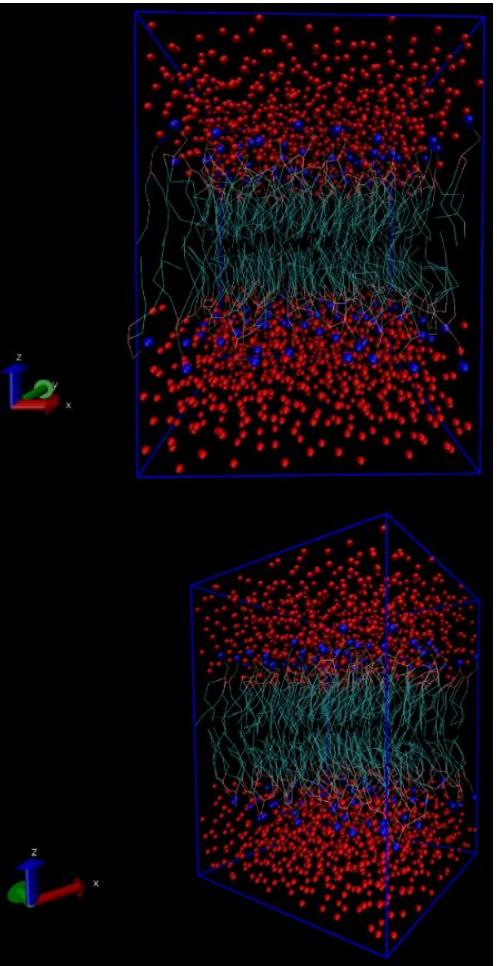
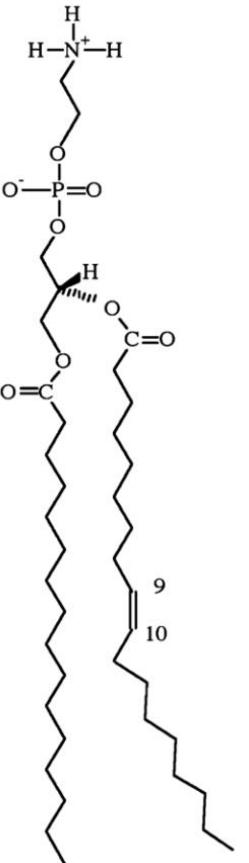
No evidence to reject the null hypothesis (groups are similar)



DFCAF en FhuE



Simulaciones de Dinámica Molecular

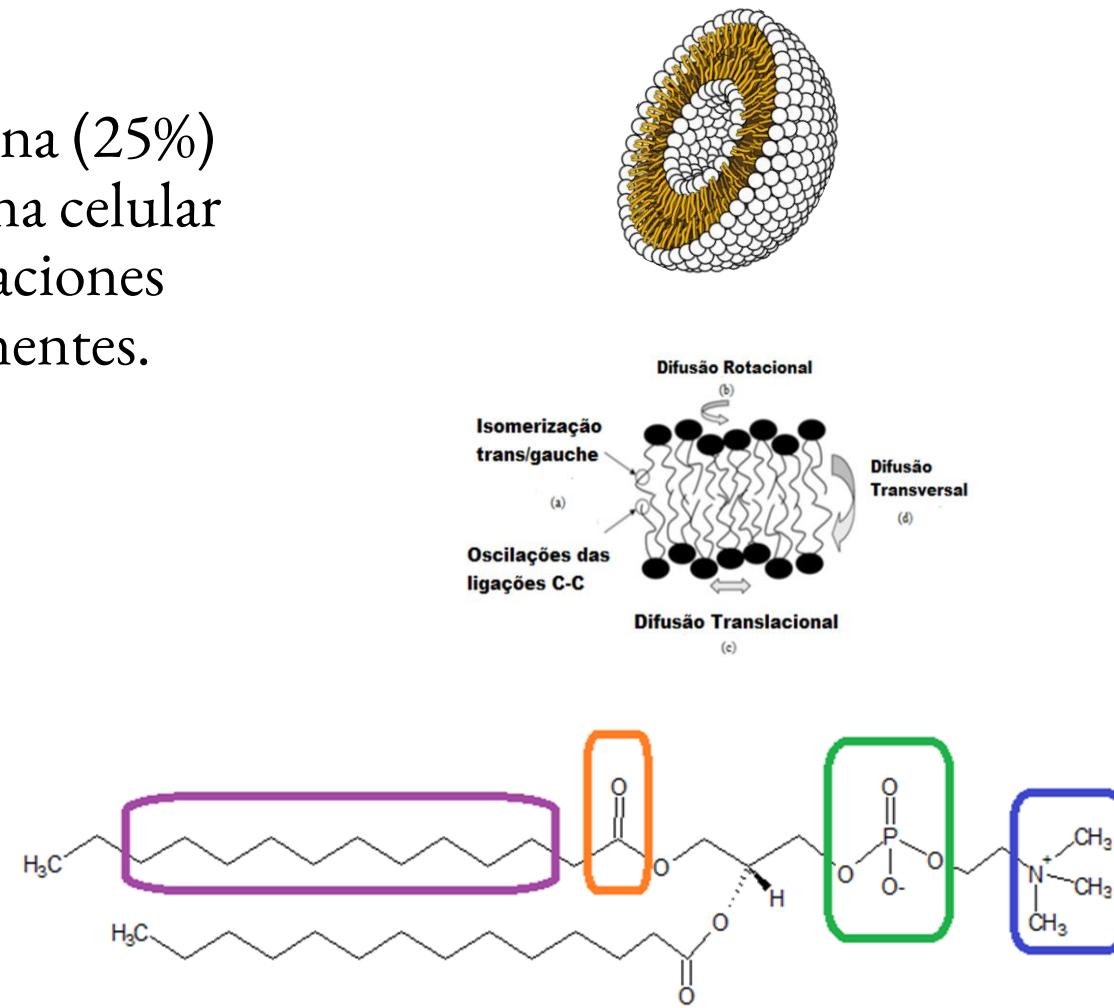


Tiempo	Inicio	Intermedio	Final
DFO			
DFCAF			

Dinámica Molecular *in vitro*

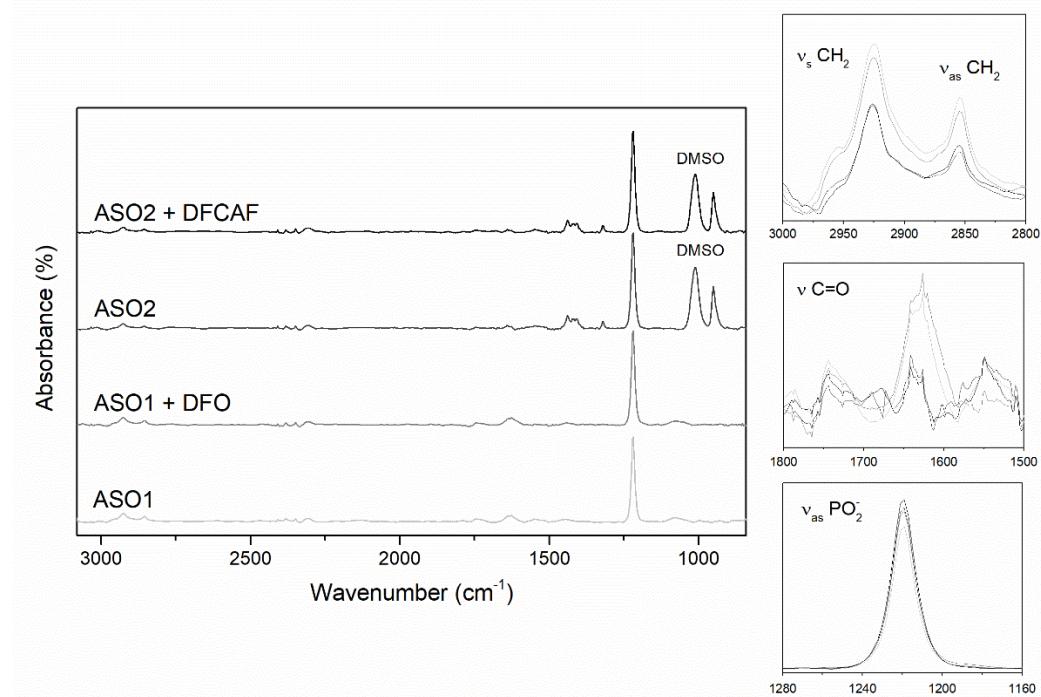
- ✓ Asolecitina de soja (ASO) - Fosfatidilcolina (25%)
- ✓ Liposomas de ASO: modelo de membrana celular
- ✓ Sustancias activas pueden provocar alteraciones en las propiedades de los lípidos componentes.

Región lipídica	Estiramientos	Número de onda (cm ⁻¹)
Polar	ν_{as} PO ₂ ν_{as} N ⁺ (CH ₃) ₃	1260-1220 970
Interface	ν C=O	1725 – 1740
Apolar	ν_s CH ₂ ν_{as} CH ₂	2850 2920



Dinámica Molecular *in vitro*

Lipid region	Stretching	<i>In vitro</i> wavenumber (cm ⁻¹)	<i>In silico</i> (cm ⁻¹)
Polar	ν_{as} PO ₂	1260-1220	1072
	ν_{as} N ⁺ (CH ₃) ₃	970	992
Interface	ν C=O	1725-1740	1766
Apolar	ν_s CH ₂	2850	2981
	ν_{as} CH ₂	2920	

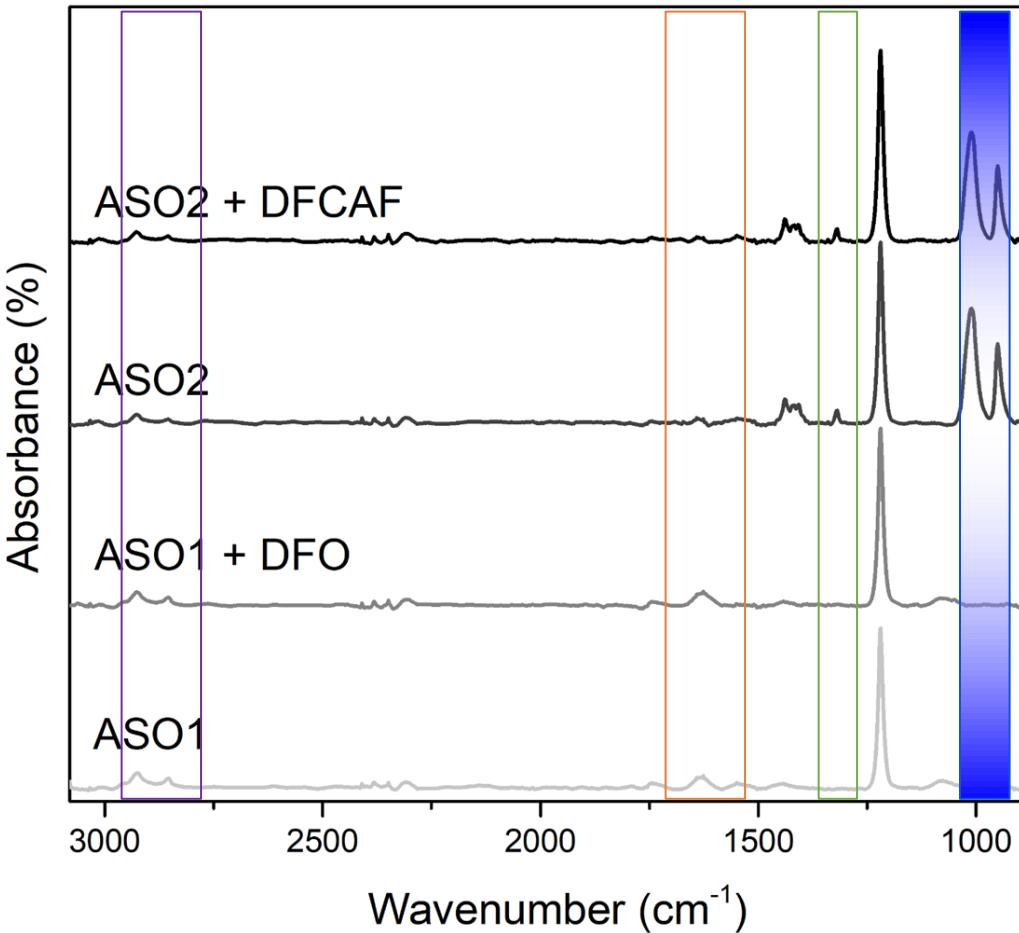
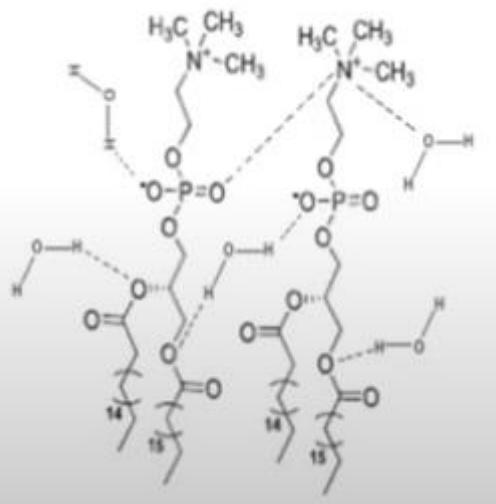


✓HATR-FTIR

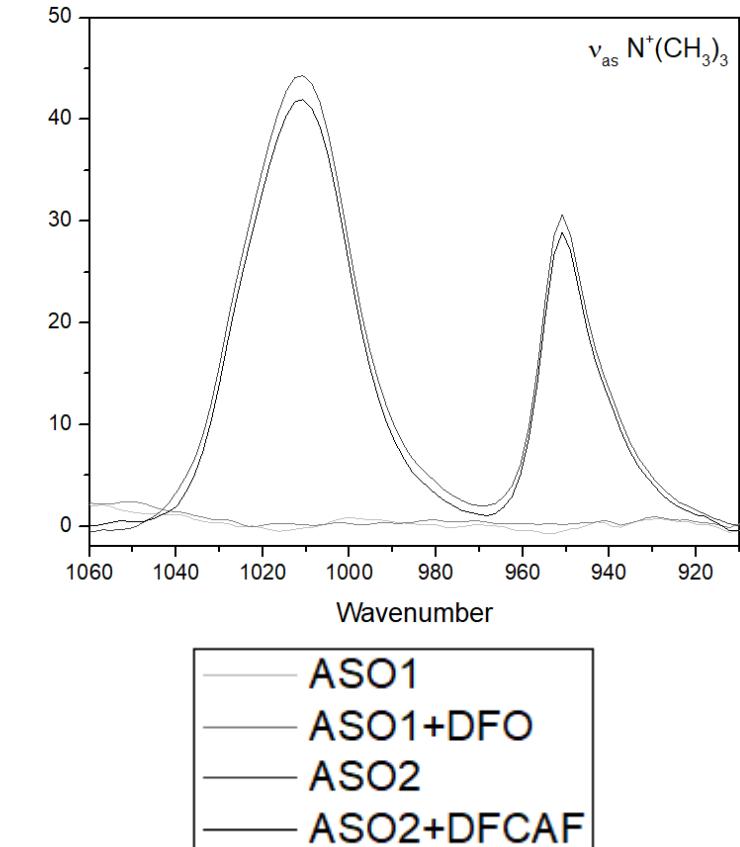
$\nu_{as} N^+(CH_3)_3$

$\nu_{as} PO_2$

$\nu C=O$



Zoom



✓HATR-FTIR

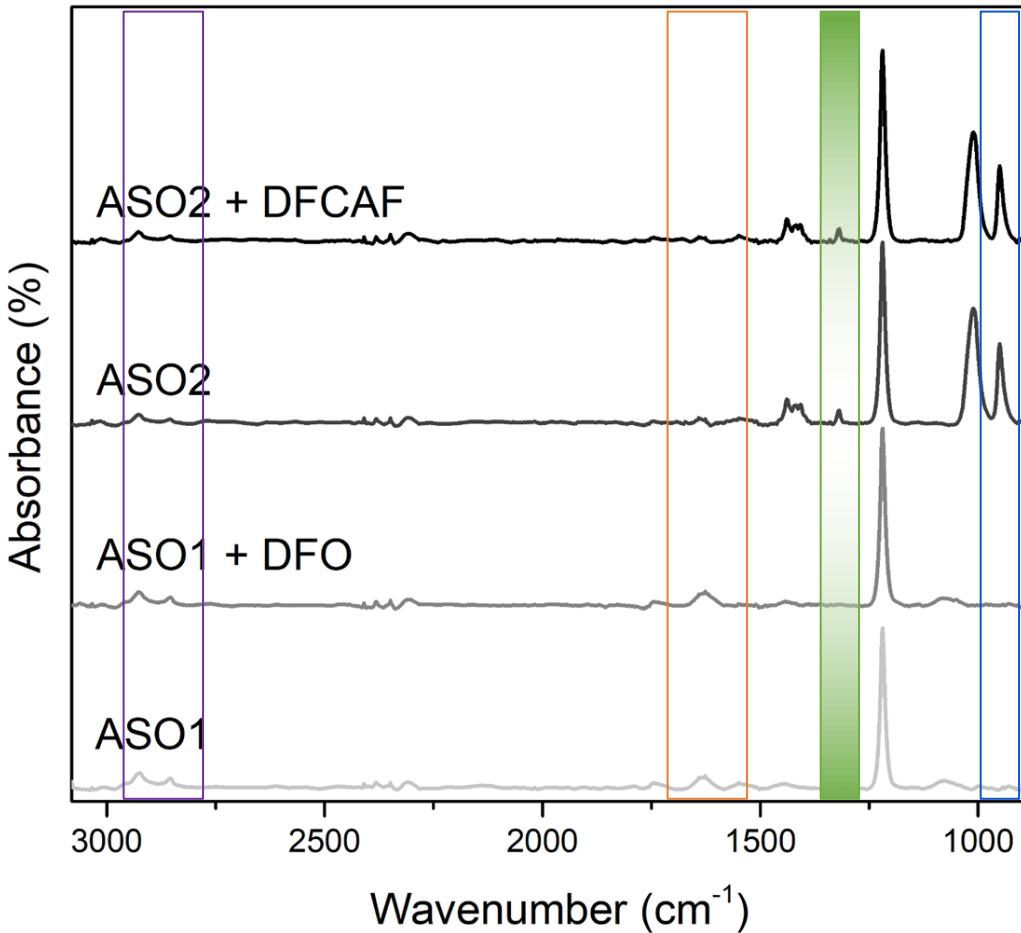
$\nu_{as} N^+(CH_3)_3$

$\nu_{as} PO_2$

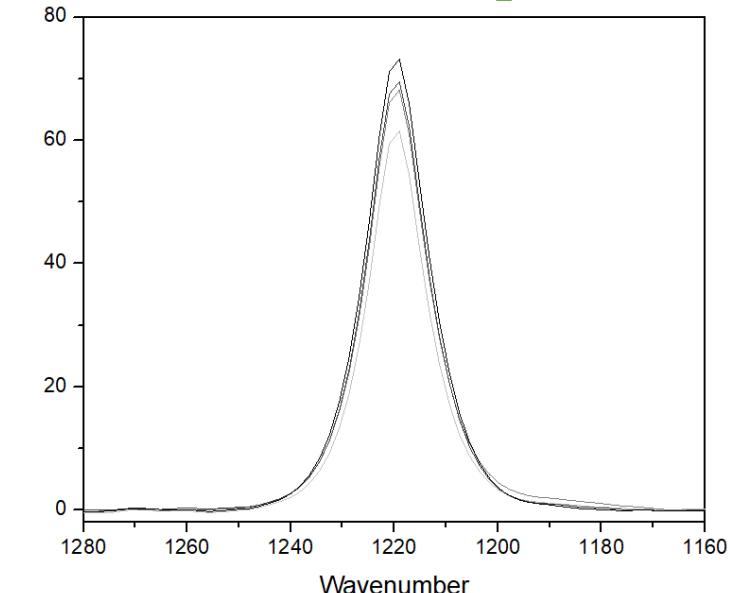
$\nu C=O$

$\nu_s CH_2$

$\nu_{as} CH_2$

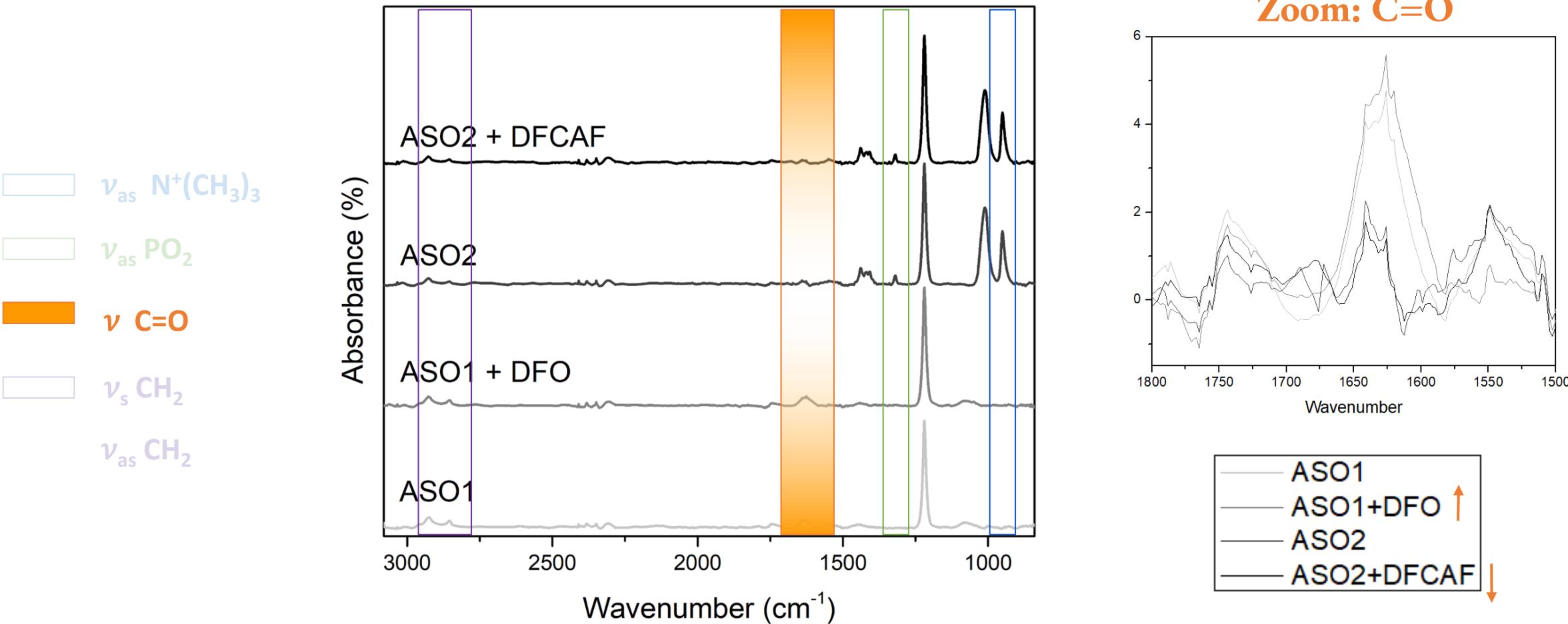


Zoom: PO_2



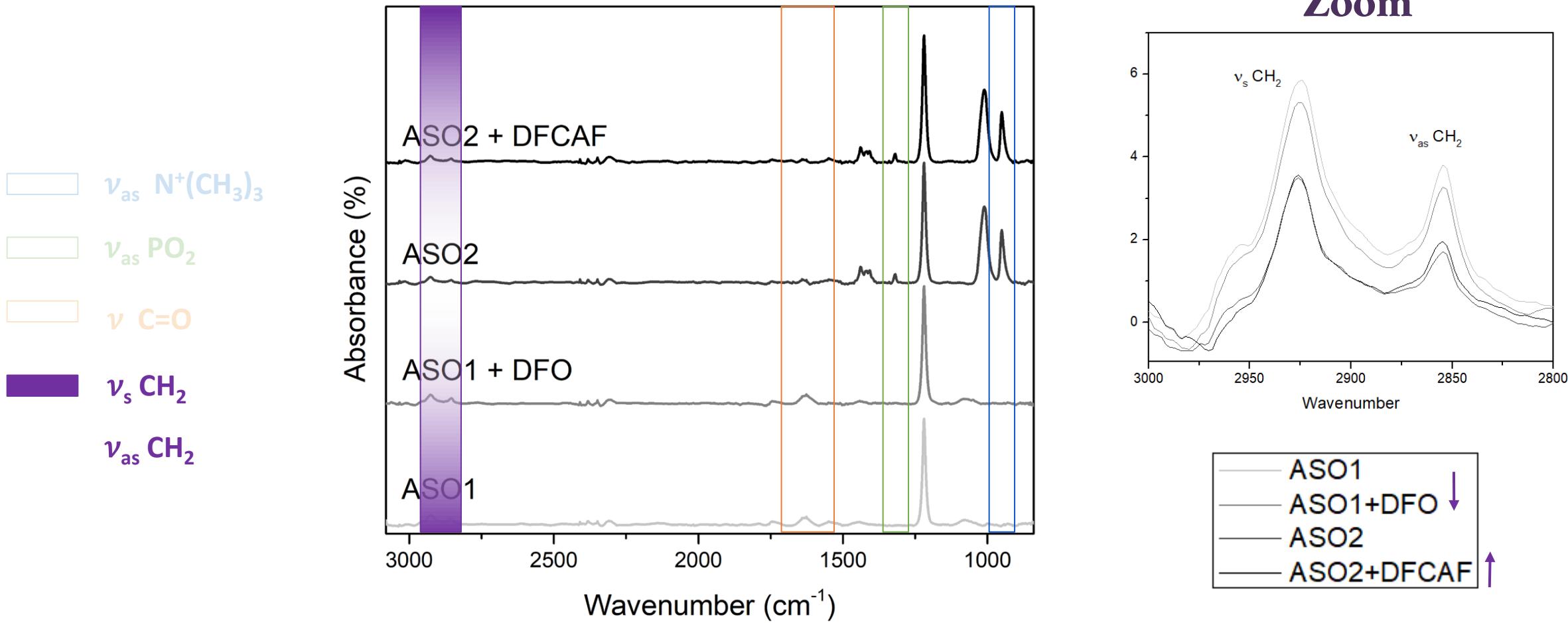
DFO aumentó discretamente la movilidad del fosfato de ASO (aumento del ancho)

✓HATR-FTIR



DFO aumenta significativamente la movilidad del grupo carbonílico en comparación con el DFCAF.

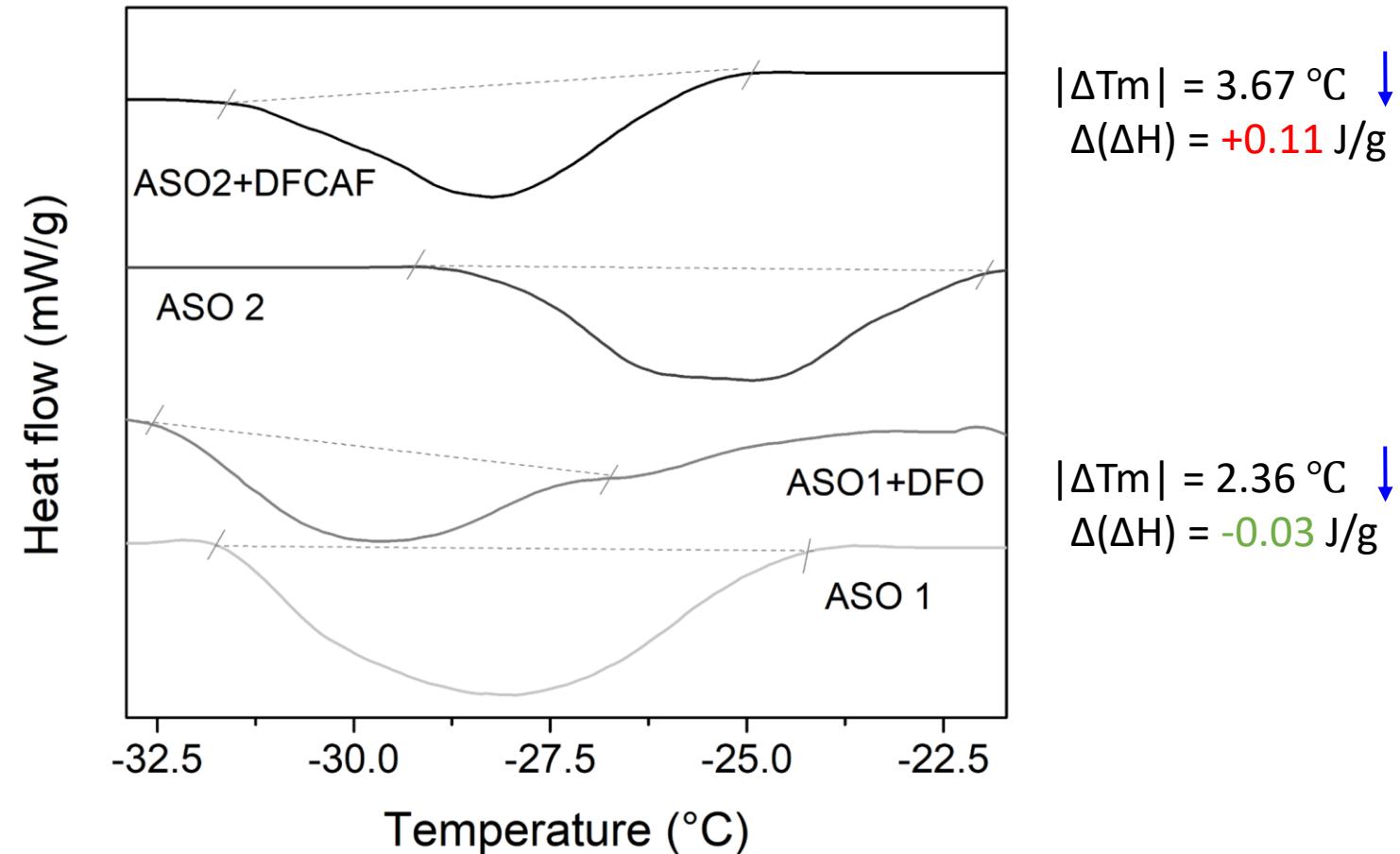
✓HATR-FTIR



DFCAF incrementa la movilidad (desordena) en la región de los metilenos (fluidifica)

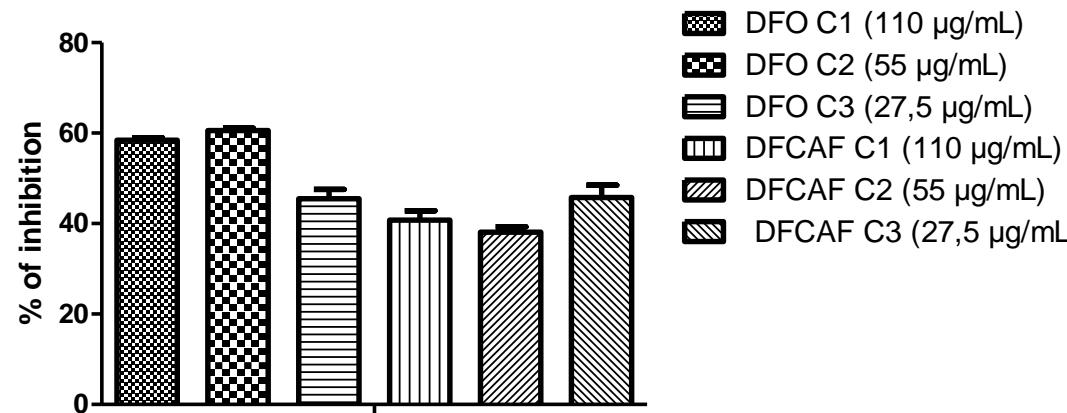
✓DSC

1. Isomerización trans-gauche:
 $\downarrow T_m \rightarrow \uparrow DFCAF$
 $\downarrow T_m \rightarrow \uparrow DFO^*$
1. $\Delta(\Delta H)$:
 $DFO < 0 \rightarrow H\text{-bonds, VdW}$
 $DFCAF > 0 \rightarrow \text{Interacciones hidrofóbicas y apilamiento } \pi$



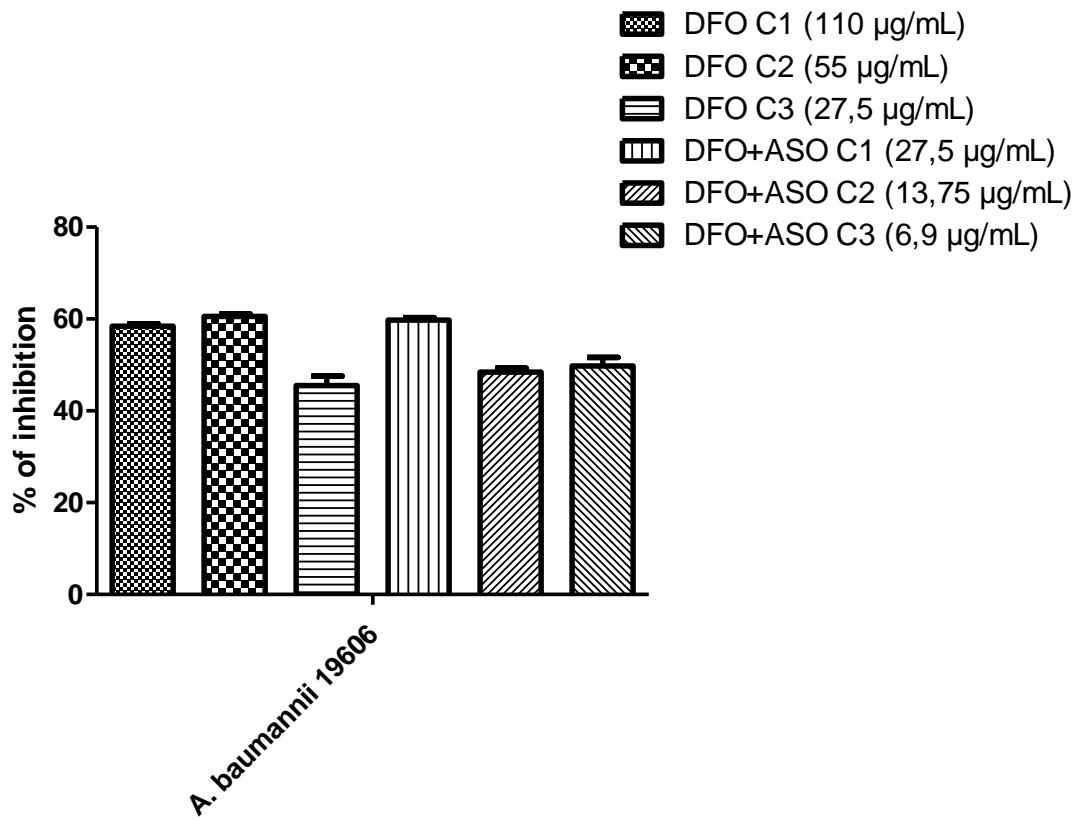
DFCAF desordena la membrana, promueve la isomerización trans-gauche y las interacciones hidrofóbicas.

✓ Ensayos *in vitro* en *Acinetobacter baumannii*



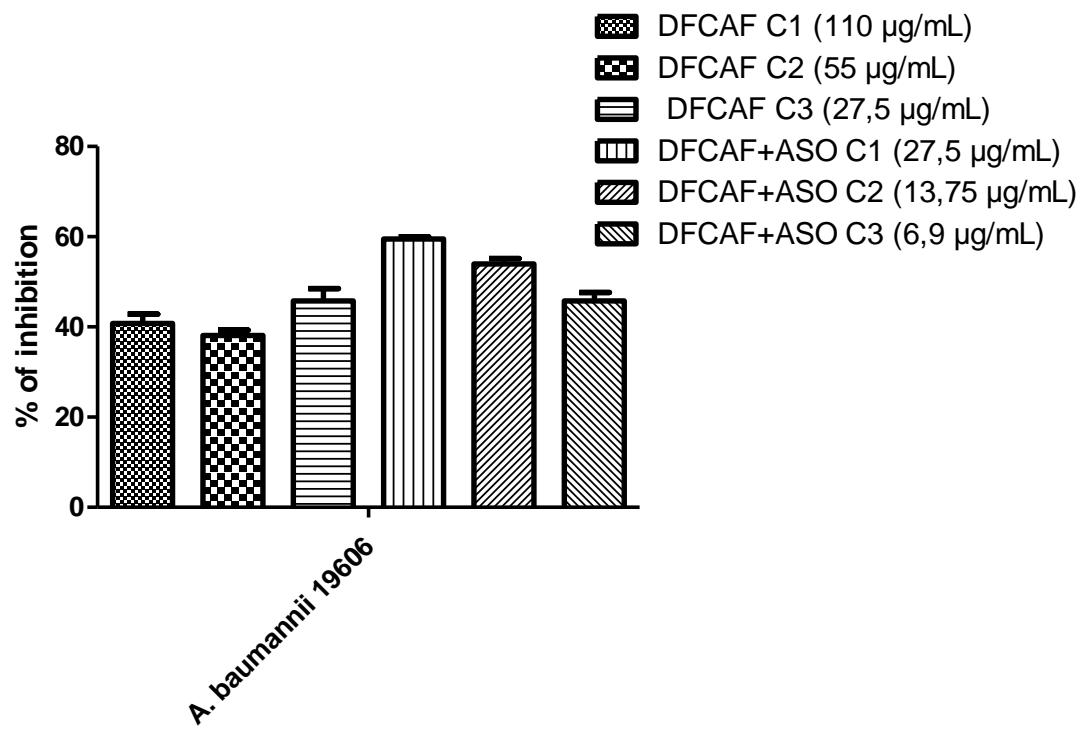
Compuesto	¿Existe diferencia significativa?
DFO C1 (110 µg/mL) vs DFCAF C1 (110 µg/mL)	Sí
DFO C2 (55 µg/mL) vs DFCAF C2 (55 µg/mL)	Sí
DFO C3 (27,5 µg/mL) vs DFCAF C3 (27,5 µg/mL)	No

✓ Ensayos *in vitro* en *Acinetobacter baumannii*



Compuesto	¿Existe diferencia significativa?
DFO C1 (110 µg/mL) vs DFO + ASO C1 (27.5 µg/mL)	No
DFO C1 (110 µg/mL) vs DFO + ASO C2 (13.75 µg/mL)	Sí **
DFO C1 (110 µg/mL) vs DFO + ASO C3 (6.9 µg/mL)	Sí *
DFO C2 (55 µg/mL) vs DFO + ASO C1 (27.5 µg/mL)	No
DFO C2 (55 µg/mL) vs DFO + ASO C2 (13.75 µg/mL)	Sí ***
DFO C2 (55 µg/mL) vs DFO + ASO C3 (6.9 µg/mL)	Sí **
DFO C3 (27.5 µg/mL) vs DFO + ASO C1 (27.5 µg/mL)	Sí ***
DFO C3 (27.5 µg/mL) vs DFO + ASO C2 (13.75 µg/mL)	No
DFO C3 (27.5 µg/mL) vs DFO + ASO C3 (6.9 µg/mL)	No

✓ Ensayos *in vitro* en *Acinetobacter baumannii*



Compuesto	¿Existe diferencia significativa?
DFCAF C1 (110 µg/mL) vs DFCAF + ASO C1 (27.5 µg/mL)	Sí ***
DFCAF C1 (110 µg/mL) vs DFCAF + ASO C2 (13.75 µg/mL)	Sí ***
DFCAF C1 (110 µg/mL) vs DFCAF + ASO C3 (6.9 µg/mL)	No
DFCAF C2 (55 µg/mL) vs DFCAF + ASO C1 (27.5 µg/mL)	Sí ***
DFCAF C2 (55 µg/mL) vs DFCAF + ASO C2 (13.75 µg/mL)	Sí ***
DFCAF C2 (55 µg/mL) vs DFCAF + ASO C3 (6.9 µg/mL)	No
DFCAF C3 (27.5 µg/mL) vs DFCAF + ASO C1 (27.5 µg/mL)	Sí ***
DFCAF C3 (27.5 µg/mL) vs DFCAF + ASO C2 (13.75 µg/mL)	Sí *
DFCAF C3 (27.5 µg/mL) vs DFCAF + ASO C3 (6.9 µg/mL)	No

Conclusiones

✓ Vía 1:

- ✓ Validación del nivel de cálculo
- ✓ Reactividad diferente entre Fe-DFO, Al-DFO y Ga-DFO
- ✓ Afinidad y estabilidad análoga em FhuE (distintas interacciones intermoleculares)
- ✓ Isómeros de Fe-DFO se acopla principalmente con GLY657 (86%), ASN662 (81%), PRO659 (87%)
- ✓ No hay diferencia significativa en el reconocimiento molecular de isómeros de Fe-DFO

✓ Vía 2:

- ✓ Nuestro modelo predice la permeabilidad DFCAF a través de la membrana celular
- ✓ DFO interactúa en la superficie de la membrana y DFCAF en la región intermembrana desordenándola.
- ✓ La administración de DFCAF en liposomas incrementa significativamente la toxicidad en biofilmes de *A. baumannii*.

Agradecimientos

