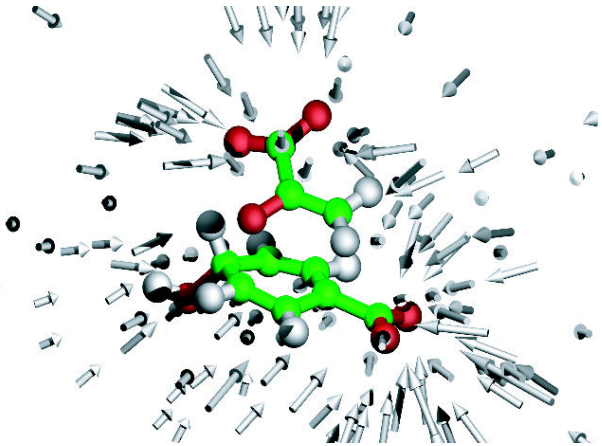
Is solvation/desolvation enough to unravel protein interactions? Examples on HIV-1 gp41-based miniprotein antibody eliciting and sGC ligand binding



Luis Agulló, Martin Floor, Jordi Villà-Freixa

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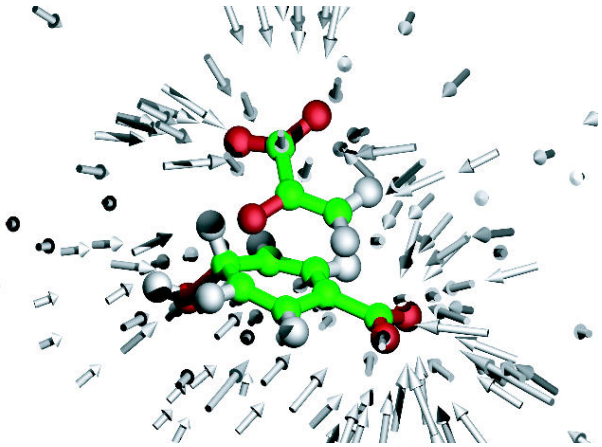
Chair on AIDS and related diseases

Two examples of molecular interactions analysis through MD



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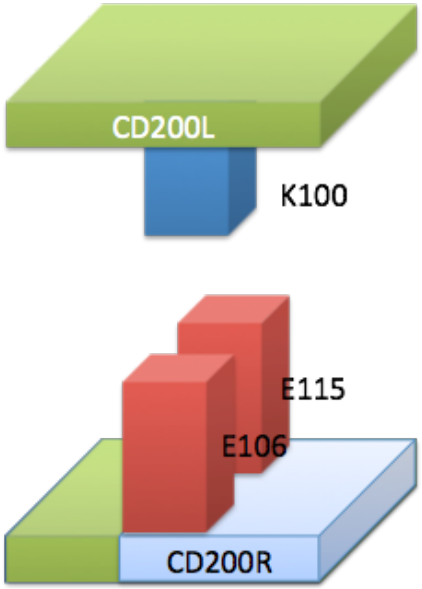
CD200-CD200R agonist search



PDLD/S-LRA

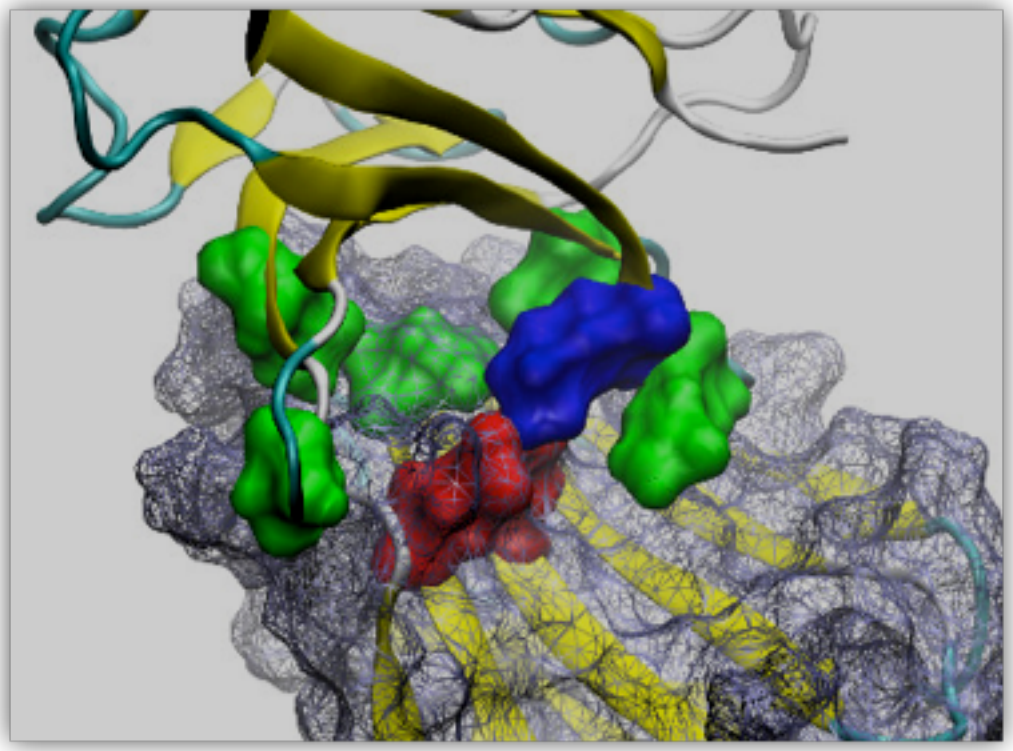


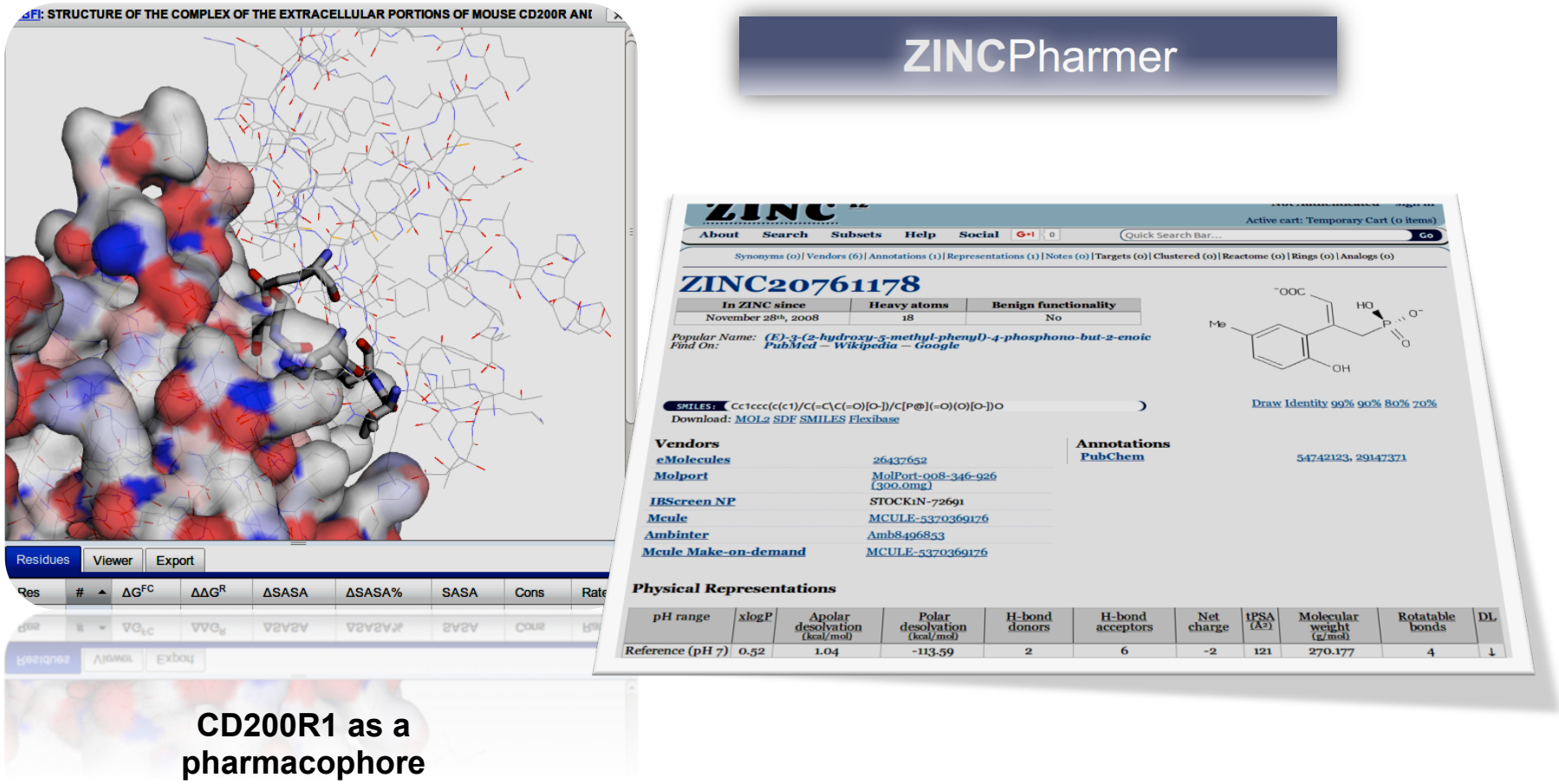
TABLE 1

Primary and Secondary Sequence Identification of CD200 Peptides

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

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

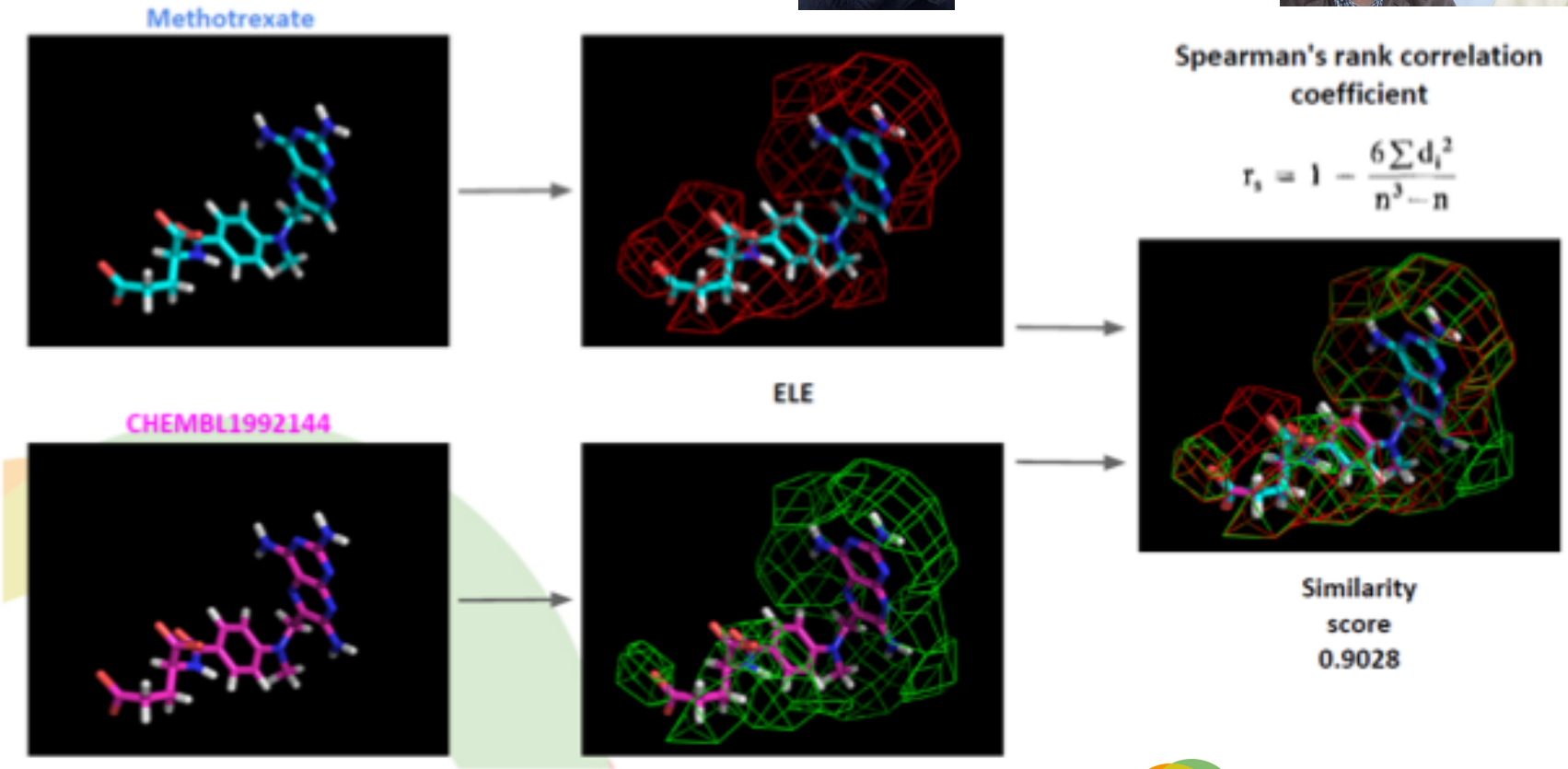
structure-based drug screening



CD200R1 as a pharmacophore

Drug repositioning

EDUARDO SANTAMARIA & ALFONS NONELL



Hurakan

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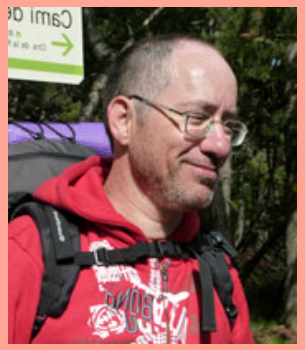
de Desarrollo Regional
"Una manera de hacer Europa"



LUIS M. MOLINOS, JORGE CARRILLO & JULIÀ BLANCO

LUIS AGULLÓ

Càtedra de la SIDA
i Malalties Relacionades
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MINISTERIO
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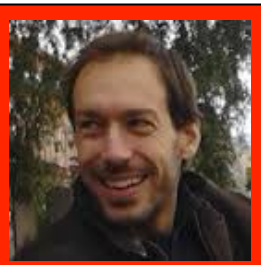

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PROTEINS
STRUCTURE ■ FUNCTION ■ BIOINFORMATICS

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-Freixa[1]

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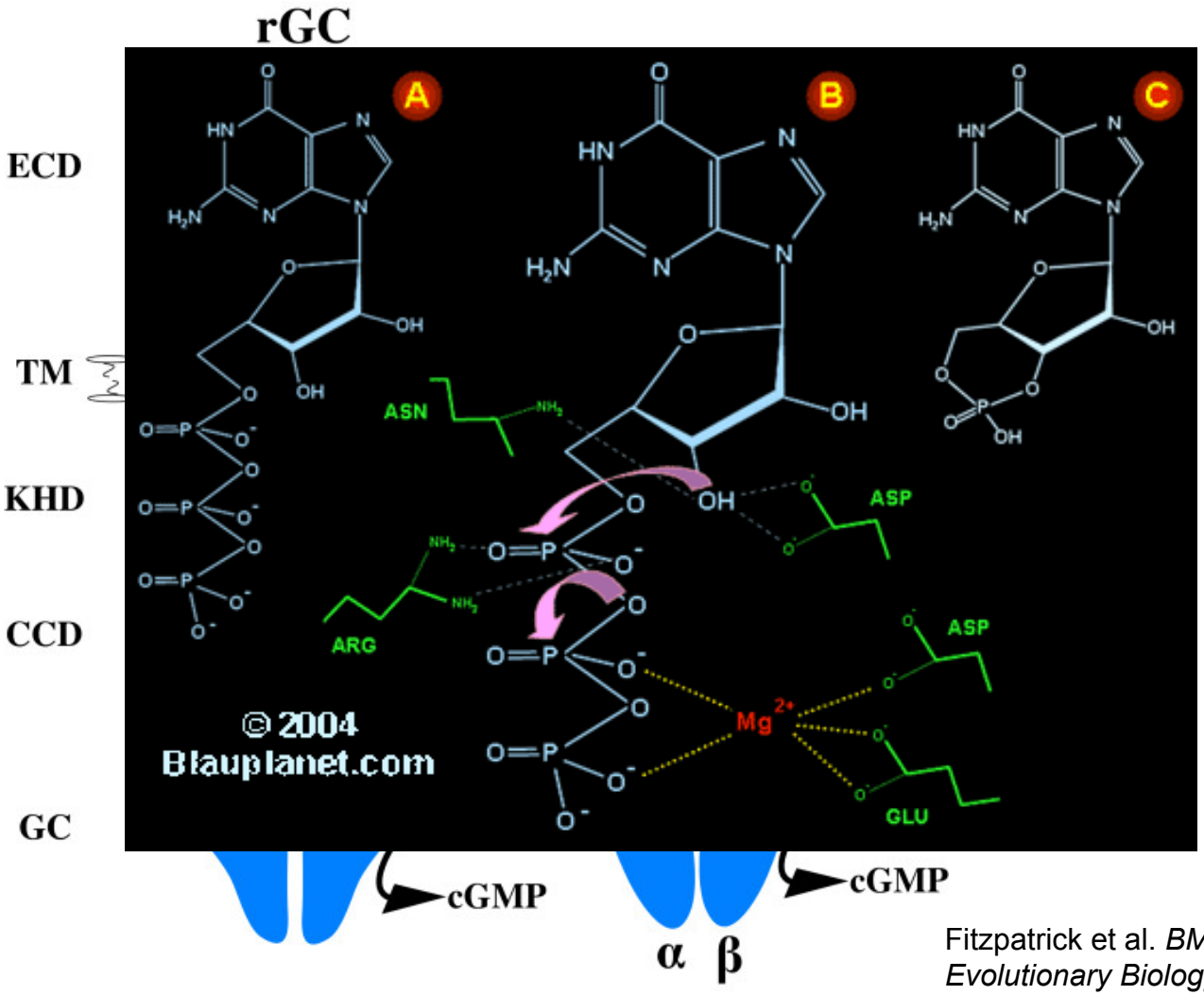
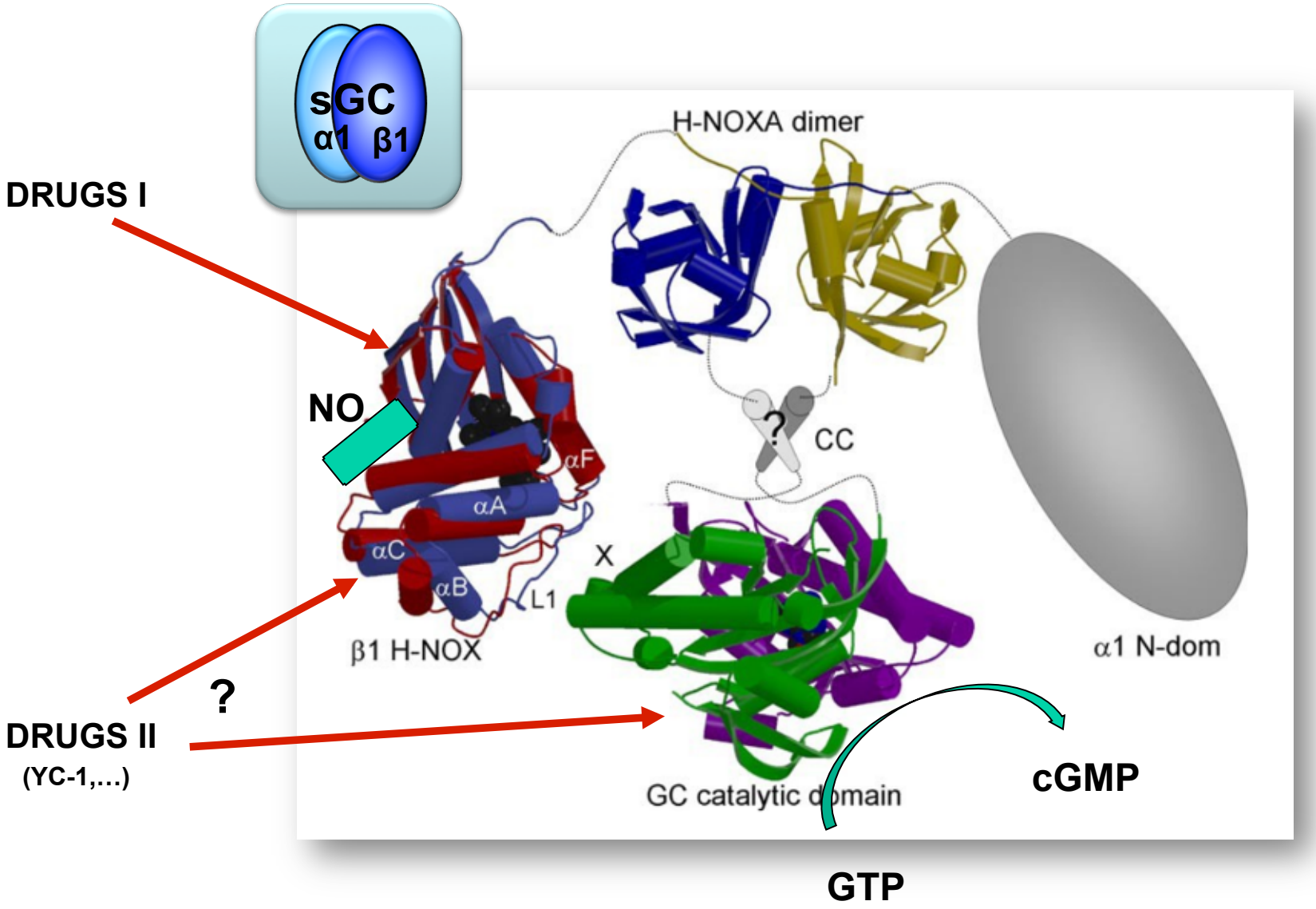
Binding into catalytic domain of soluble guanylate cyclase



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

Binding into catalytic domain of soluble guanylate cyclase



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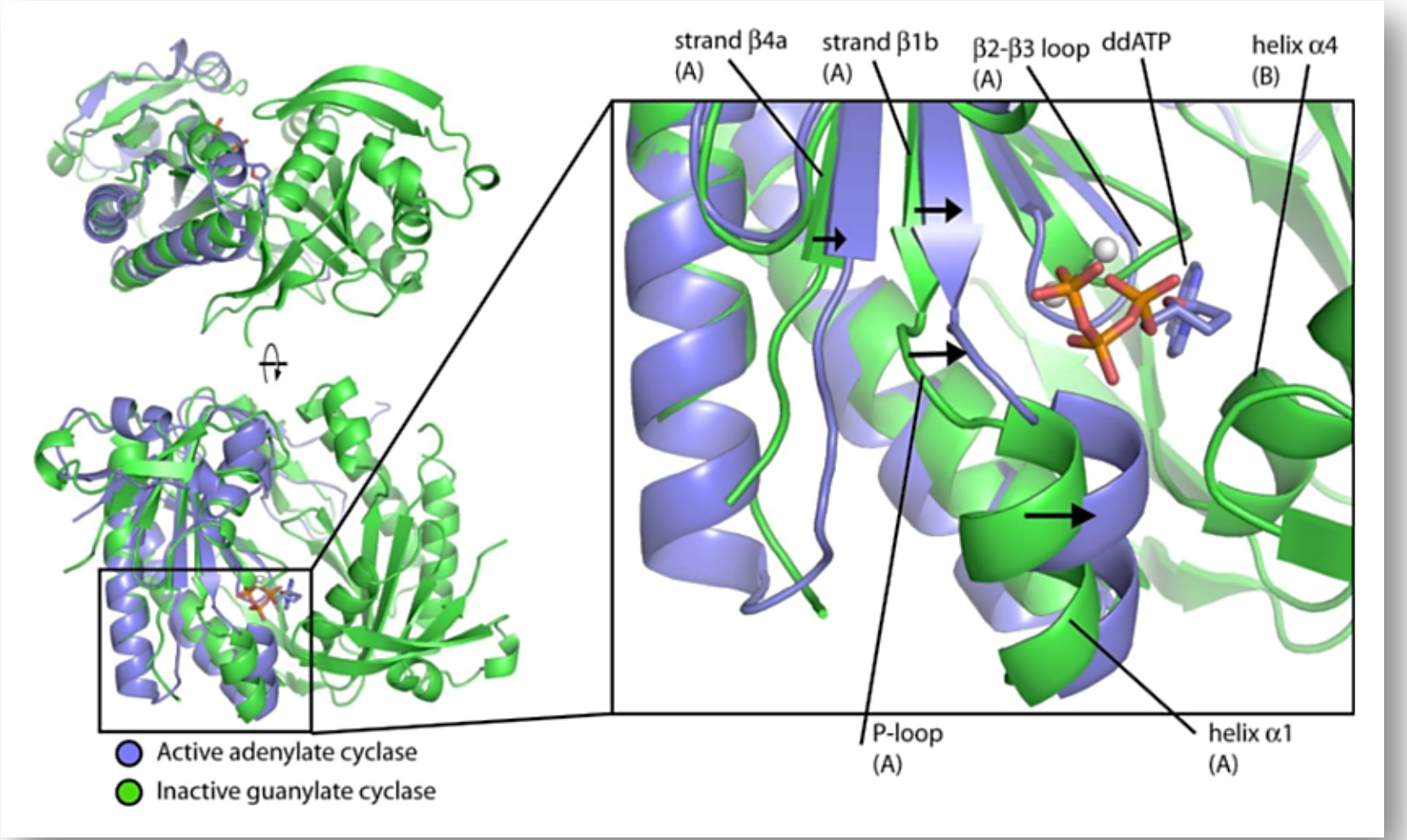
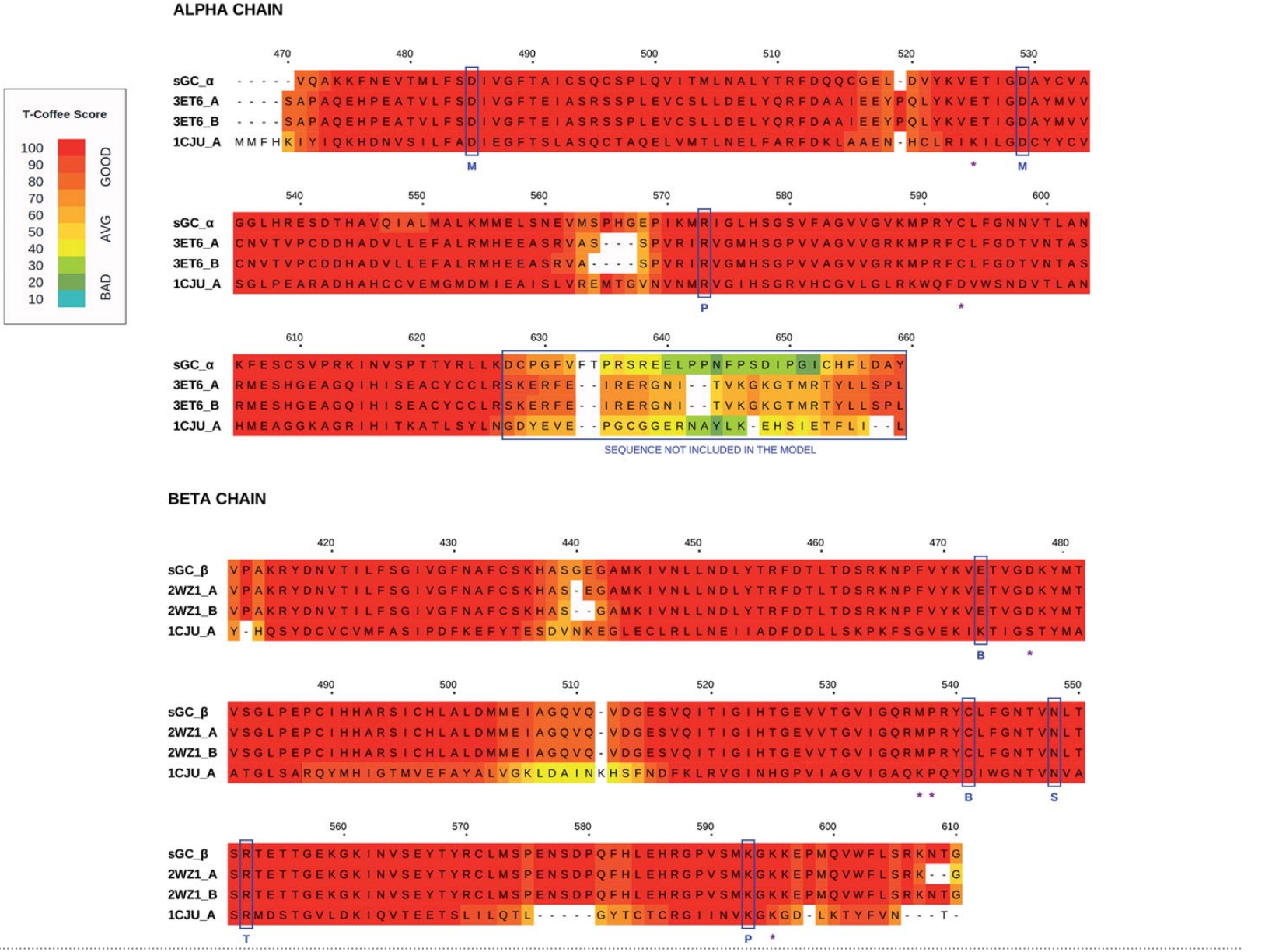
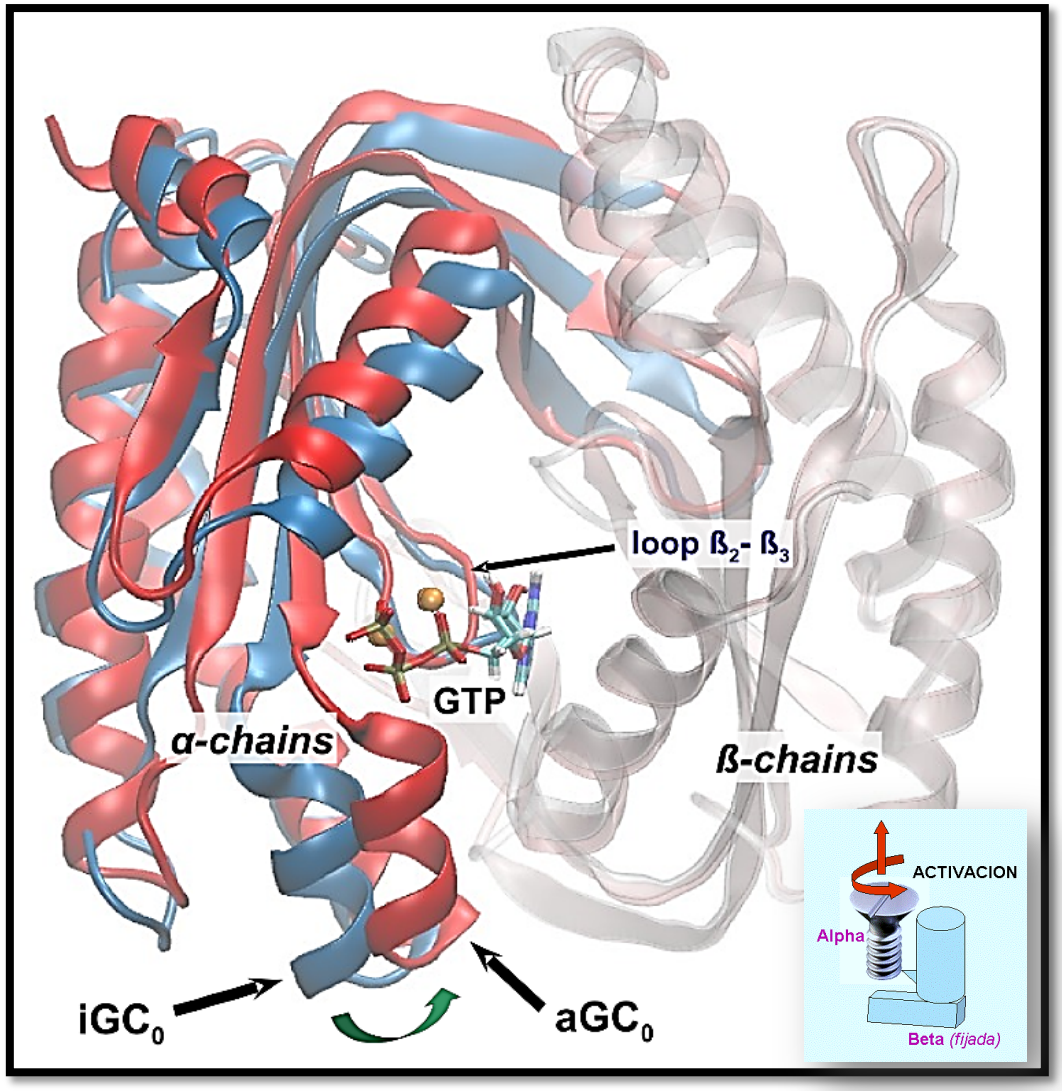


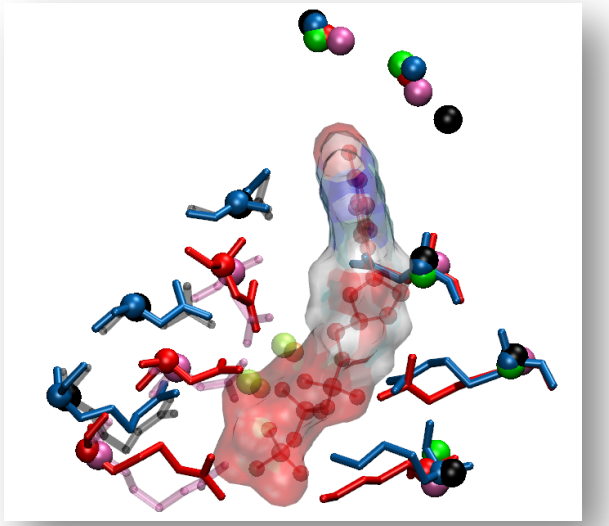
Winger et al. *BMC Structural Biology* 2008, 8:42

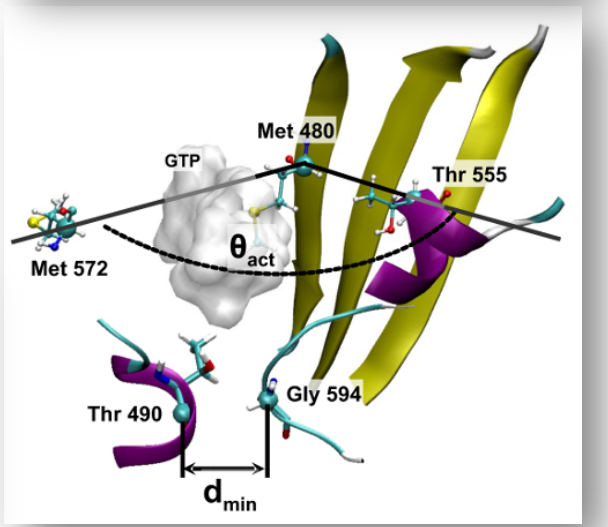
Binding into catalytic domain of soluble guanylate cyclase



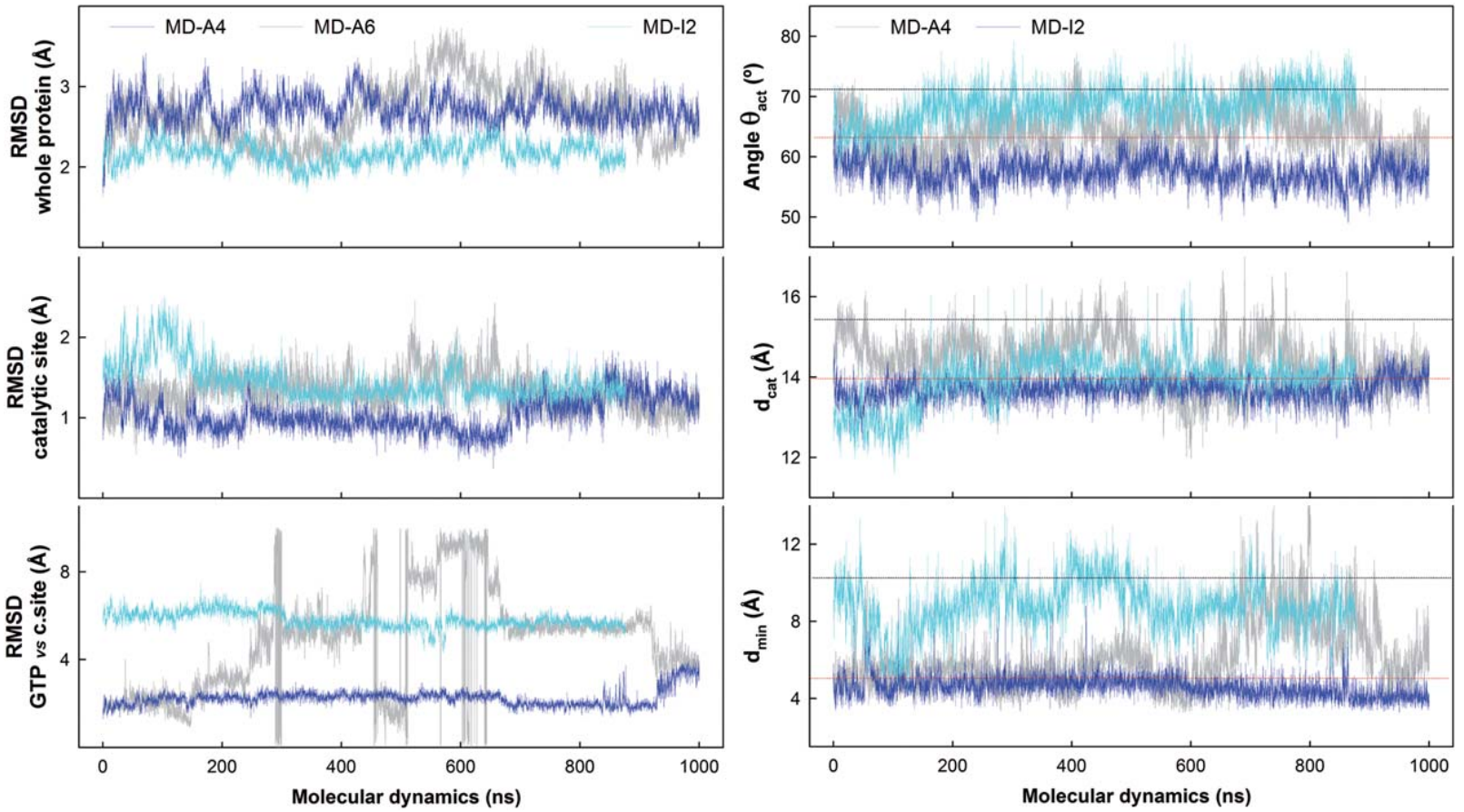
Binding into catalytic domain of soluble guanylate cyclase





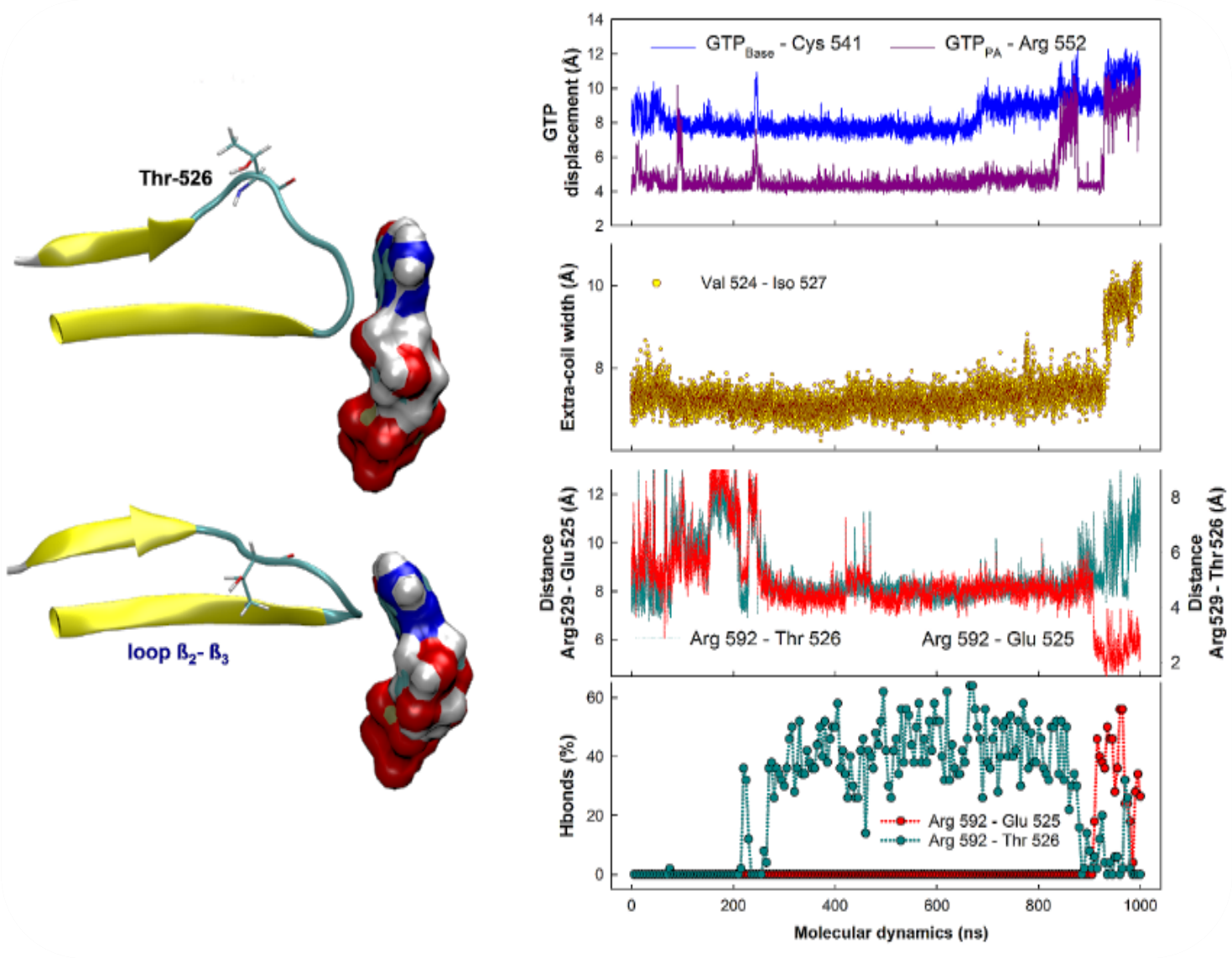


Binding into catalytic domain of soluble guanylate cyclase



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



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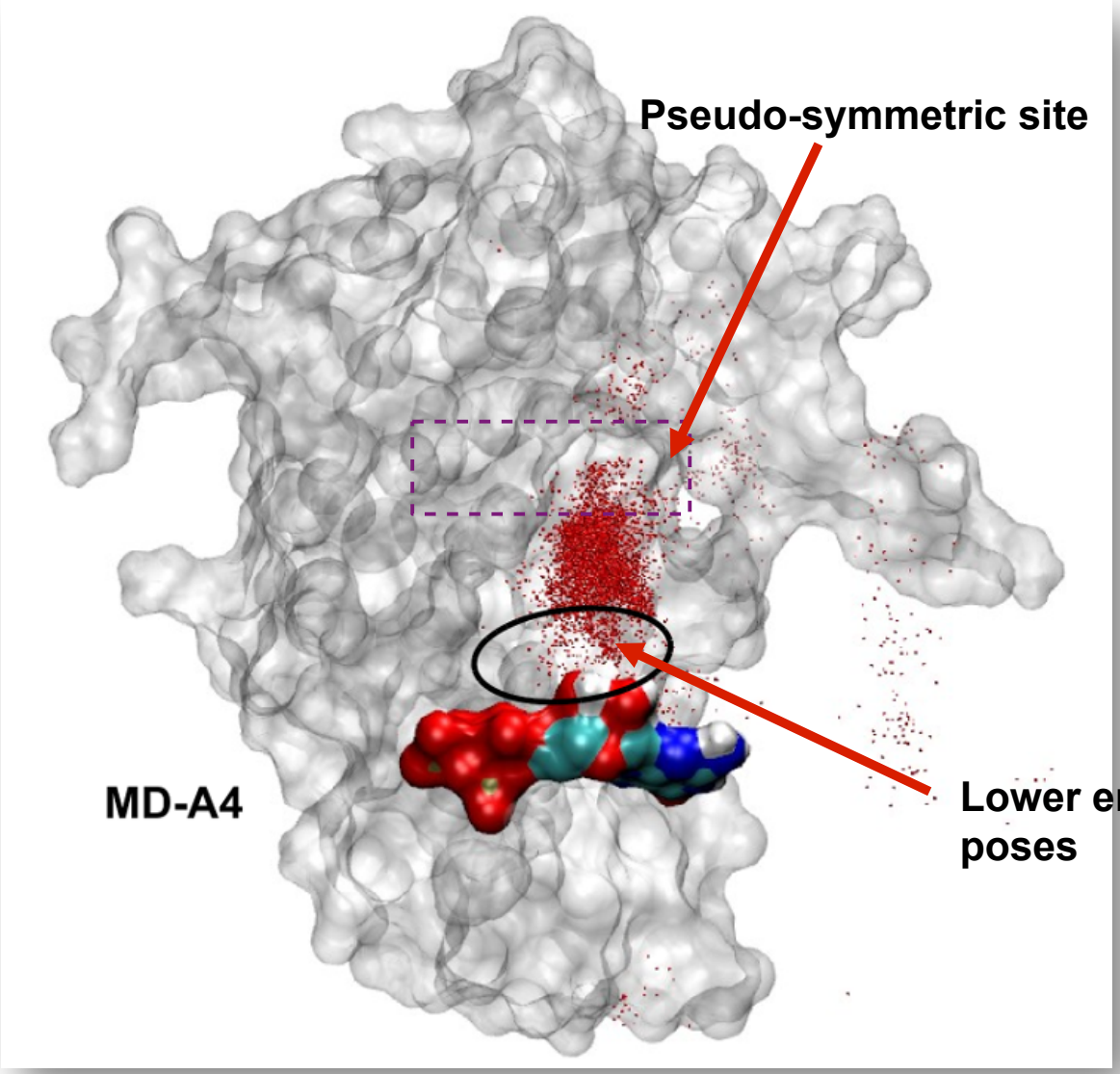
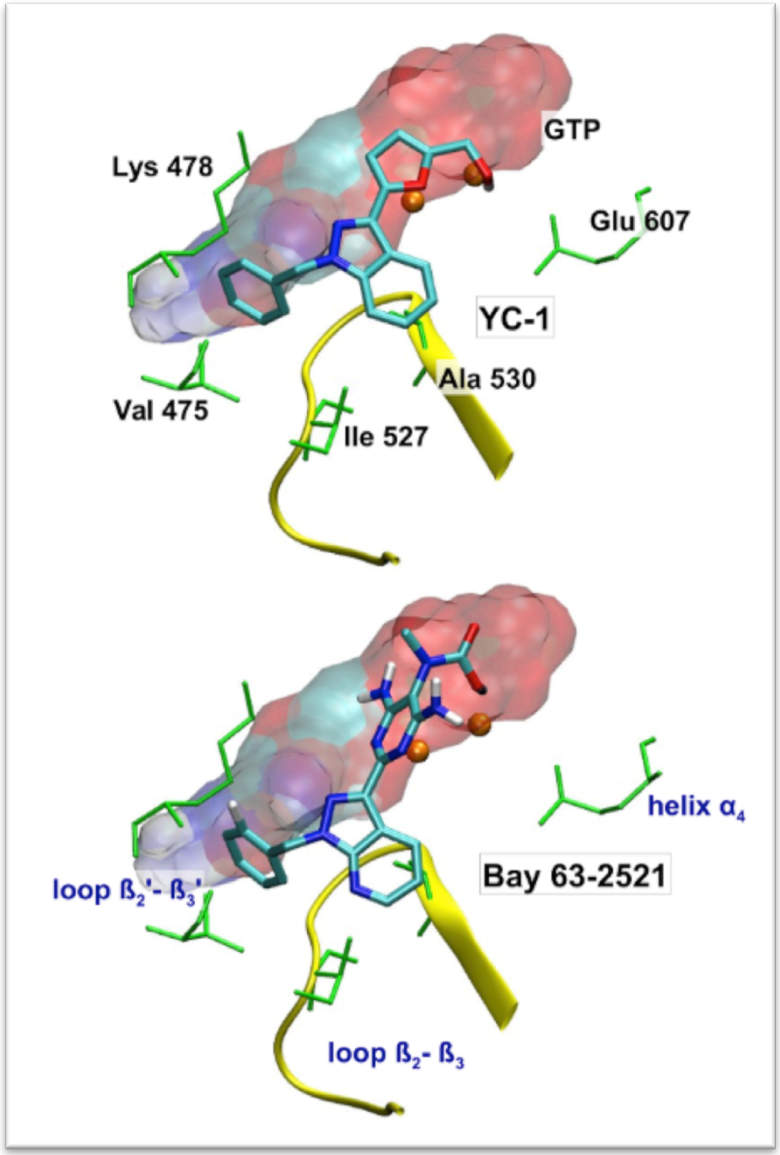


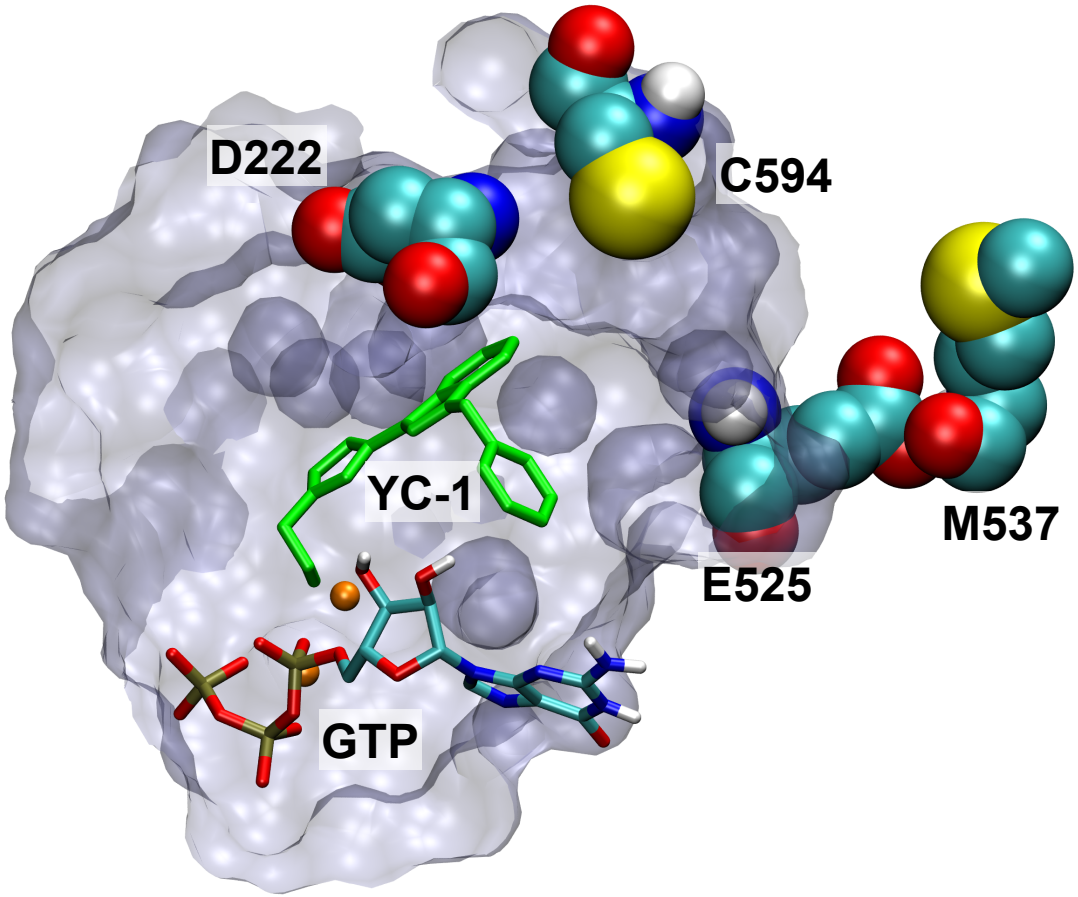
Binding into catalytic domain of soluble guanylate cyclase



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 1_7 (best binding energy for YC-1) resulted in poses very similar to that observed for YC-1.

	ΔG (Kcal/mol)[a]	
Drug	**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)

PROTEINS
STRUCTURE ■ FUNCTION ■ BIOINFORMATICS

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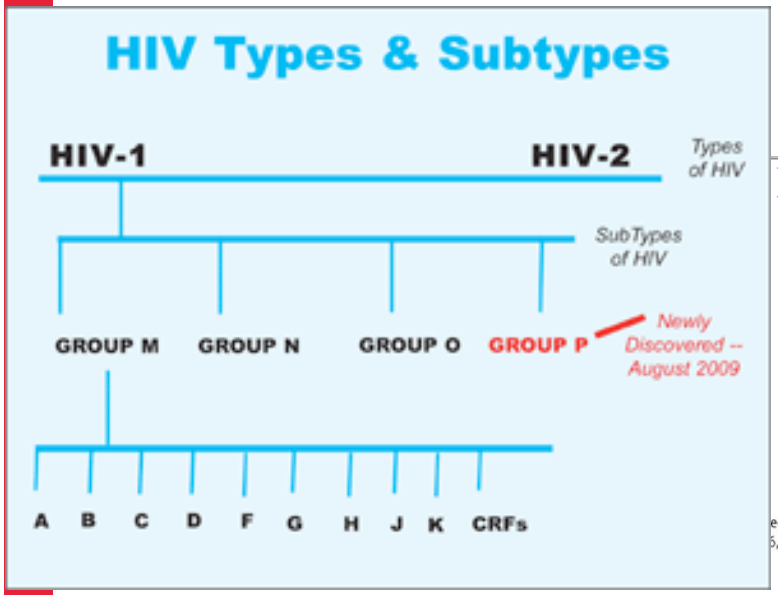
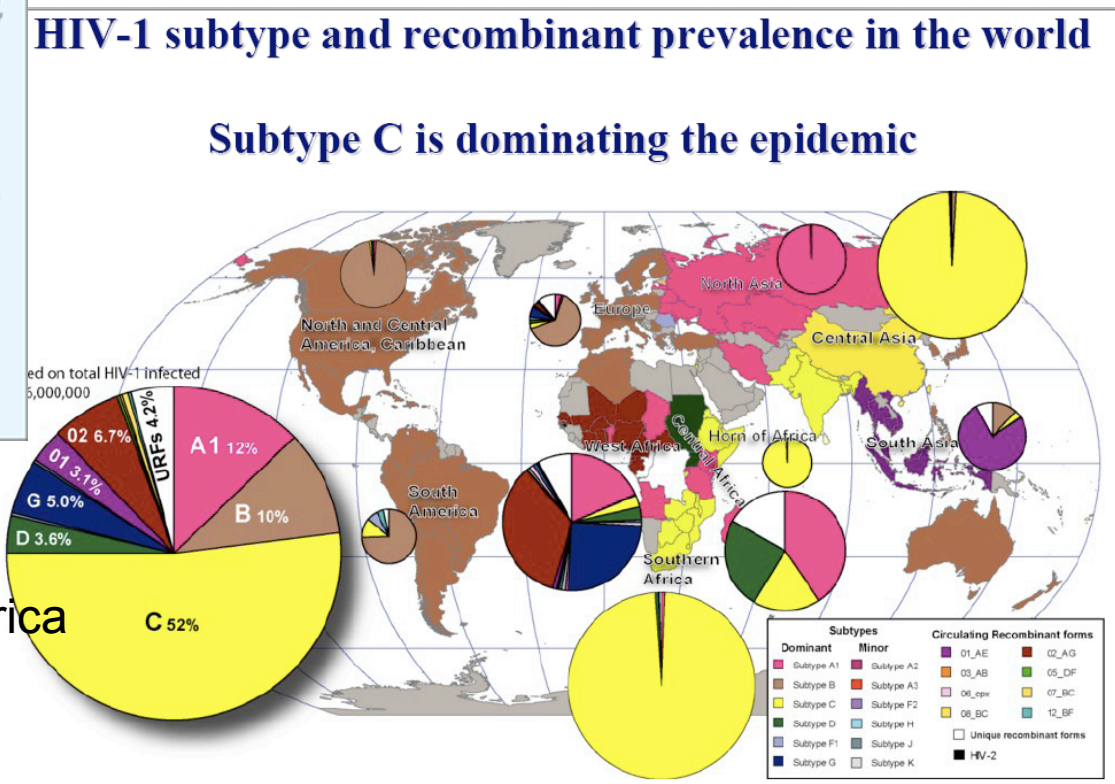
HIV variability

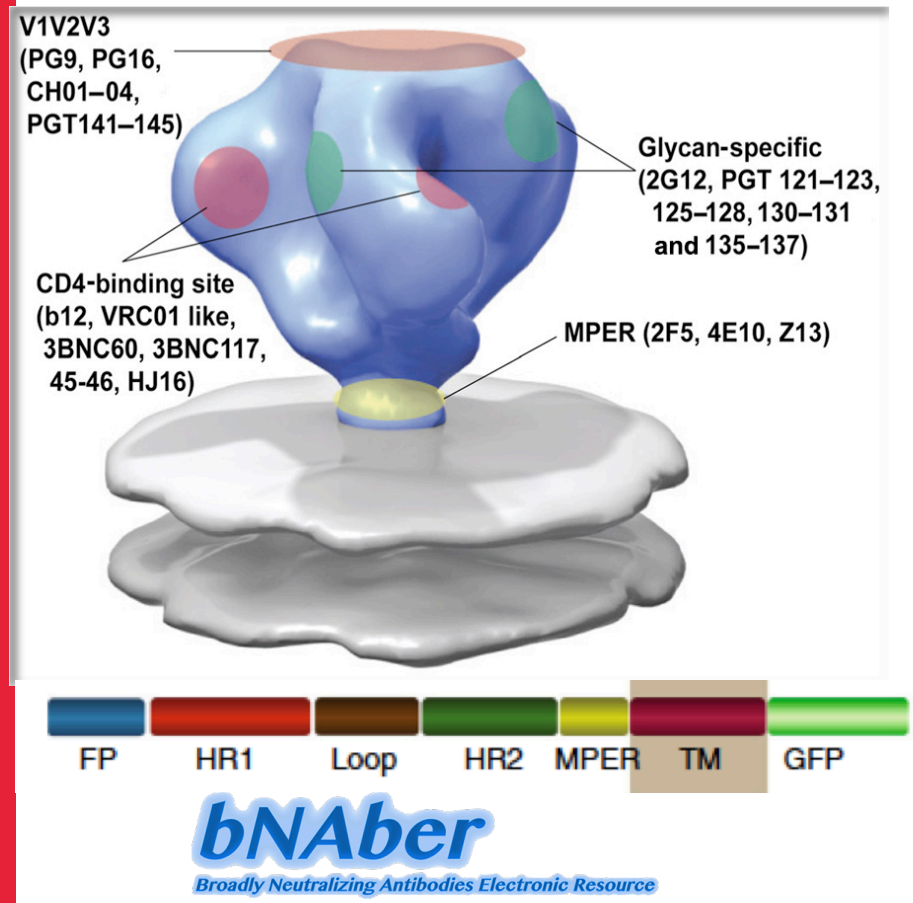


Càtedra de la SIDA i Malalties Relacionades — catSIDA



Subgroup A	Russia
Subgroup B	Europe, America
Subgroup C	Africa, Asia
Recomb AE	Asia
Recomb AG	Africa

Regions of HIV envelope protein show different variability



Càtedra de la SIDA i Malalties Relacionades — catSIDA

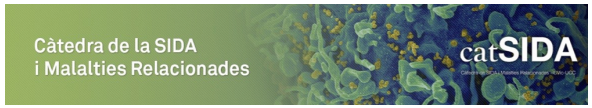
GP41 is the transmembrane subunit of the HIV envelope glycoprotein

Gp41 contsins severals regions:

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

Consensus sequence of the fragment HR2 MPER-TM of gp41

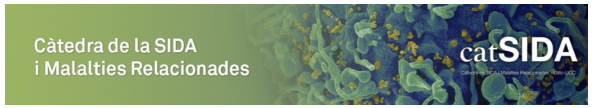
HR2 **helical region 2 of gp41**
MPER **Membrane proxinal extracellular region of gp41 (LOW VARIABILITY)**
TM **Transmembrane region of gp41** (lowest variability, inaccessible to Ab)



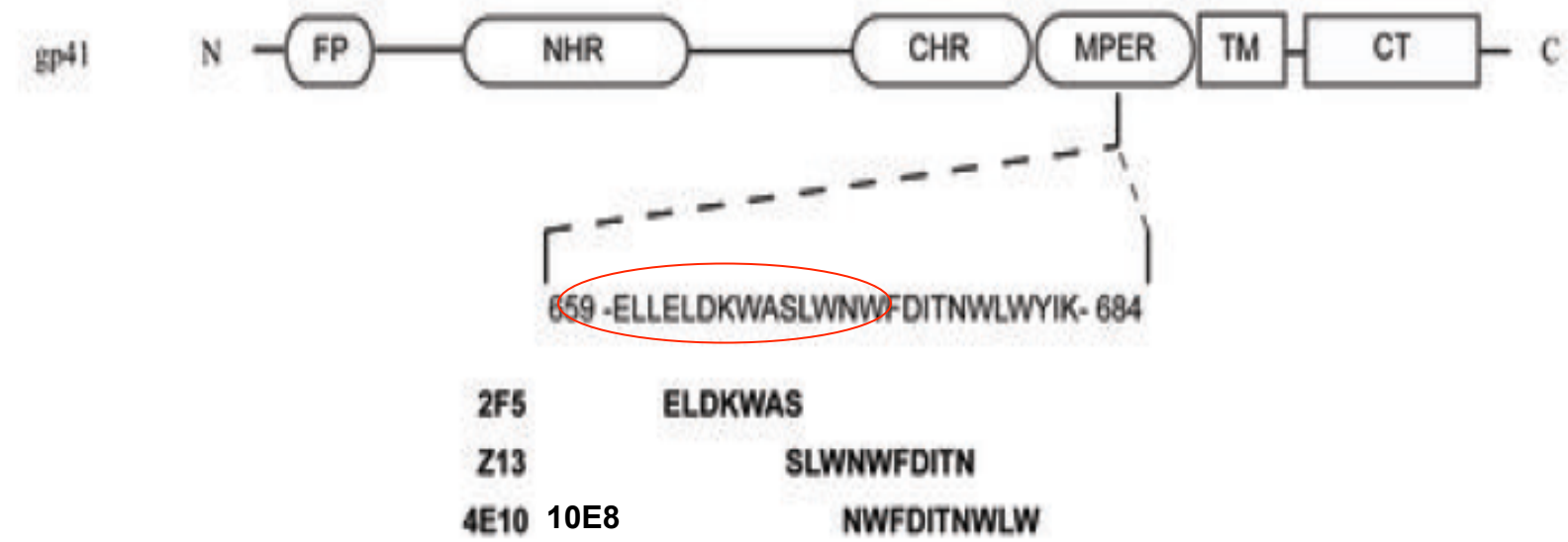
MIN immunogen sequence

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

HR2 helical region 2 of gp41
MPER Membrane proxinal extracellular region of gp41
TM Transmembrane region of gp41
Additional sequences for purification or identification



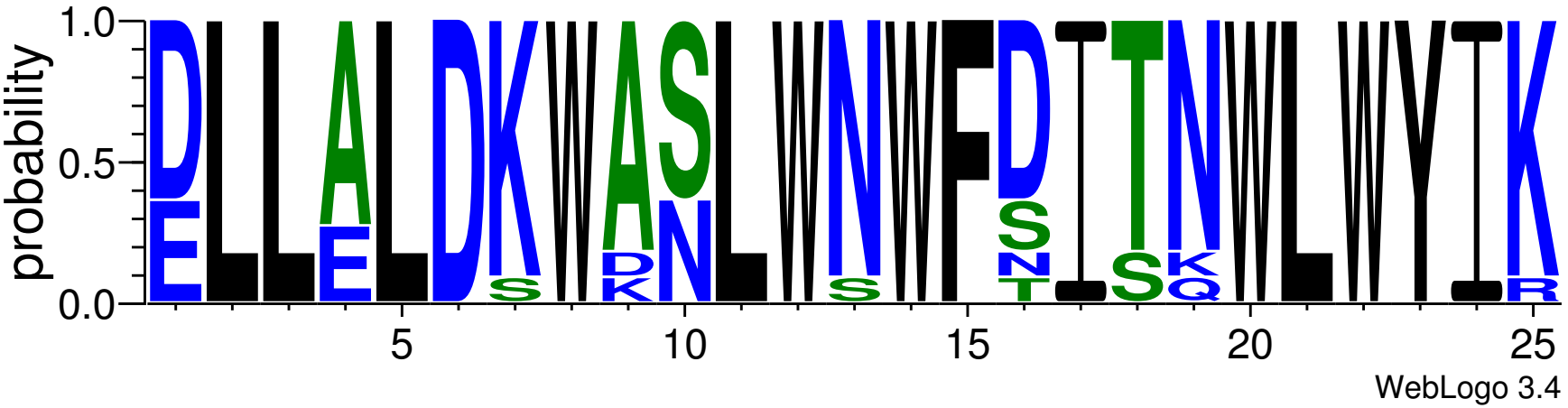
DIFFERENT EPITOPES OF MPER ARE TARGETED BY NEUTRALIZING ANTIBODIES



Càtedra de la SIDA i Malalties Relacionades

catSIDA

MPER SEQUENCE PROBABLITY FOR ALL HIV SUBTYPES

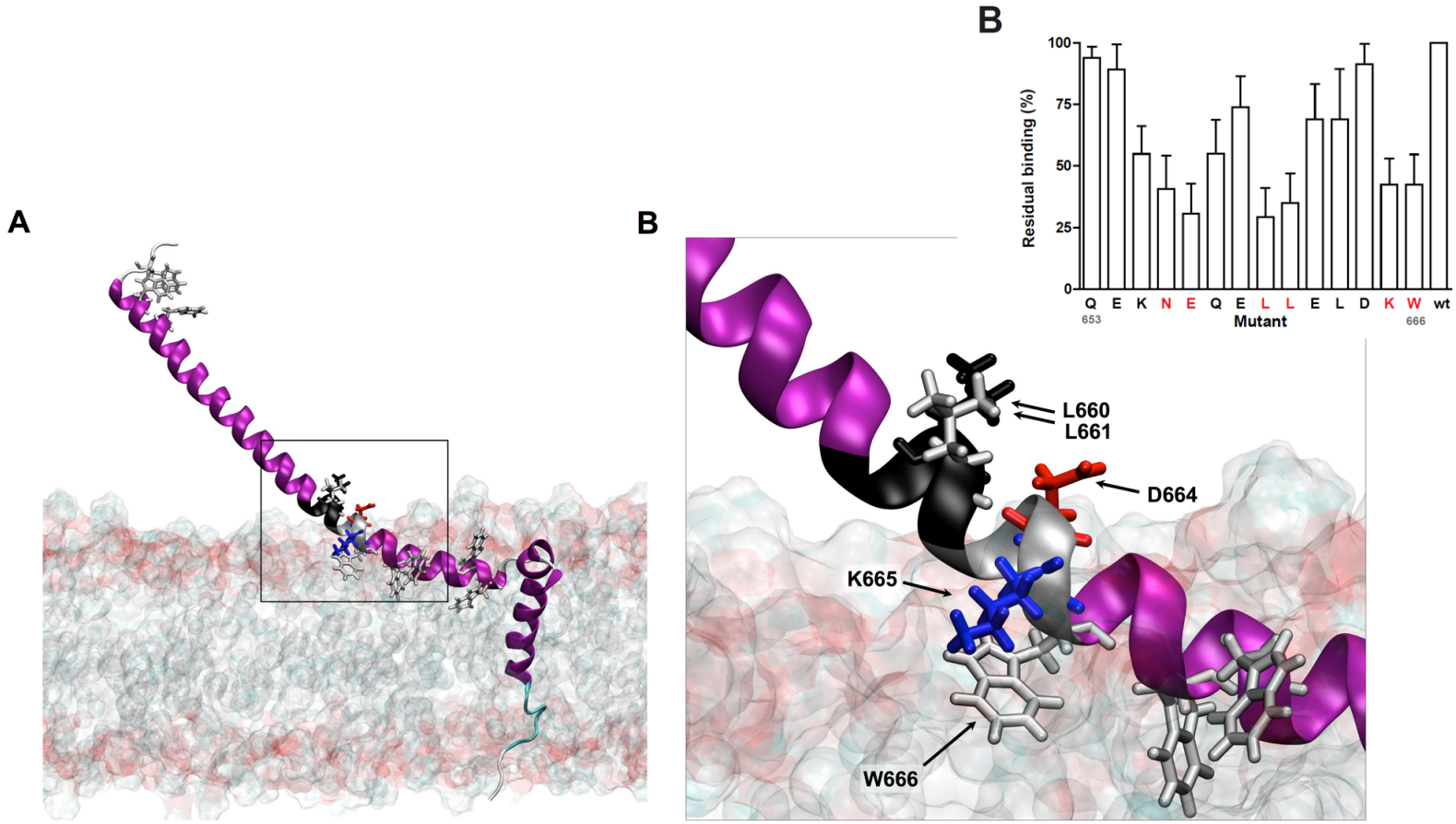


MPER SEQUENCE PROBABLITY FOR MOST PREVALENT HIV SUBTYPES (A, B, C, AE, AG)

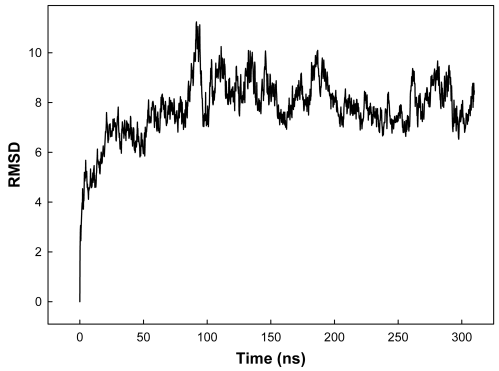
2F5

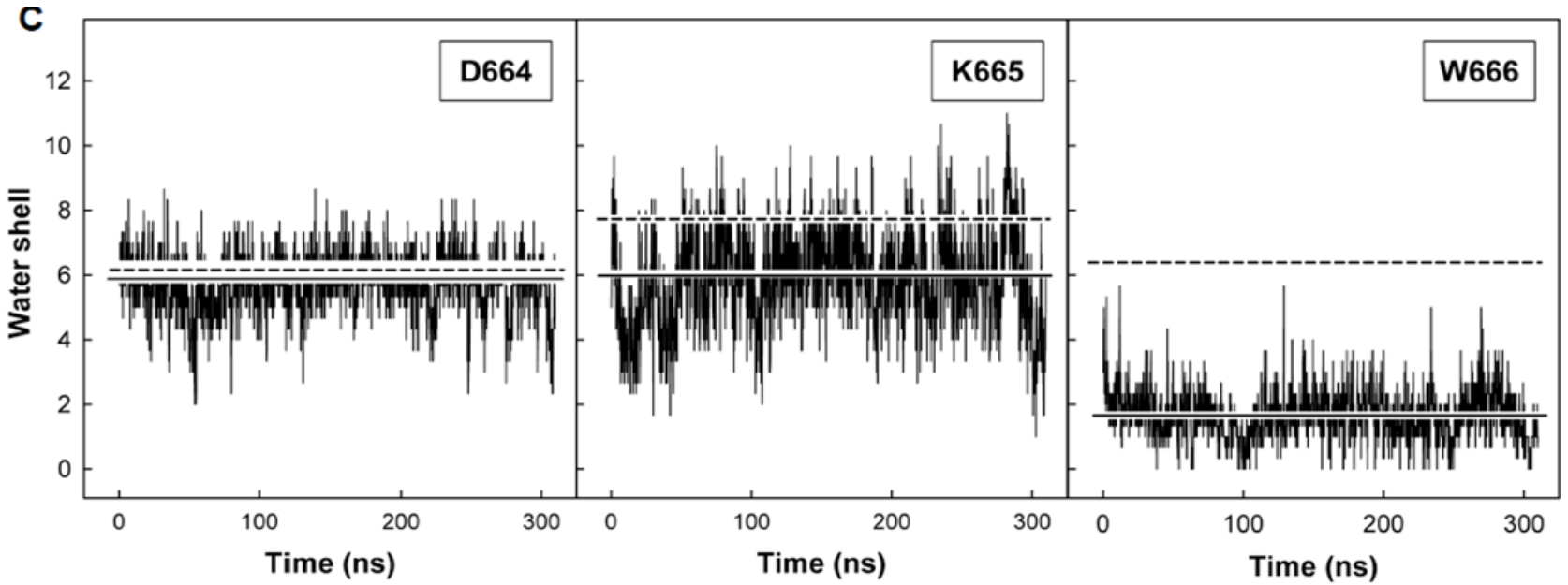
8E10

Relevant residues lie in the membrane interface



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

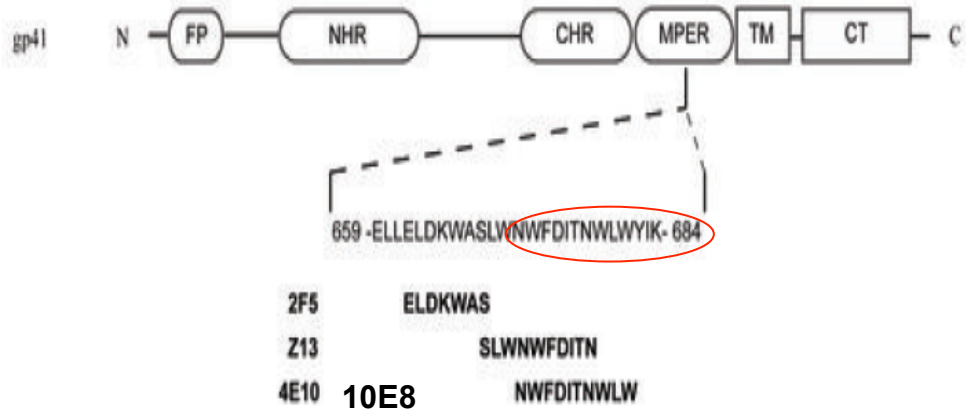




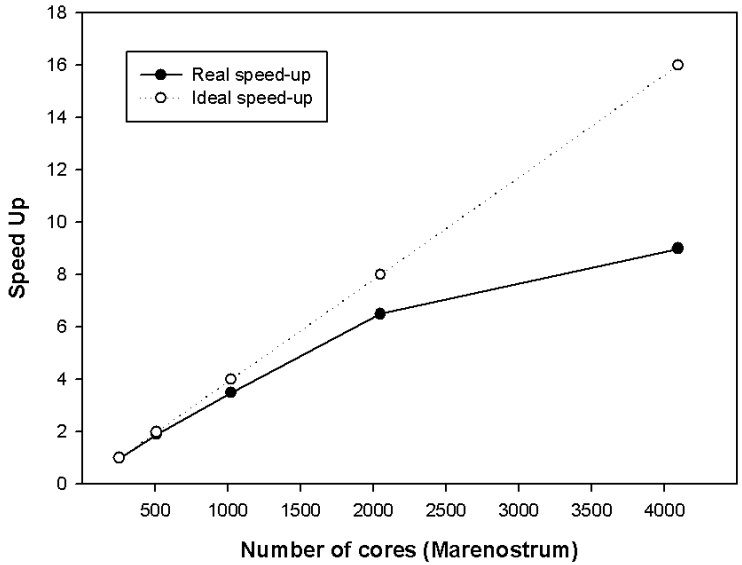
Next steps

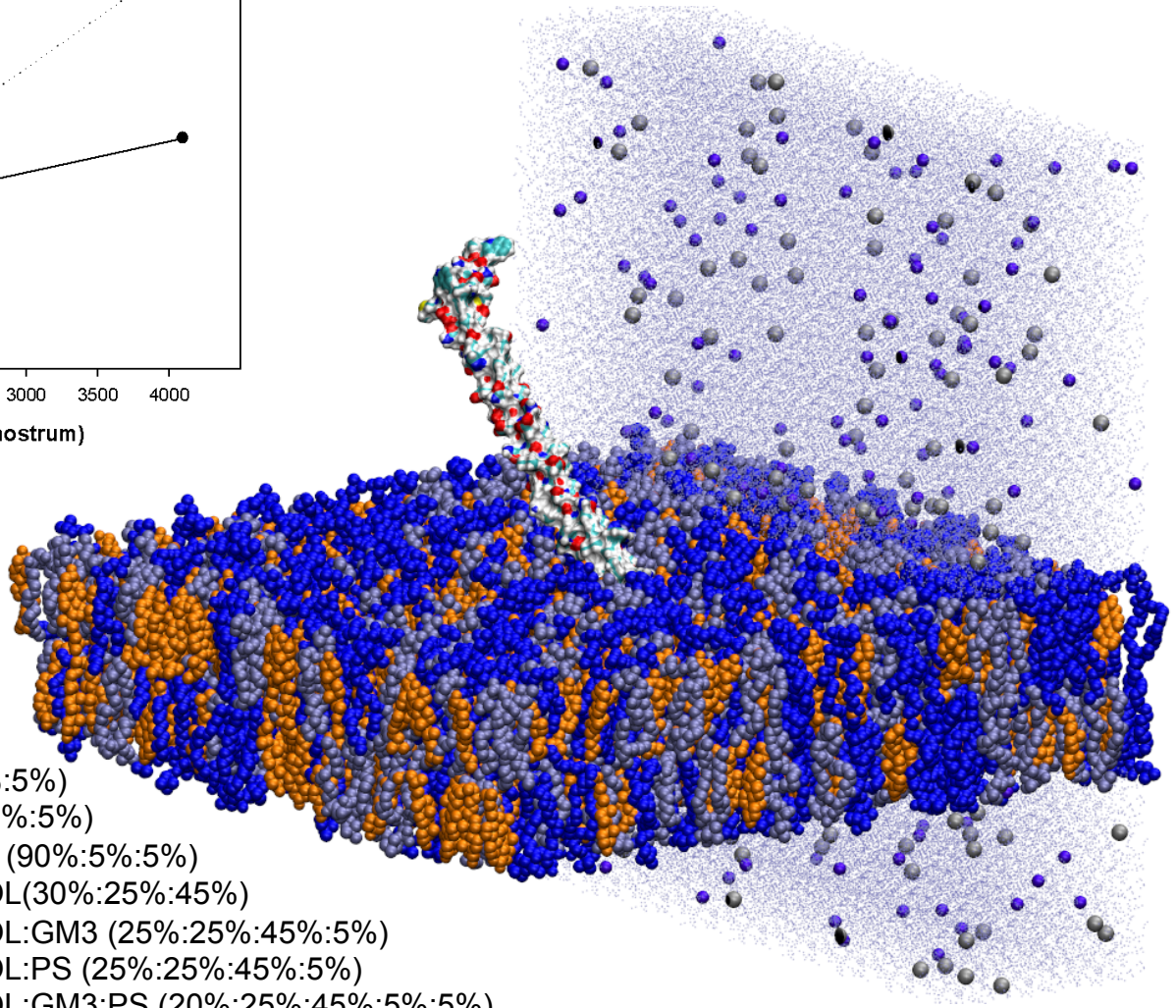
HIV-1 Broadly Neutralizing Antibody Extracts Its Epitope from a Kinked gp41 Ectodomain Region on the Viral Membrane

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,*]
[1]Department of Biological Chemistry and Molecular Pharmacology
[2]Department of Medicine
[3]Department of Pediatrics
Harvard Medical School, Boston, MA 02115, USA
[4]Cancer Vaccine Center
[5]Laboratory of Immunobiology and Department of Medical Oncology
Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, USA
[6]These authors contributed equally to this work.
[7]Present 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!