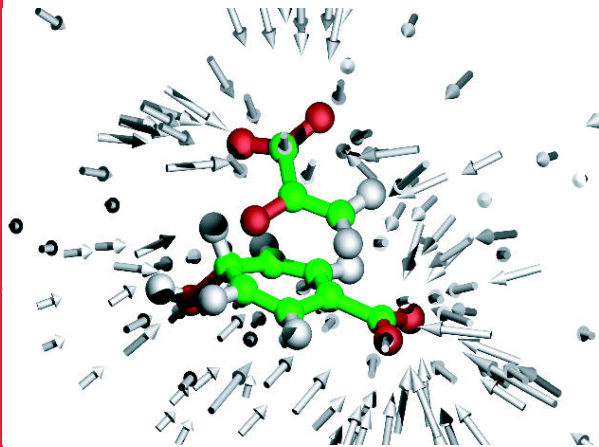


Is solvation/desolvation enough to unravel protein interactions? Examples on HIV-1 gp41-based miniprotein antibody eliciting and sGC ligand binding

Luis Agulló, Martin Floor, Jordi Villà-Freixa

Computational Biochemistry and Biophysics lab



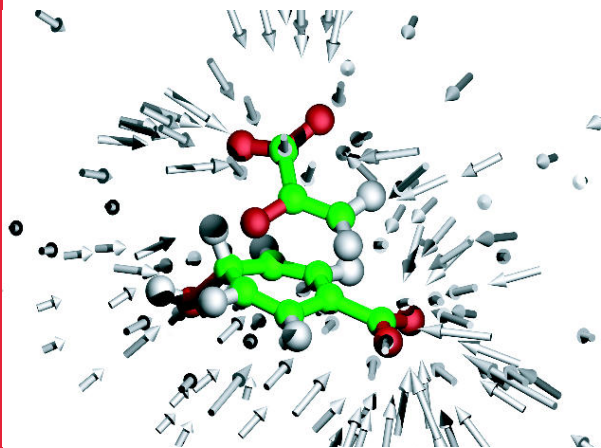
Luis M. Molinos, Jorge Carrillo, Julià Blanco

Chair on AIDS and related diseases

Two examples of molecular interactions analysis through MD

Luis Agulló, Martin Floor, Jordi Villà-Freixa

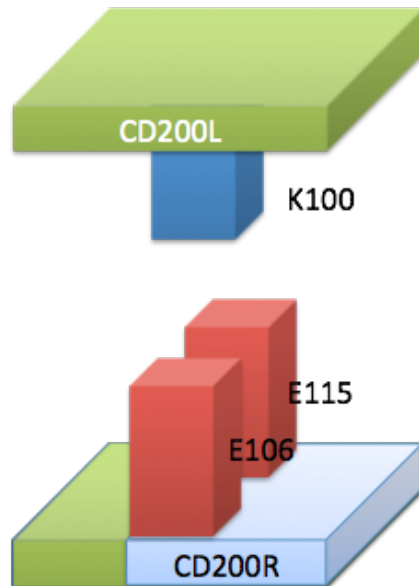
Computational Biochemistry and Biophysics lab



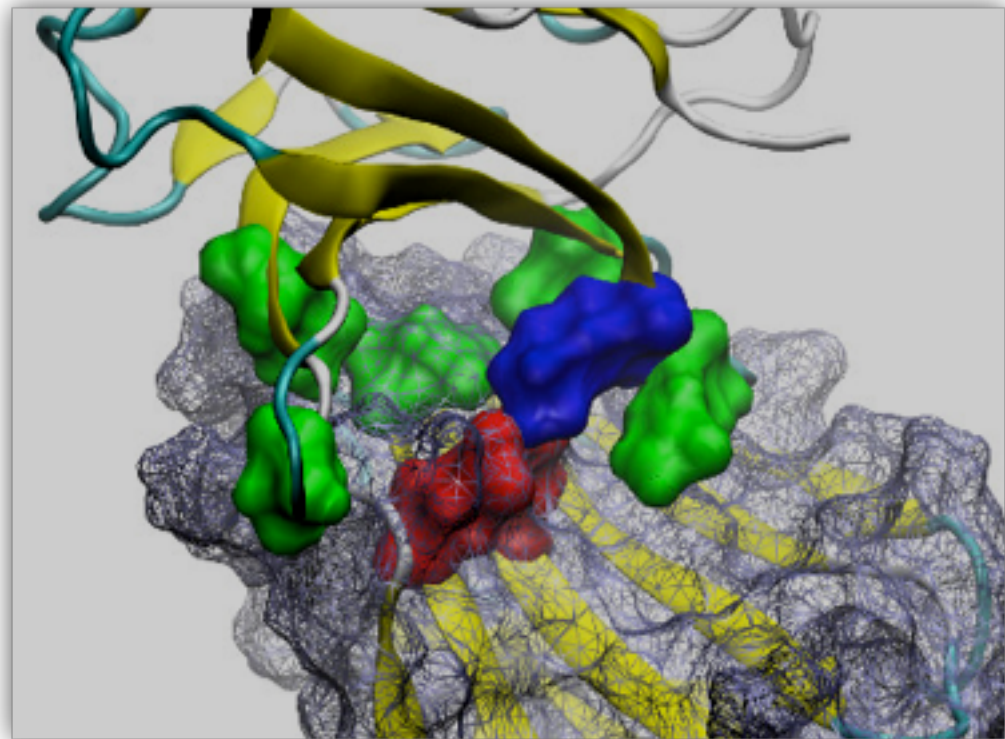
Luis M. Molinos, Jorge Carrillo, Julià Blanco

Chair on AIDS and related diseases

CD200-CD200R agonist search



PDLD/S-LRA



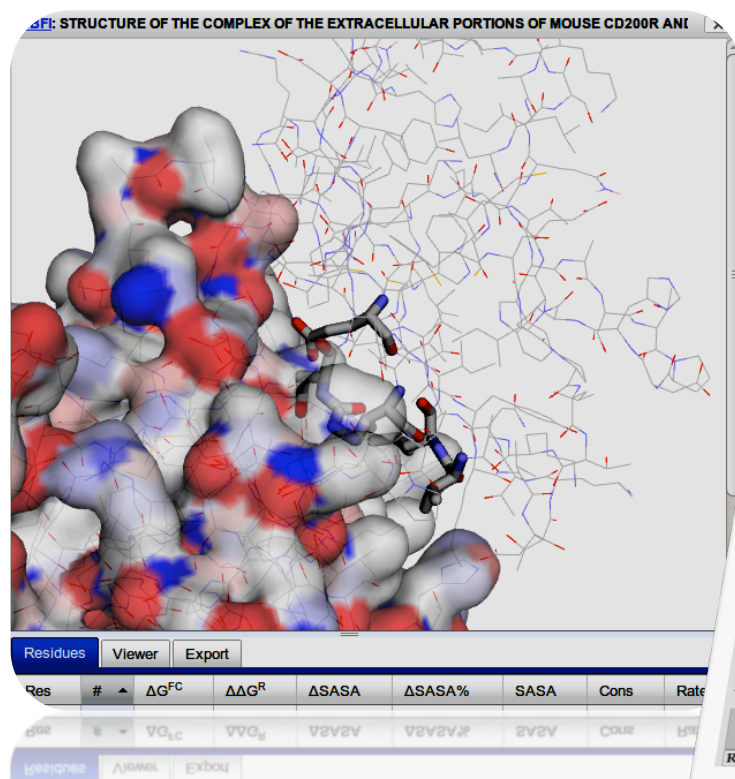
K100 vs E106 + E115 \approx 65% of total E_{interact}
CD200R1-CD200

TABLE 1

Primary and Secondary Sequence Identification of CD200 Peptides

Peptide sequence	Identification no.	Secondary sequence region
TASLRCSLKTSQE	#4004	CDR1 \rightarrow FR2
RCSLKTSQE	#6061	CDR1 \rightarrow FR2
SPENMVTYSKT	#4005	FR2 \rightarrow CDR2
ENMVTYSKT	#6060	FR2 \rightarrow CDR2 and C'-face
TYSKTHGVVTQ	#4012	CDR2 \rightarrow FR3
YKDRINVTE	#6062	C'-face
TELGLWNSSIT	#8000	Control peptide
NTIGDGGCY	#6059	F-face
LFNTFGSQKVSQT	#4013	CDR3
SQKVSGTACLTY	#4006	CDR3 \rightarrow

structure-based drug screening



CD200R1 as a
pharmacophore

ZINCPharmer

ZINC

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Synonyms (0) Vendors (6) Annotations (1) Representations (1) Notes (0) Targets (0) Clustered (0) Reactome (0) Rings (0) Analogs (0)

ZINC20761178

In ZINC since	Heavy atoms	Benign functionality
November 28 th , 2008	18	No

Popular Name: *(E)-3-(2-hydroxy-5-methyl-phenyl)-4-phosphono-but-2-enoic*
Find On: PubMed - Wikipedia - Google

SMILES: Cc1ccc(c(c1)/C=C/C(=O)[O-])/C[P@]([O-])([O-])O
Download: MOL2 SDF SMILES Flexibase

Draw Identity 99% 90% 80% 70%

Vendors

eMolecules	Molport	IBScreen NP	Mcule	Ambinter	Mcule Make-on-demand
26437652	MolPort-008-346-926 (300.0mg)	STOCKIN-72691	MCULE-5370369176	Amb8496833	MCULE-5370369176

Annotations
PubChem 54742123, 29147371

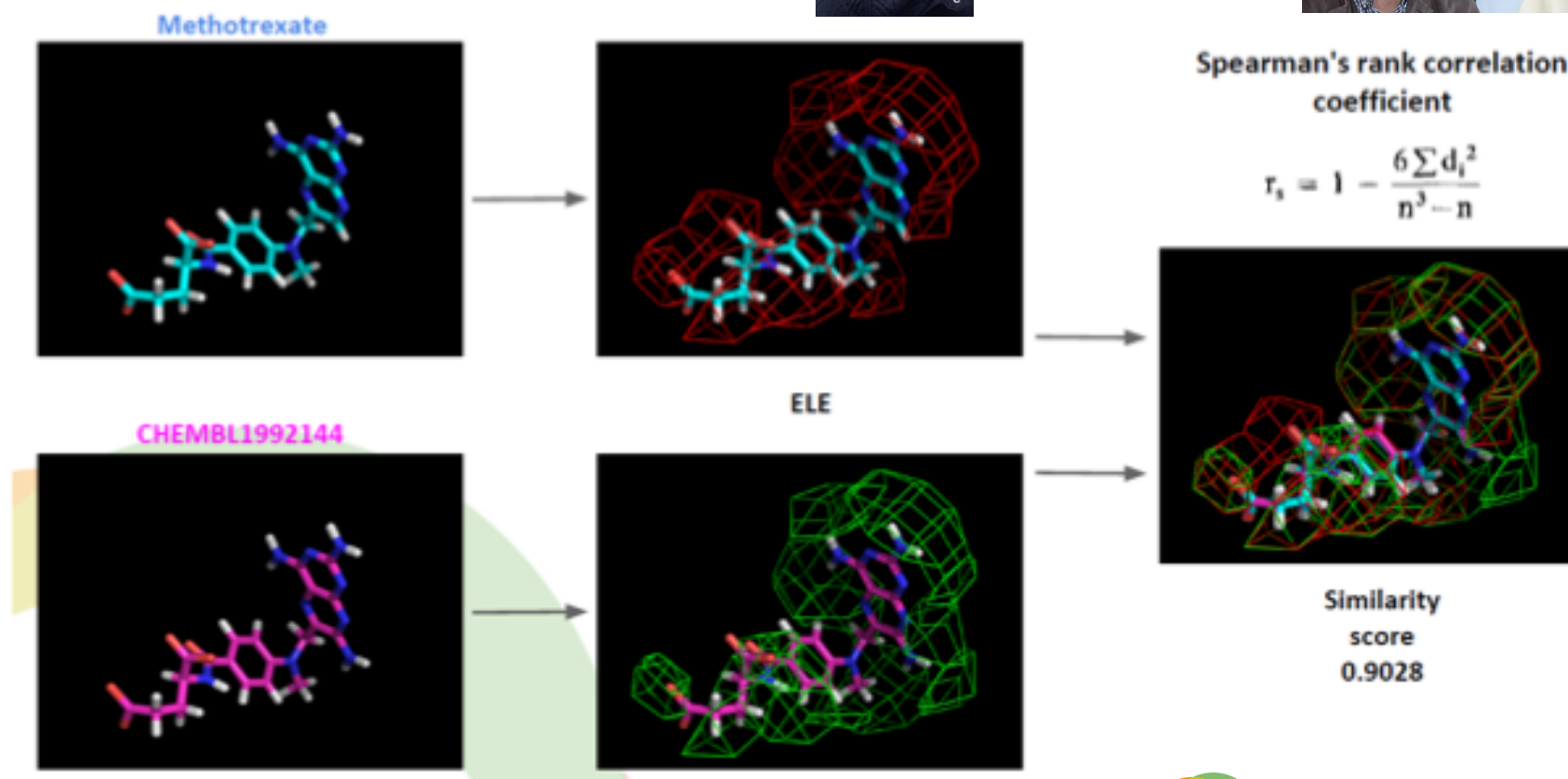
Physical Representations

pH range	xlogP	Apolar desolvation (kcal/mol)	Polar desolvation (kcal/mol)	H-bond donors	H-bond acceptors	Net charge	tPSA (Å²)	Molecular weight (g/mol)	Rotatable bonds	DL
Reference (pH 7)	0.52	1.04	-113.59	2	6	-2	121	270.177	4	↓

Drug repositioning



EDUARDO
SANTAMARIA &
ALFONS NONELL



Mind
theByte

SaaS
for Drug
Discovery



LUIS M. MOLINOS, JORGE CARRILLO & JULIÀ BLANCO



Unión Europea

Fondo Europeo
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"Una manera de hacer Europa"



LUIS AGULLÓ

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MINISTERIO
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GERALD ZAPATA
& MARTIN FLOOR

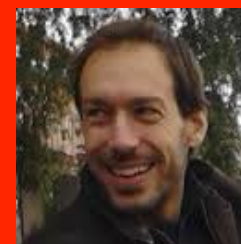


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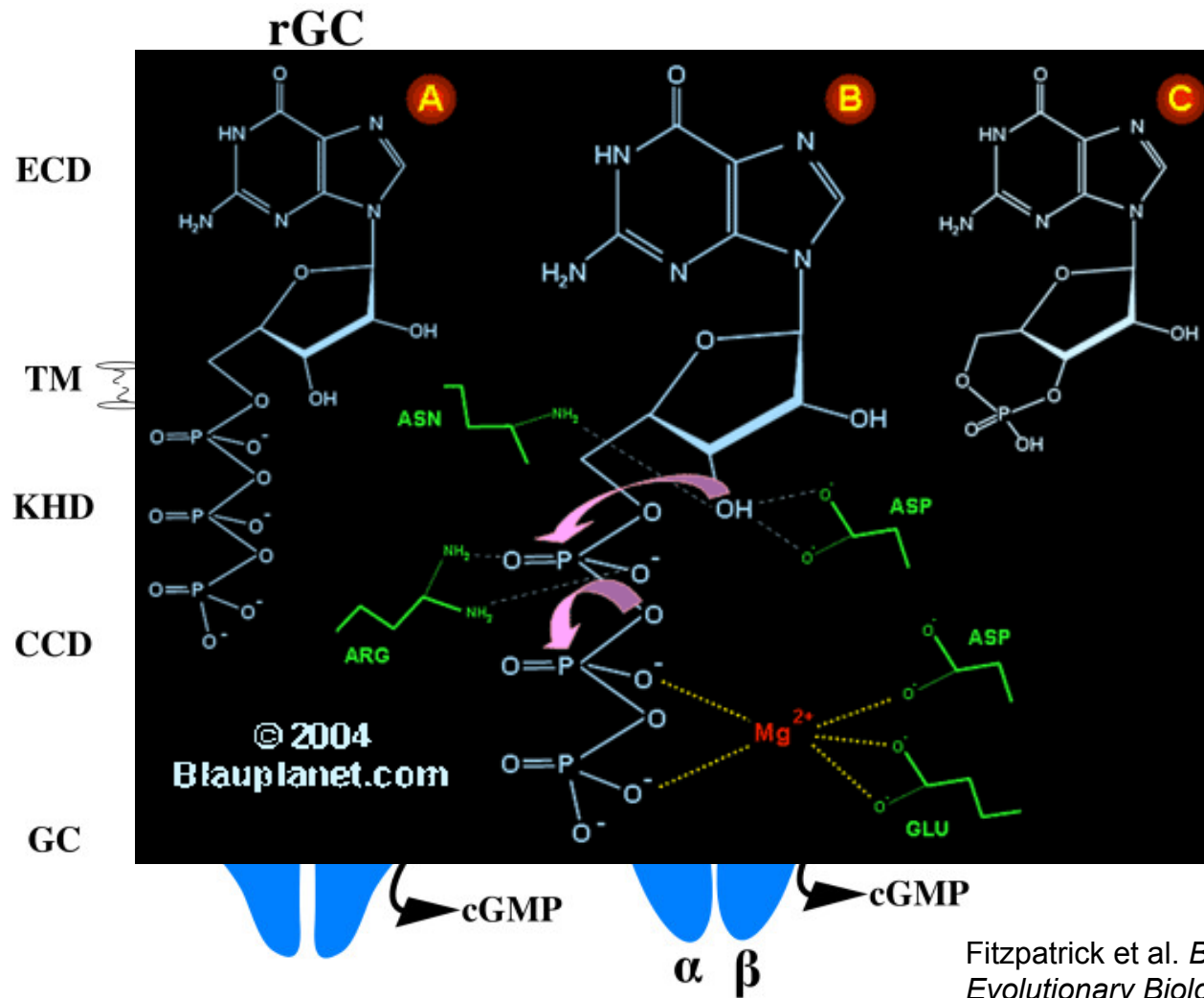
Computational exploration of the binding mode of heme-dependent stimulators into the active catalytic domain of soluble guanylate cyclase

Luis Agulló,^{1*} Ignasi Buch,² Hugo Gutiérrez-de-Terán,³ David Garcia-Dorado,⁴ and Jordi Villà-Freixa¹

Proteoliposomal formulations of an HIV-1 gp41-based miniprotein elicit a lipid-dependent immunodominant response overlapping the 2F5 binding motif

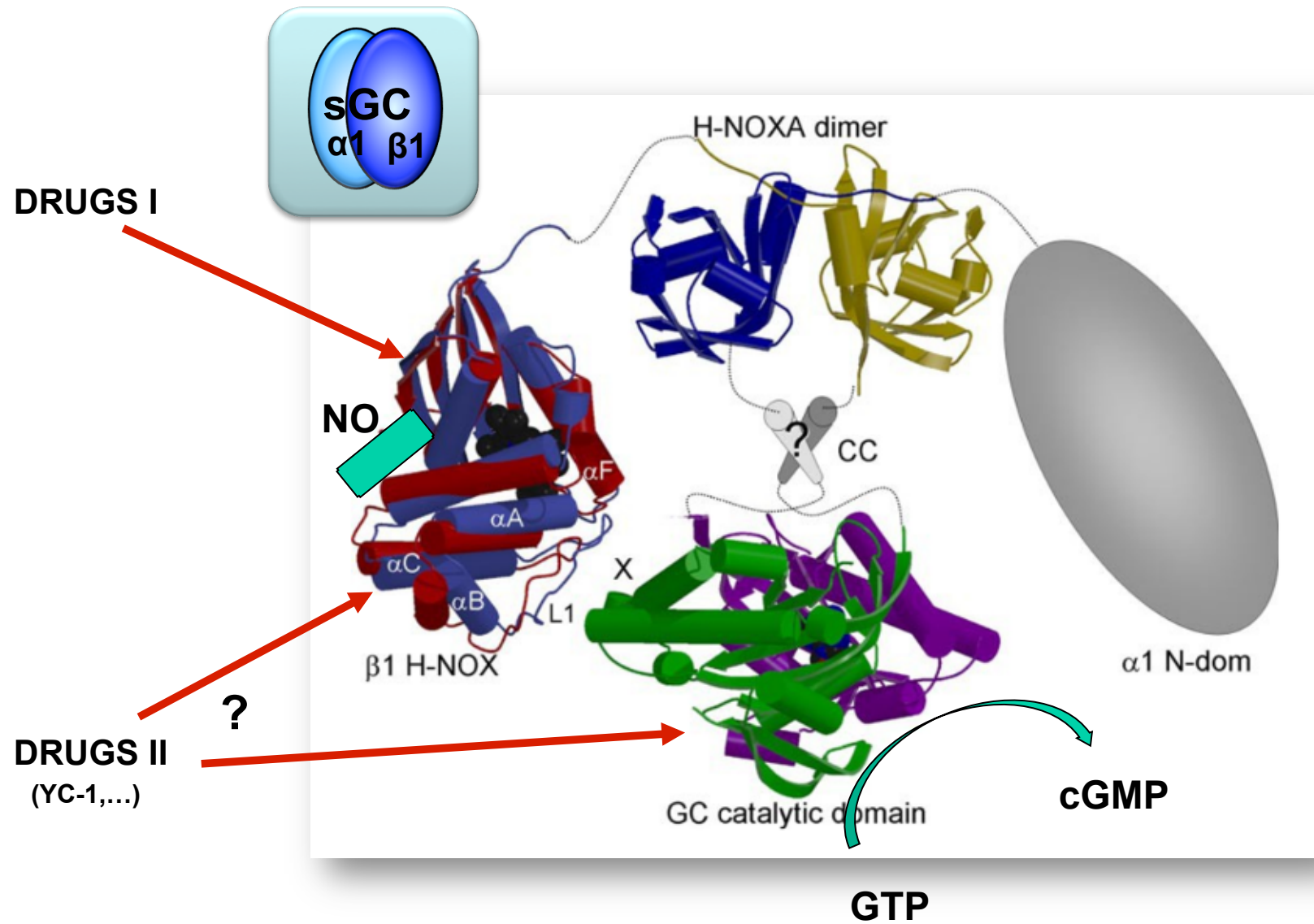
Luis M Molinos-Albert¹, Eneritz Bilbao², Luis Agulló³, Silvia Marfil¹, Elisabet García¹, Maria Luisa Rodríguez de la Concepción¹, Nuria Izquierdo-Useros¹, Cristina Vilaplana⁴, F.-Xabier Contreras^{2,5}, Martin Floor^{3,6}, Pere J Cardona⁴, Javier Martinez-Picado^{1,7,8}, Bonaventura Clotet^{1,8,9}, Jordi Villà-Freixa³, Maier Lorizate^{2*}, Jorge Carrillo^{1,#}, Julià Blanco^{1,8,#*}

Binding into catalytic domain of soluble guanylate cyclase

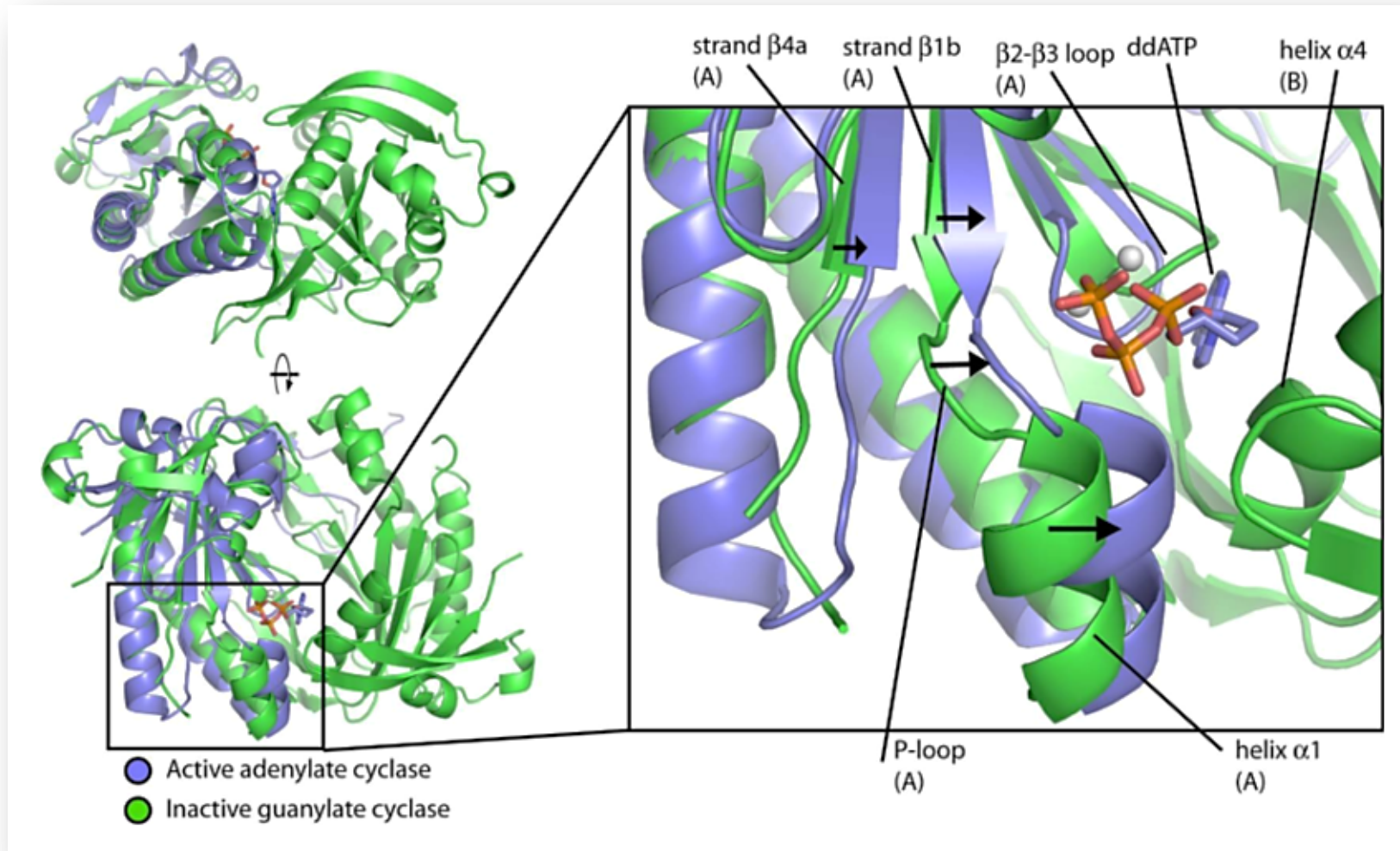


Fitzpatrick et al. *BMC Evolutionary Biology* (2006)

Binding into catalytic domain of soluble guanylate cyclase



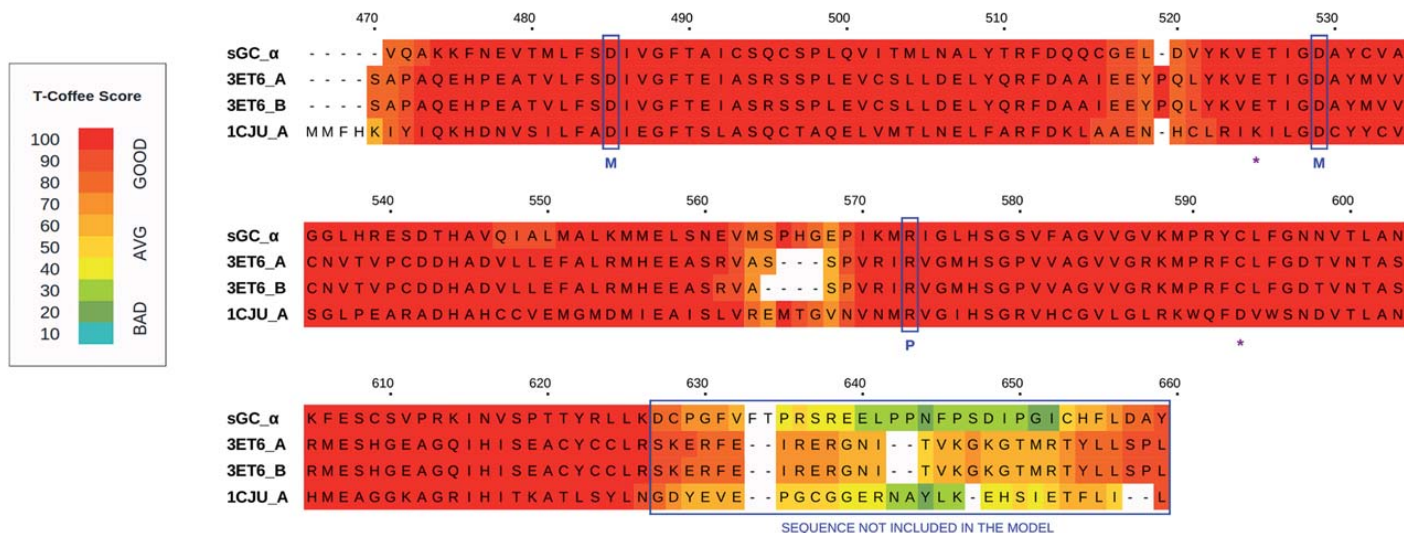
Binding into catalytic domain of soluble guanylate cyclase



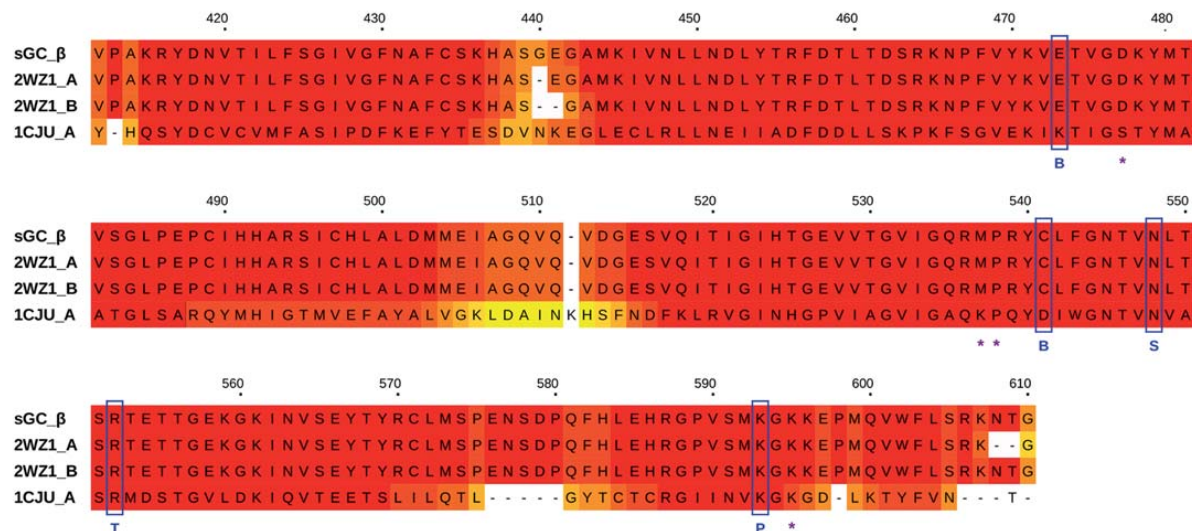
Winger et al. *BMC Structural Biology* 2008, 8:42

Binding into catalytic domain of soluble guanylate cyclase

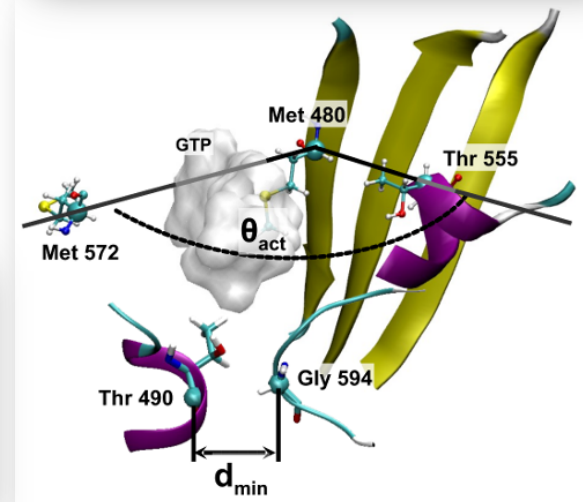
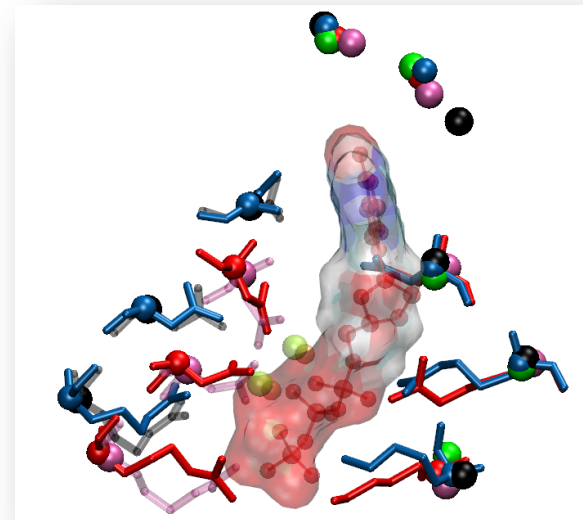
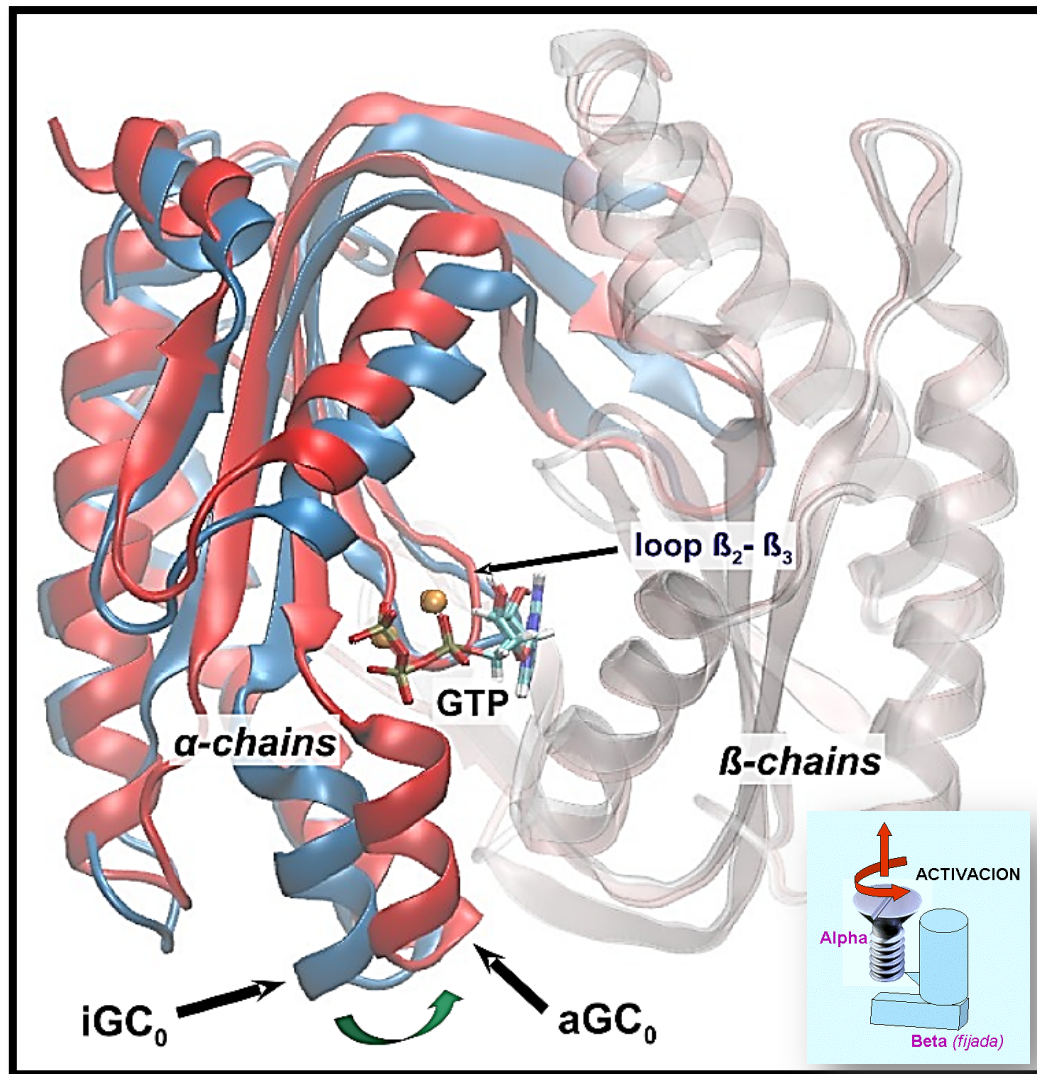
ALPHA CHAIN



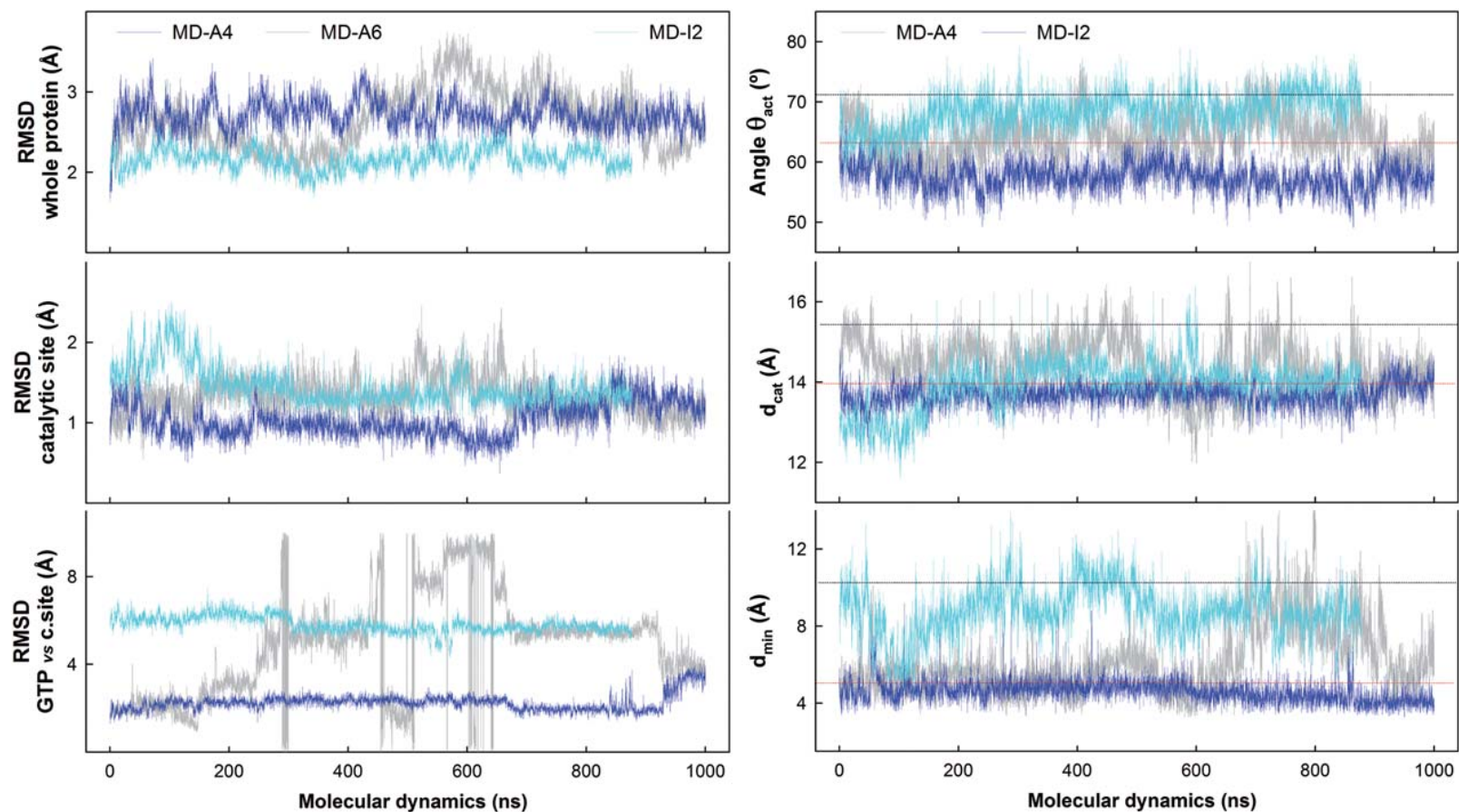
BETA CHAIN



Binding into catalytic domain of soluble guanylate cyclase

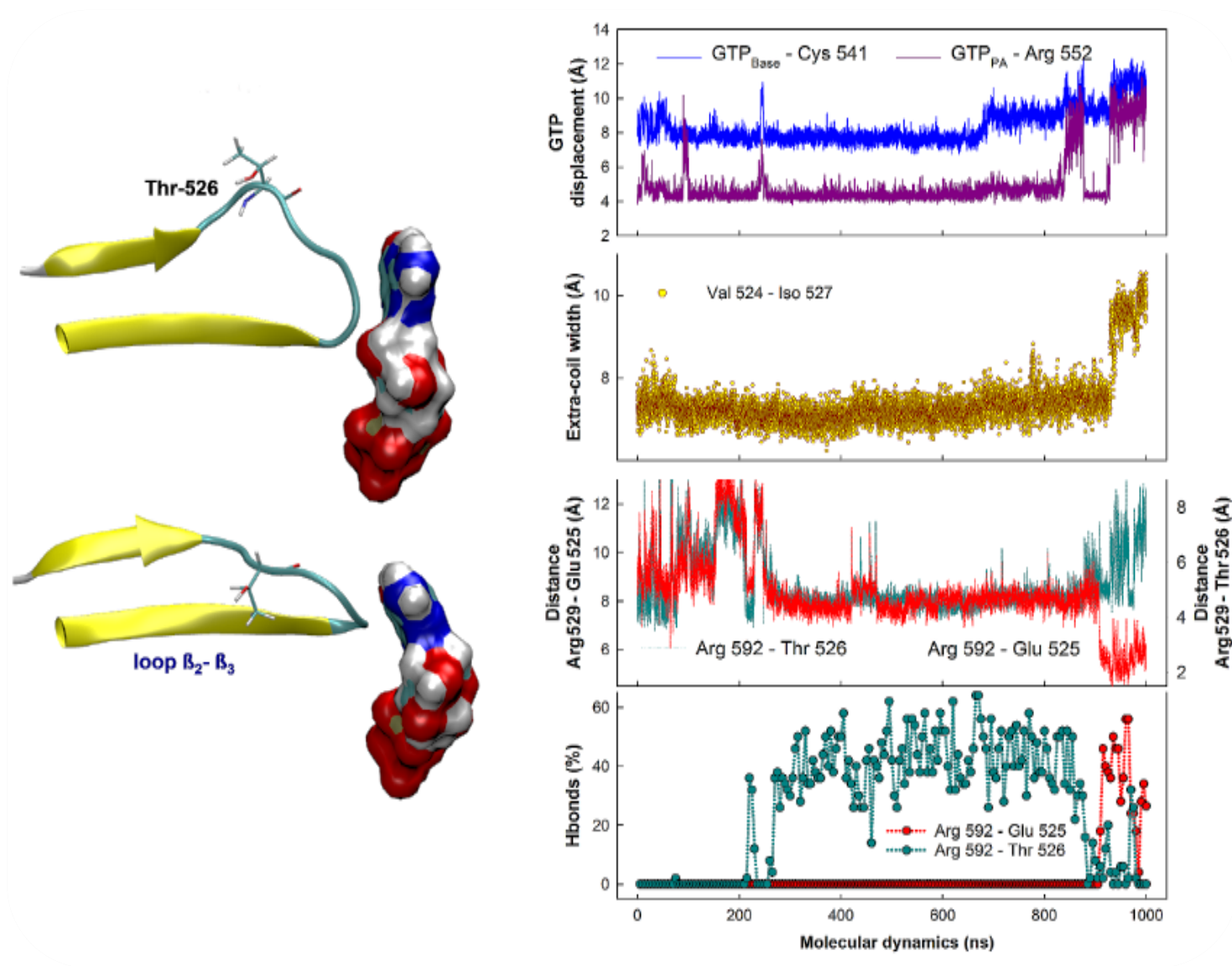


Binding into catalytic domain of soluble guanylate cyclase

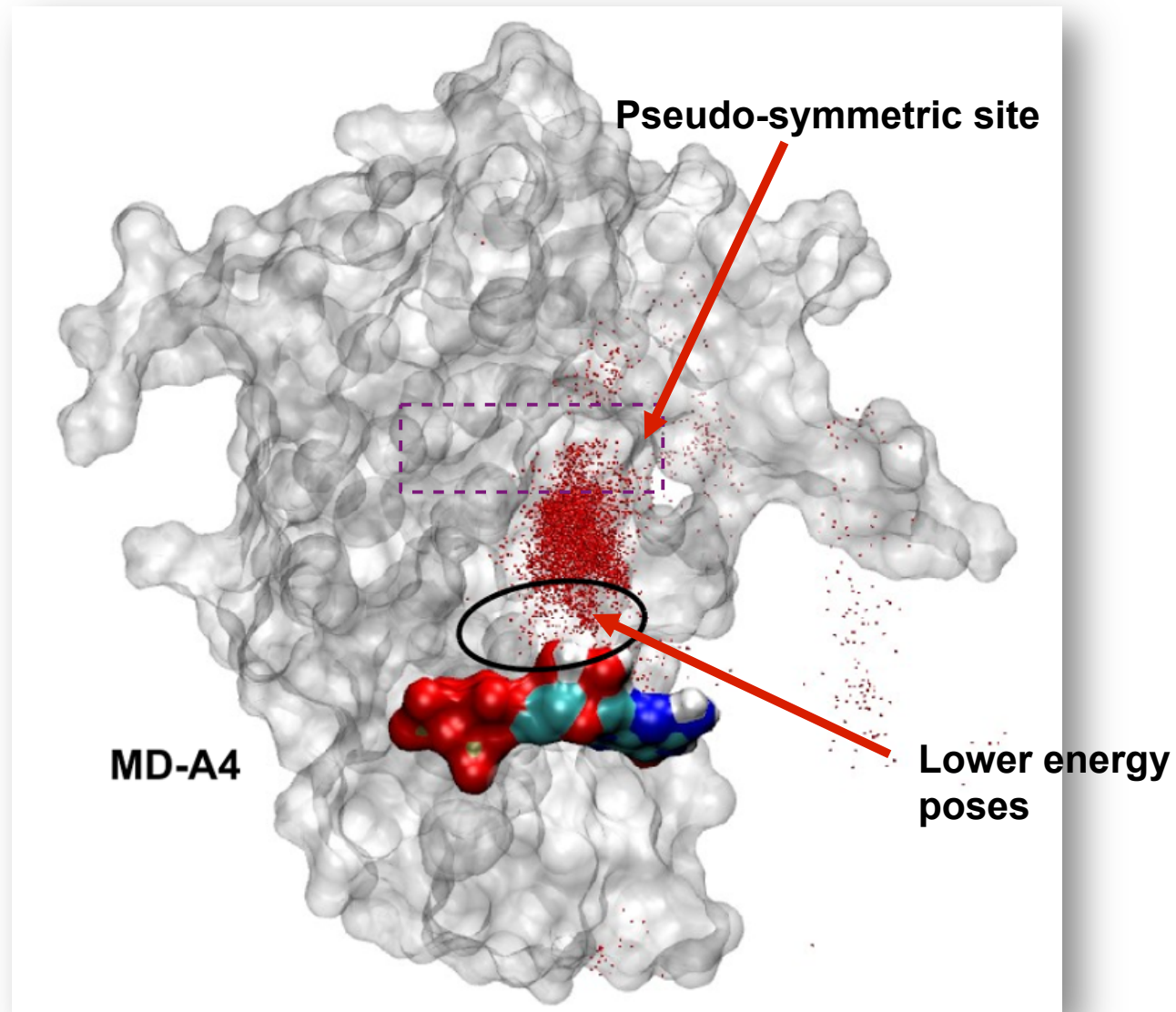


Structure ^a	θ_{act} (°)	d_{cat} (Å)	d_{min} (Å)
3ET6	71	15.5	10.3
1CJU	63	14.7	5.1
iGC ₀	71	15.5	9.1
aGC ₀	63	14.8	4.7
3UVJ	64	14.7	8.2

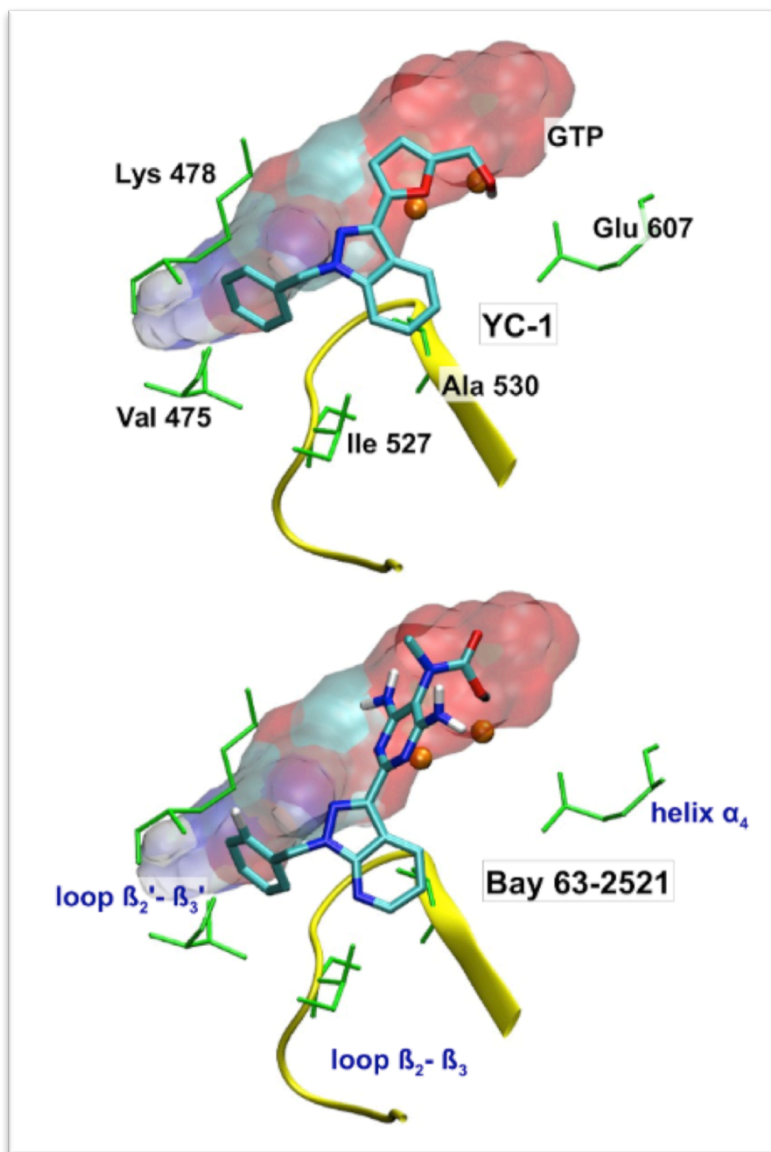
Binding into catalytic domain of soluble guanylate cyclase



Binding into catalytic domain of soluble guanylate cyclase



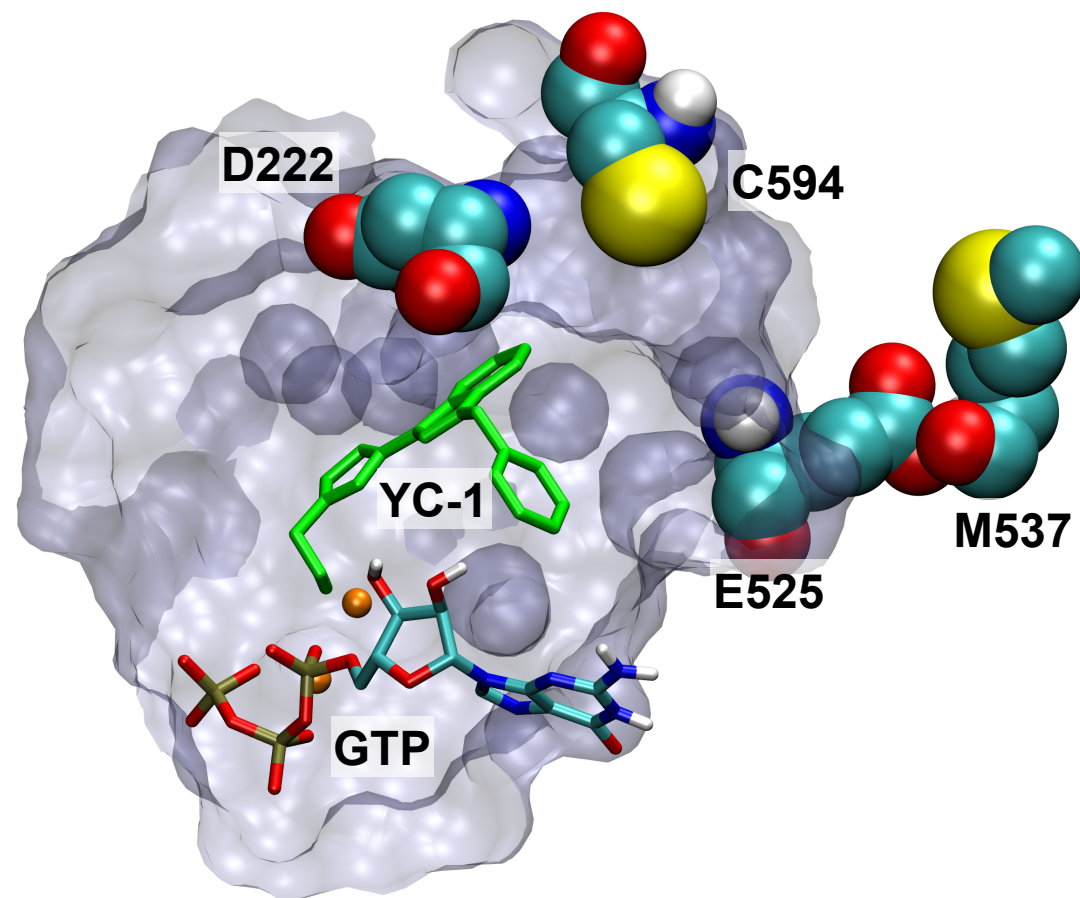
Binding into catalytic domain of soluble guanylate cyclase



Docking of other heme-dependent drugs (**Bay 41-2272**, **Bay 41-8543**, **Bay 63-2521**) and less studied stimulators (**A350619**, **A778935**, **Benzydamine**, **CFM-1571**) to the protein structure used for cluster 1₇ (best binding energy for YC-1) resulted in poses very similar to that observed for YC-1.

Drug	ΔG (Kcal/mol) ^a	
	Uncharged	Ionized
YC-1	-8.6	-
BAY 41-2272	-9.1	-
BAY 41-8543	-9.5	-9.9
BAY 63-2521	-9.4	-9.7
CFM-1571	-7.4	-8.1
A-350619	-7.3	-7.6
A-778935	-7.2	-7.3

Binding into catalytic domain of soluble guanylate cyclase



Lamothe et al. *Biochemistry* 2004,43:3039

Conclusions

- Potential high affinity binding site for YC-1 (and other sGC stimulators) on the catalytic domain of sGC
- This site is exclusively accessible on 'active' models (and even in these models only occasionally)
- It is located on the interphase of the dimer (interacting with both subunits)
- It is not on the pseudo-symmetric site (then it would interfere with the binding of ATP to that site; Marletta's group)
- It is compatible with some of the available mutational data in the bibliography (*Beuve's Group*)
- This potential location is particularly interesting because the drug would interacting with:
 - (1) loop β_2 - β_3 , in the α subunit
 - (2) two arginines (connecting catalytic and pseudo-symmetric sites)
 - (3) Mg^{2+} (critical for the cyclization reaction)

Computational exploration of the binding mode of heme-dependent stimulators into the active catalytic domain of soluble guanylate cyclase

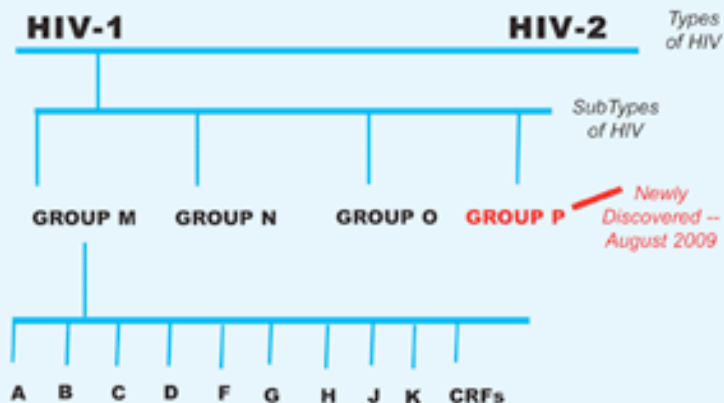
Luis Agulló,^{1*} Ignasi Buch,² Hugo Gutiérrez-de-Terán,³ David Garcia-Dorado,⁴ and Jordi Villà-Freixa¹

Proteoliposomal formulations of an HIV-1 gp41-based miniprotein elicit a lipid-dependent immunodominant response overlapping the 2F5 binding motif

Luis M Molinos-Albert¹, Eneritz Bilbao², Luis Agulló³, Silvia Marfil¹, Elisabet García¹, Maria Luisa Rodríguez de la Concepción¹, Nuria Izquierdo-Useros¹, Cristina Vilaplana⁴, F.-Xabier Contreras^{2,5}, Martin Floor^{3,6}, Pere J Cardona⁴, Javier Martinez-Picado^{1,7,8}, Bonaventura Clotet^{1,8,9}, Jordi Villà-Freixa³, Maier Lorizate^{2*}, Jorge Carrillo^{1,#}, Julià Blanco^{1,8,#*}

HIV variability

HIV Types & Subtypes

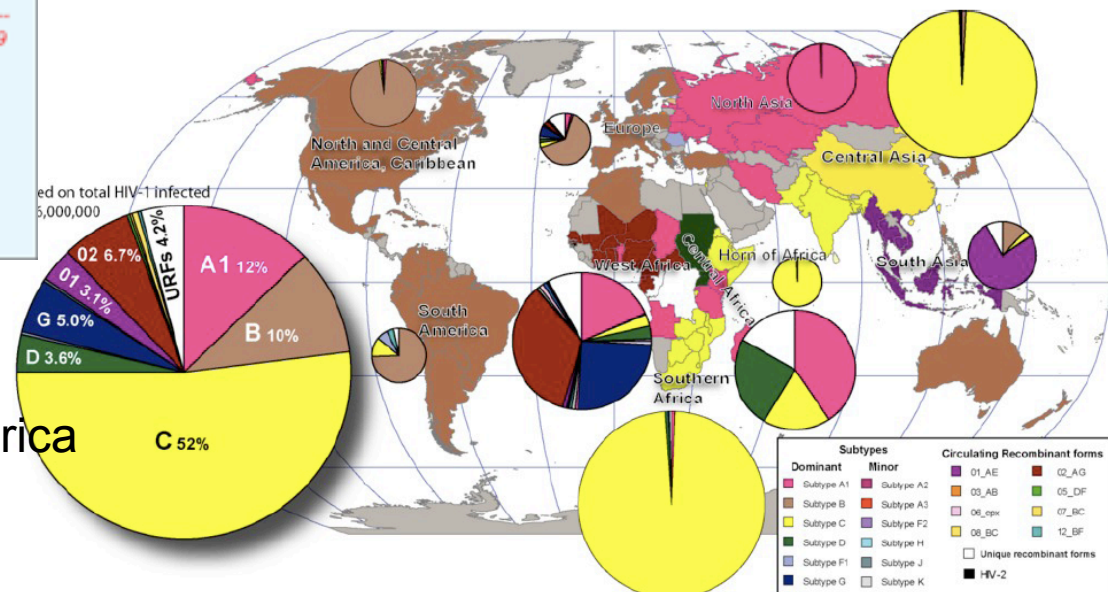


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HIV-1 subtype and recombinant prevalence in the world

Subtype C is dominating the epidemic



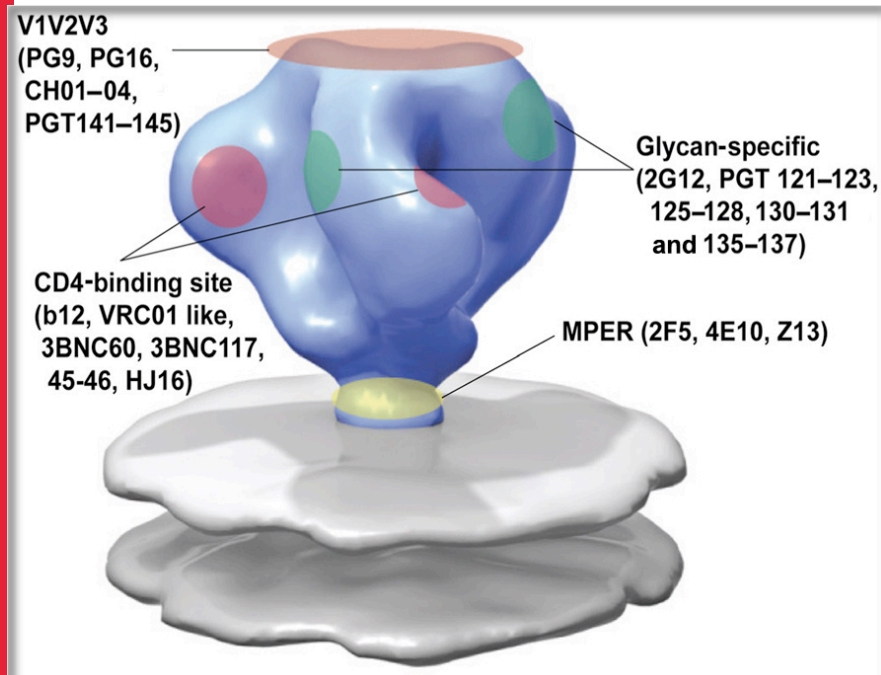
Subgroup A
Subgroup B
Subgroup C
Recomb AE
Recomb AG

Russia
Europe, America
Africa, Asia
Asia
Africa

Regions of HIV envelope protein show different variability

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bNAber

Broadly Neutralizing Antibodies Electronic Resource

GP41 is the transmembrane subunit of the HIV envelope glycoprotein

Gp41 contains several regions:

FP fusion peptide
HR1 Helicoidal region 1
Loop Immunodominant region
HR2 Helicoidal region 2
MPER membrane proximal extracellular region
TM transmembrane domain
Cyt Intracellular tail

Consensus sequence of the fragment HR2 MPER-TM of gp41

CON	EIWDNMTWME	WDKEINNYTD	IIYSLIEESQ	NQOEKNEQEL	LALDKWASLW	NWFDITNWLW	YIKIFIMIVG	GLIGLRIVFA	VLSIV
A1	EIWDNMTWLQ	WDKEISNYTH	IIYNLIEESQ	NQOEKNEQDL	LALDKWANLW	NWFDISNWLW	YIKIFIMIVG	GLIGLRIVFA	VLSVI
A2	EIWNNMTWLQ	WDKEISNYTN	IIYKLLEESQ	NQOEKNEQDL	LALDKWANLW	NWFNITNWLW	YIRIFIMIVG	GLIGLRIVIA	IISV
B	EIWDNMTWME	WEREIDNYTS	LIYTLIEESQ	NQOEKNEQEL	LELDKWASLW	NWFDITNWLW	YIKIFIMIVG	GLVGLRIVFA	VLSIV
C	DIWDNMTWMQ	WDREISNYTD	TIYRLLEDSQ	NQOEKNEKDL	LALDSWKNLW	NWFDITNWLW	YIKIFIMIVG	GLIGLRIIFA	VLSIV
D	EIWNNMTWME	WEREIDNYTG	LIYSLIEESQ	NQOEKNEQEL	LELDKWASLW	NWFSITQWLW	YIKIFIMIVG	GLIGLRIVFA	VLSIV
F1	EIWNNMTWME	WEKEISNYSN	IIYRLIEESQ	NQOEKNEQEL	LALDKWASLW	NWFDISNWLW	YIKIFIMIVG	GLIGLRIVFA	VLSIV
F2	EIWDNMTWMQ	WEKEISNYTD	TIYRLIEDAQ	NQOEKNEQDL	LALDKWDNLW	SWFTITNWLW	YIKIFIMIVG	GLIGLRIVFA	VLSV
G	EIWDNMTWIE	WEREISNYTQ	QIYSLIEESQ	NQOEKNEQDL	LALDKWASLW	NWFDITKWLW	YIKIFIMIVG	GLIGLRIVFA	VLSIV
H	EIWDNMTWME	WDKQINNYTE	EIYRLLEVSQ	TQOEKNEQDL	LALDKWASLW	NWFSITNWLW	YIKIFIMIVG	GLIGLRIIFA	VLSIV
AE	EIWNNMTWIE	WEREISNYTN	QIYEILTESQ	NQQDRNEKDL	LELDKWASLW	NWFDITNWLW	YIKIFIMIVG	GLIGLRIIFA	VLSIV
AG	DIWDNMTWLQ	WDKEISNYTD	IIYNLIEESQ	NQOEKNEQDL	LALDKWASLW	NWFDITNWLW	YIKIFIMIVG	GLIGLRIVFA	VLTI

HR2 helical region 2 of gp41

MPER Membrane proximal extracellular region of gp41 (**LOW VARIABILITY**)

TM Transmembrane region of gp41 (lowest variability, inaccessible to Ab)

MIN immunogen sequence

1 MIWNNMTWME WDREINNYTS LIHSLIEESQ NQOEKNEQEL **LELDKWASLW** NWFNITNWLW
 61 YIKLFIMIVG GLVGLRIVFA VLSIVNRAGG GGKGQDNSAD IQHSGGRSSL EGPRFEGKPI
 121 PNPLLGLDST RTGHHHHHH

HR2

helical region 2 of gp41

MPER

Membrane proximal extracellular region of gp41

TM

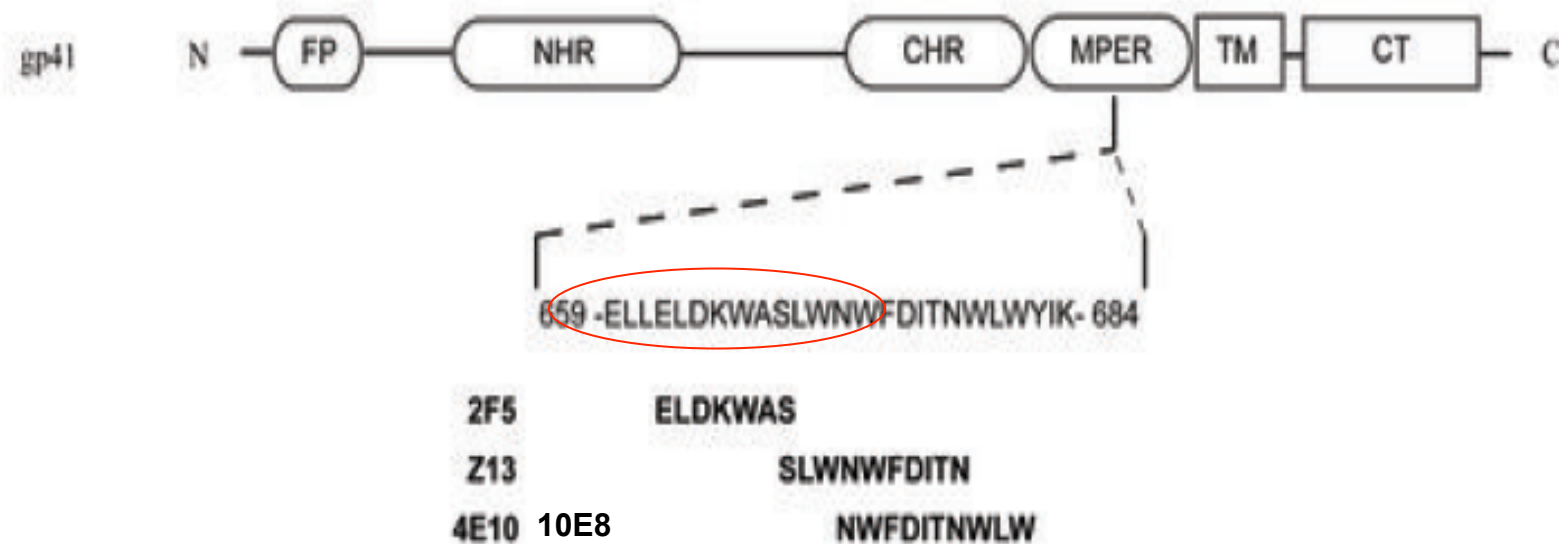
Transmembrane region of gp41

Additional sequences for purification or identification

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DIFFERENT EPITOPES OF MPER ARE TARGETED BY NEUTRALIZING ANTIBODIES



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(A, B, C, AE, AG)



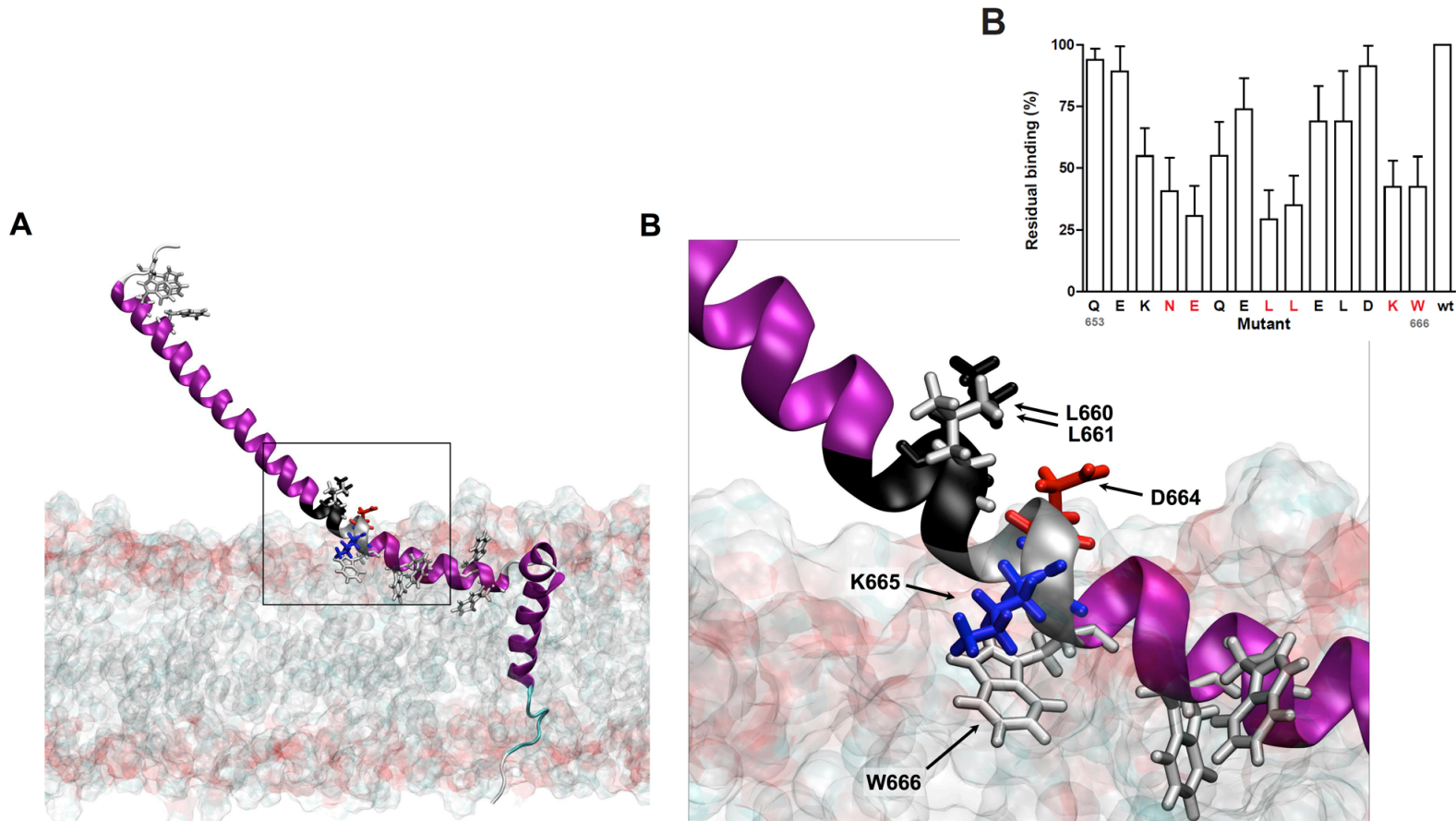
2F5

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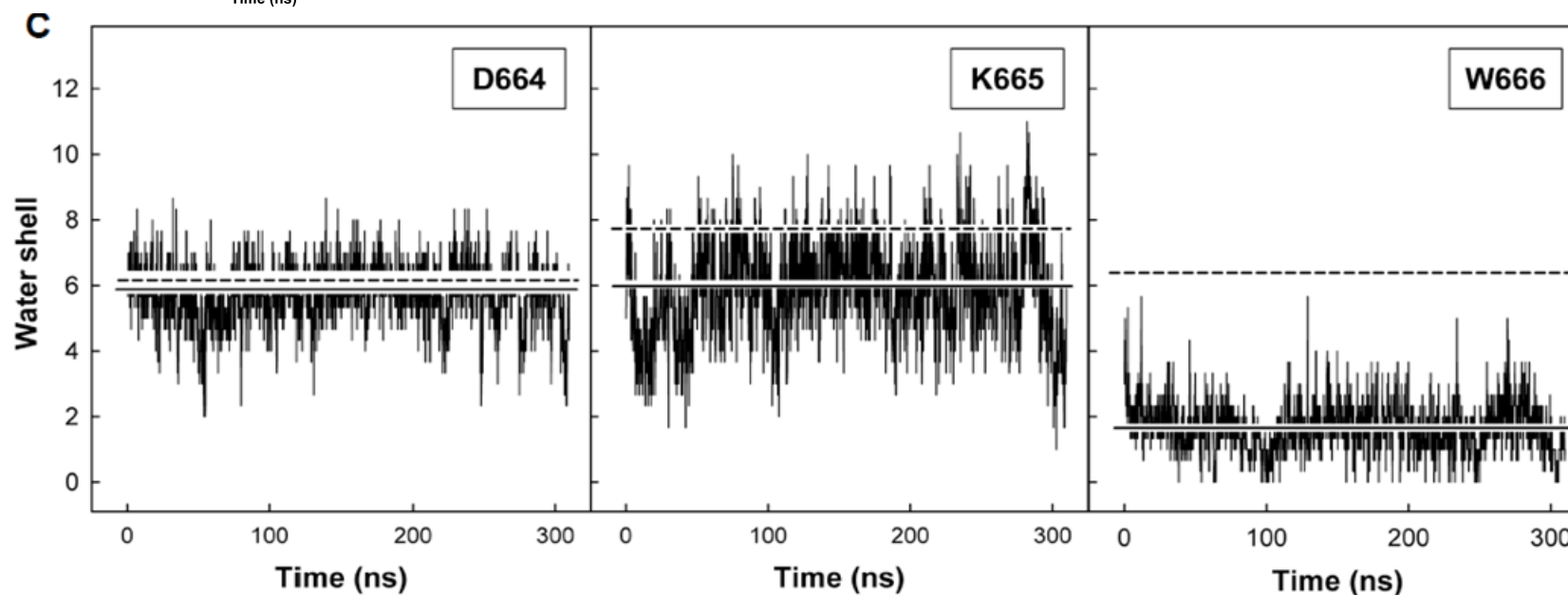
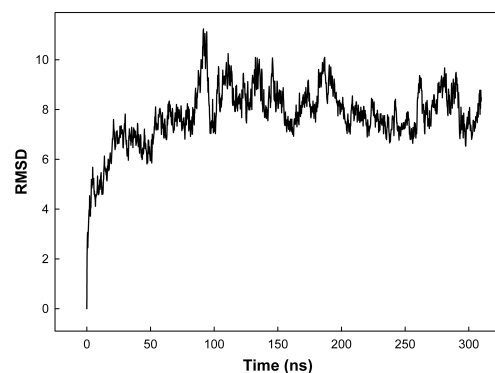
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Relevant residues lie in the membrane interface



The degree of exposure to solvent may induce selectivity by eliciting Ab's



HIV-1 Broadly Neutralizing Antibody Extracts Its Epitope from a Kinked gp41 Ectodomain Region on the Viral Membrane

Next steps

Zhen-Yu J. Sun,^{1,6} Kyoung Joon Oh,^{2,4,6,7} Mikyung Kim,^{2,5,6} Jessica Yu,⁵ Vladimir Brusic,^{2,4} Likai Song,^{2,4} Zhisong Qiao,^{2,5} Jia-huai Wang,^{1,3,5} Gerhard Wagner,¹ and Ellis L. Reinherz^{2,4,5,*}

¹Department of Biological Chemistry and Molecular Pharmacology

²Department of Medicine

³Department of Pediatrics

Harvard Medical School, Boston, MA 02115, USA

⁴Cancer Vaccine Center

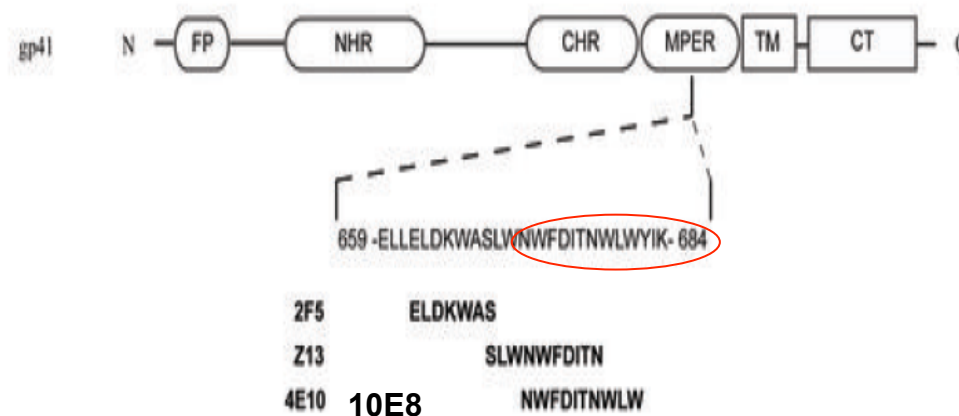
⁵Laboratory of Immunobiology and Department of Medical Oncology
Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, USA

⁶These authors contributed equally to this work.

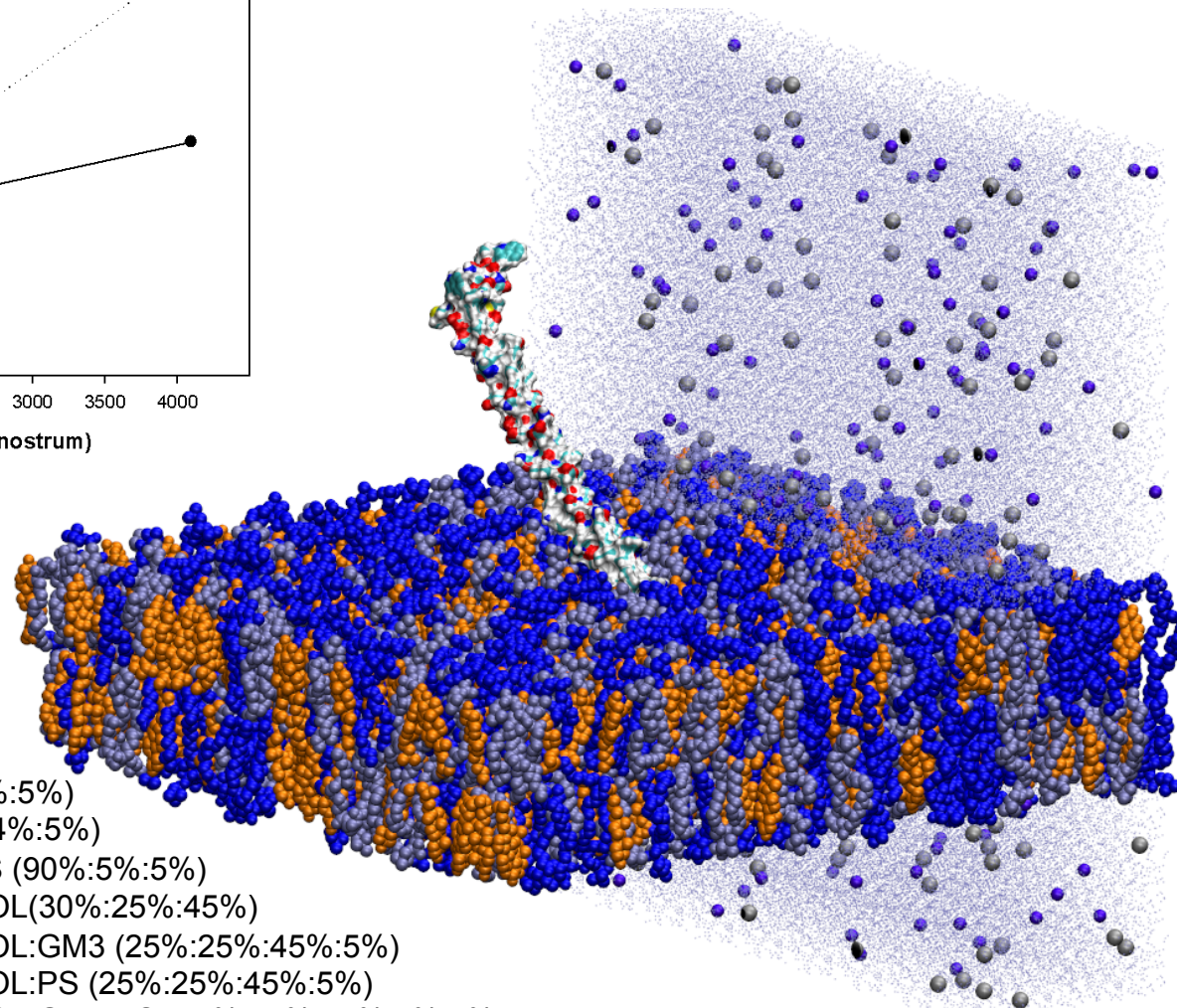
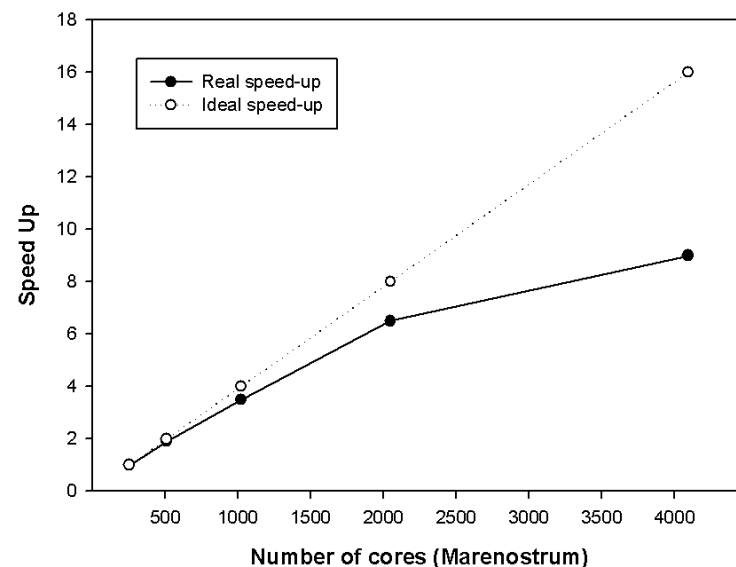
⁷Present address: Department of Biochemistry & Molecular Biology, Rosalind Franklin
3333 Green Bay Road, North Chicago, IL 60064, USA.

*Correspondence: ellis_reinherz@dfci.harvard.edu

DOI 10.1016/j.immuni.2007.11.018



Next steps



1. Proteoliposomes POPC (100%)
2. Proteoliposomes POPC:PS (94%:5%)
3. Proteoliposomes POPC:GM3 (94%:5%)
4. Proteoliposomes POPC:GM3:PS (90%:5%:5%)
5. Proteoliposomes POPC:SM:CHOL(30%:25%:45%)
6. Proteoliposomes POPC:SM:CHOL:GM3 (25%:25%:45%:5%)
7. Proteoliposomes POPC:SM:CHOL:PS (25%:25%:45%:5%)
8. Proteoliposomes POPC:SM:CHOL:GM3:PS (20%:25%:45%:5%:5%)

Conclusions

- Simple desolvation criteria could help understanding interaction of MPER with broadly neutralizing antibody 2F5
- More powerful MD runs needed to excerpt the mechanism of action of Ab's affecting transmembrane region
- Need for the analysis of the effect of different lipid compositions of the membrane

Thank you for your attention!