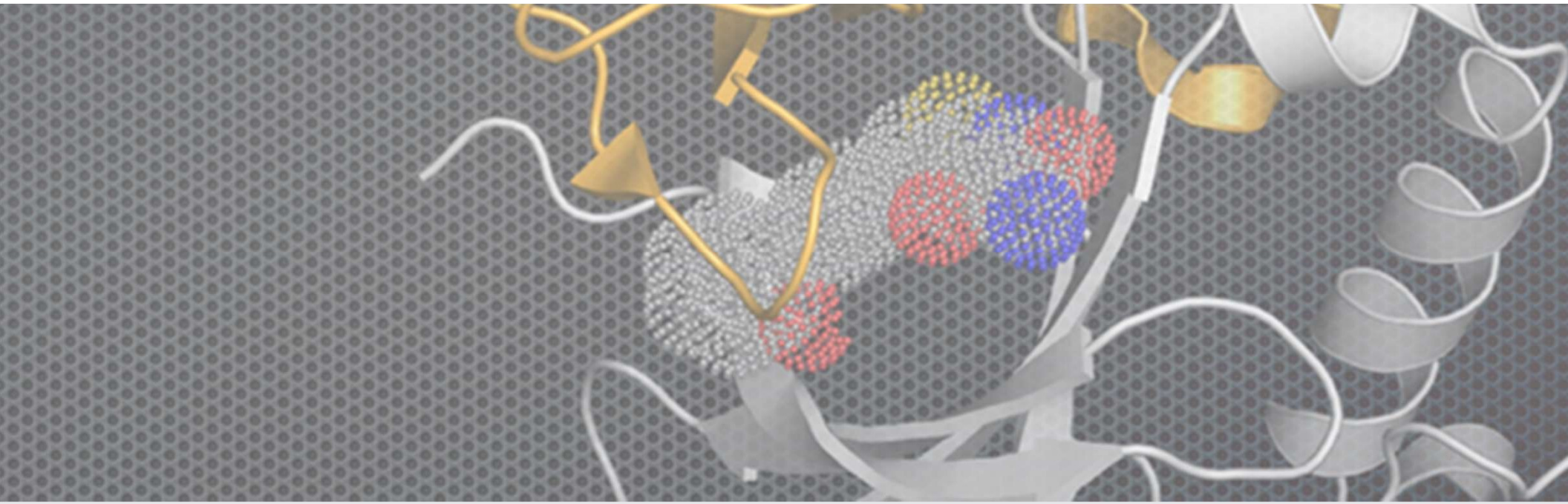


UNDERSTANDING THE MECHANISM OF DIRECT ACTIVATION OF AMPK :

TOWARD A FINE ALLOSTERIC TUNING OF THE KINASE ACTIVITY

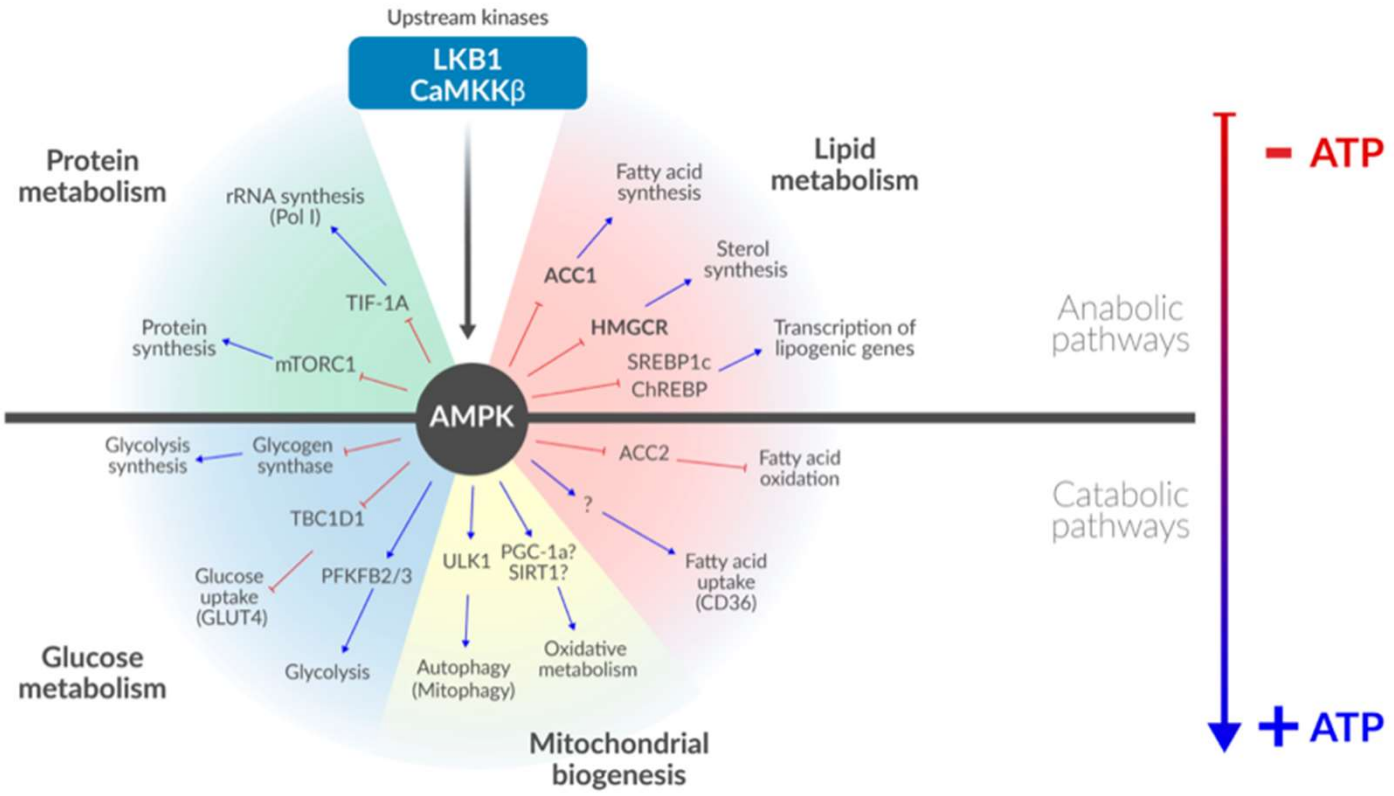
Carolina Estarellas

in collaboration with

Computational Biology, Chemistry & Gastronomy Group
Faculty of Pharmacy and Food Science
University of Barcelona

SCIENTIFIC CONTEXT: AMP-activated protein kinase

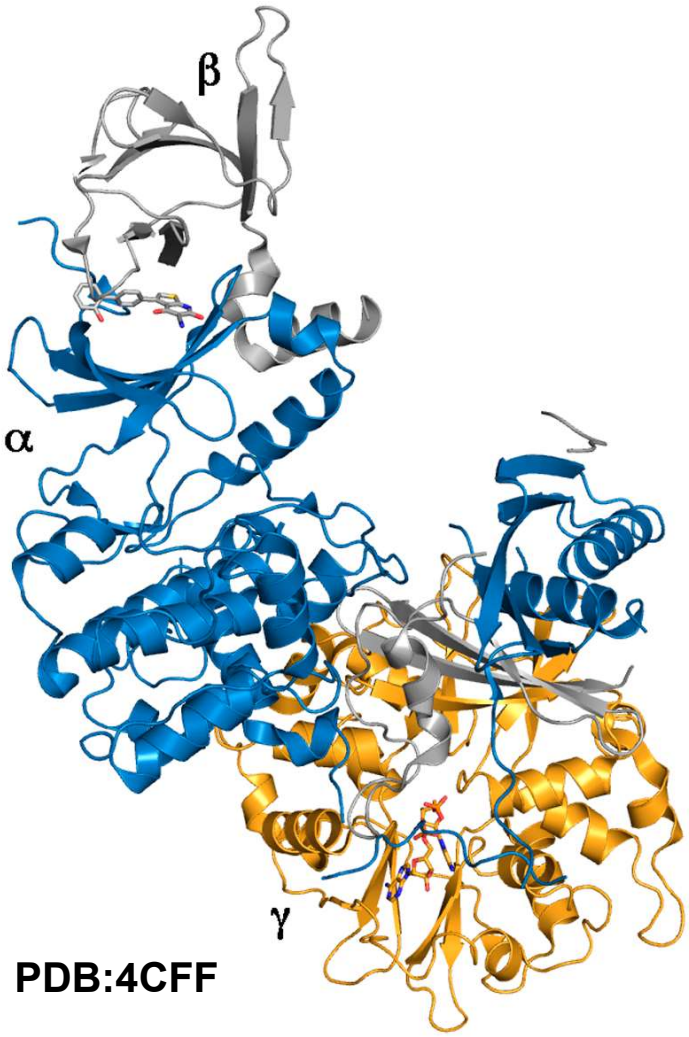
- Regulator of cellular energy homeostasis (AMP/ATP ratio);
- It switches the cellular metabolism from anabolic to catabolic mode;
- It works by phosphorylating approximately 30 well-characterized targets.

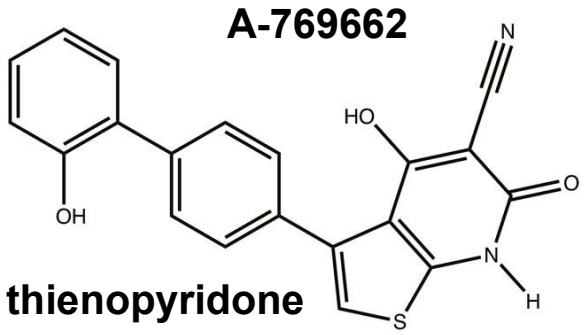


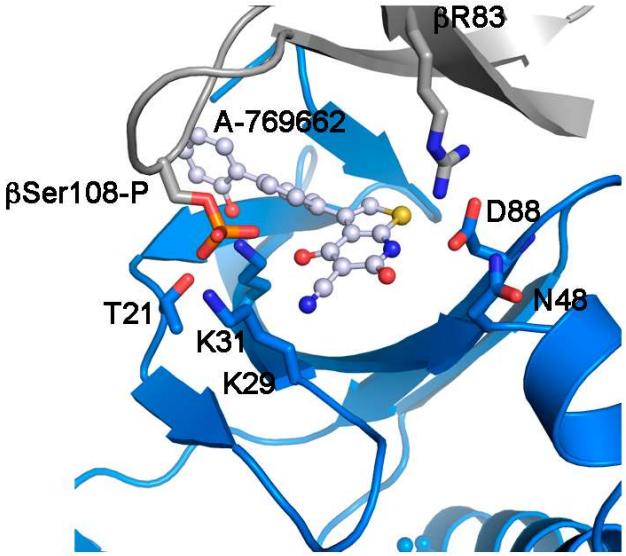
Potential therapeutic target to combat metabolic disorders such as diabetes type 2 and obesity

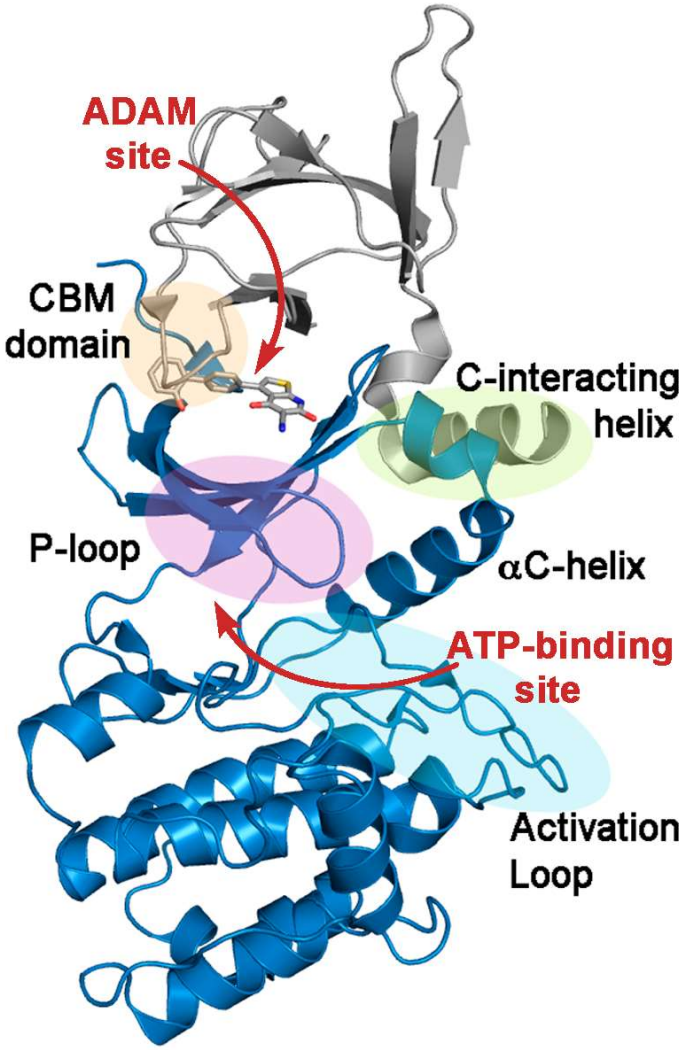
Nat Cell Biol. **2011**, *13*, 1016-23. *BMC Biology* **2013**, *11*, 36. *Chem Biol.* **2014**, *21*, 619-27

STRUCTURAL DETAILS



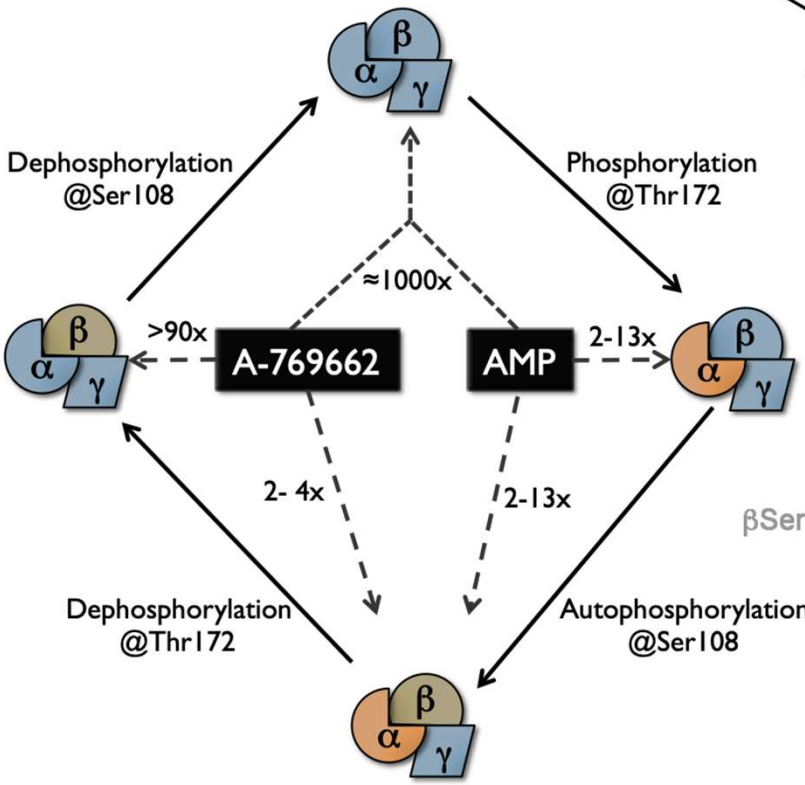


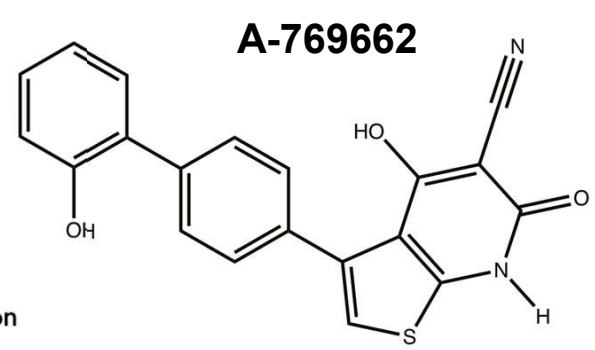


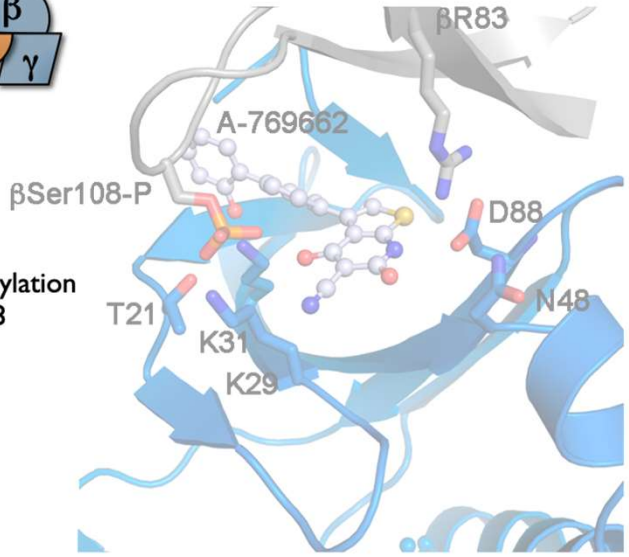


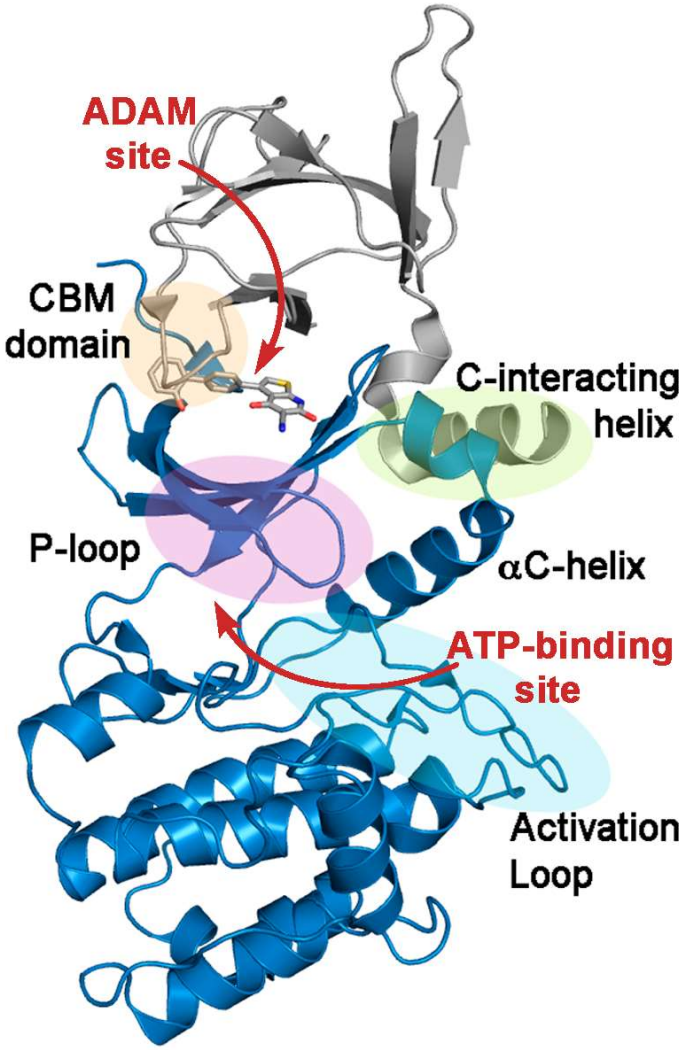
 Nat Cell Biol. **2011**, *13*, 1016-23. *BMC Biology* **2013**, *11*, 36. *Chem Biol.* **2014**, *21*, 619-27

STRUCTURAL DETAILS









Nat Cell Biol. **2011**, *13*, 1016-23. *BMC Biology* **2013**, *11*, 36. *Chem Biol.* **2014**, *21*, 619-27

OBJECTIVES

Understanding the allosteric mechanism that modulates the direct activation of AMPK by small compounds

What?

- provide a molecular basis that enables the study of other activators,
- gaining insight into the different sensitivity of AMPK isoforms, and
- improve the design of new drugs with an improved therapeutic profile against AMPK, including natural endogenous metabolites.

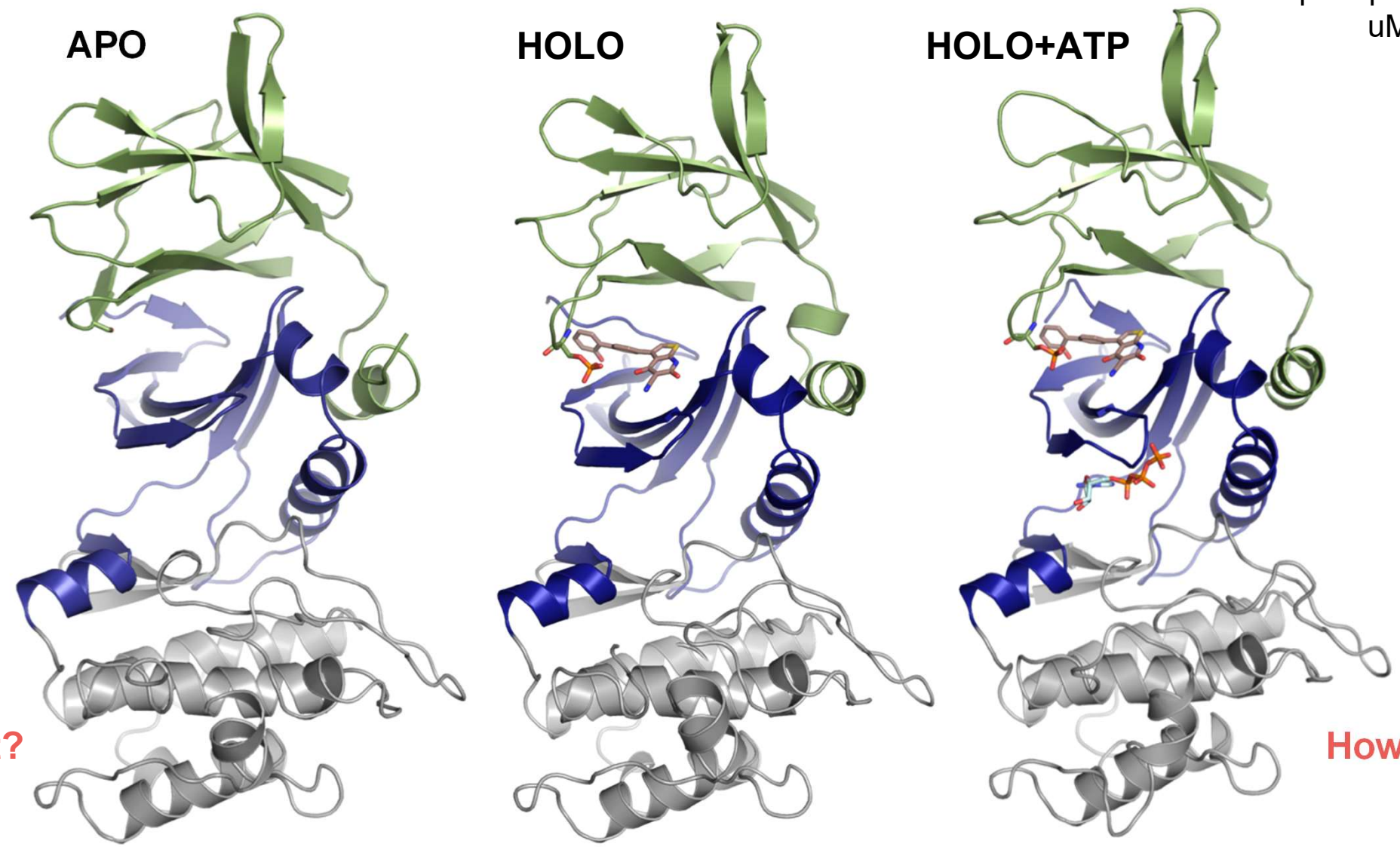
How?

- the molecular factors that govern ligand binding,
- the relationships between activator binding and structural/dynamical changes in the protein, and
- the impact of these changes in the enzyme activity.

OBJECTIVES

Understanding the allosteric mechanism that modulates the direct activation of AMPK by small compounds

3 replicas/system
1μs/replica
uMD

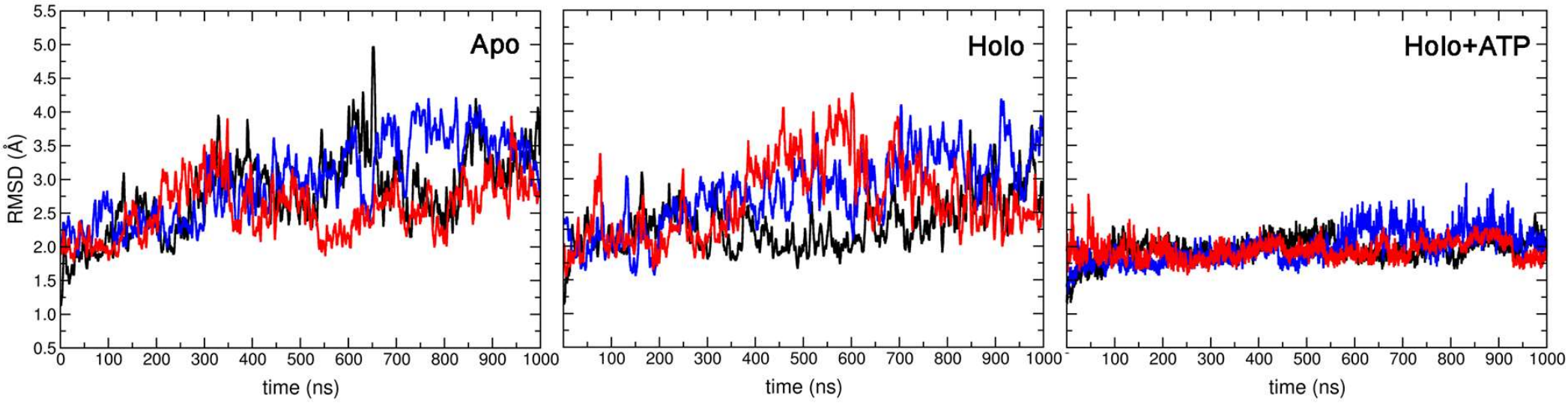


What?

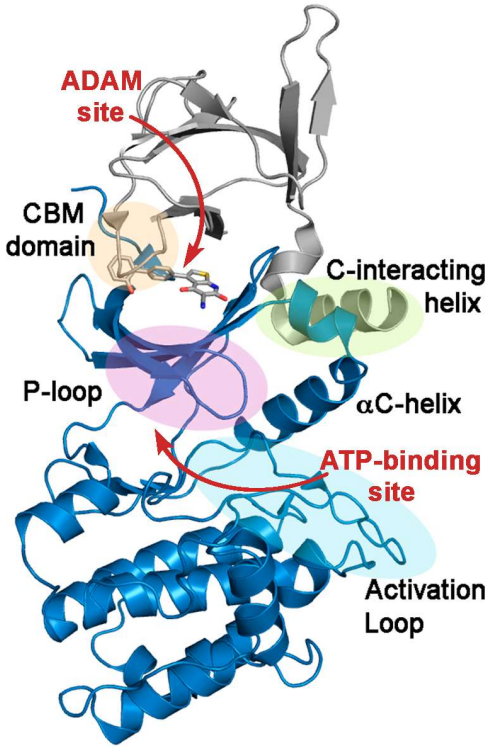
How?

RESULTS -I: Structural Stability

RMSD

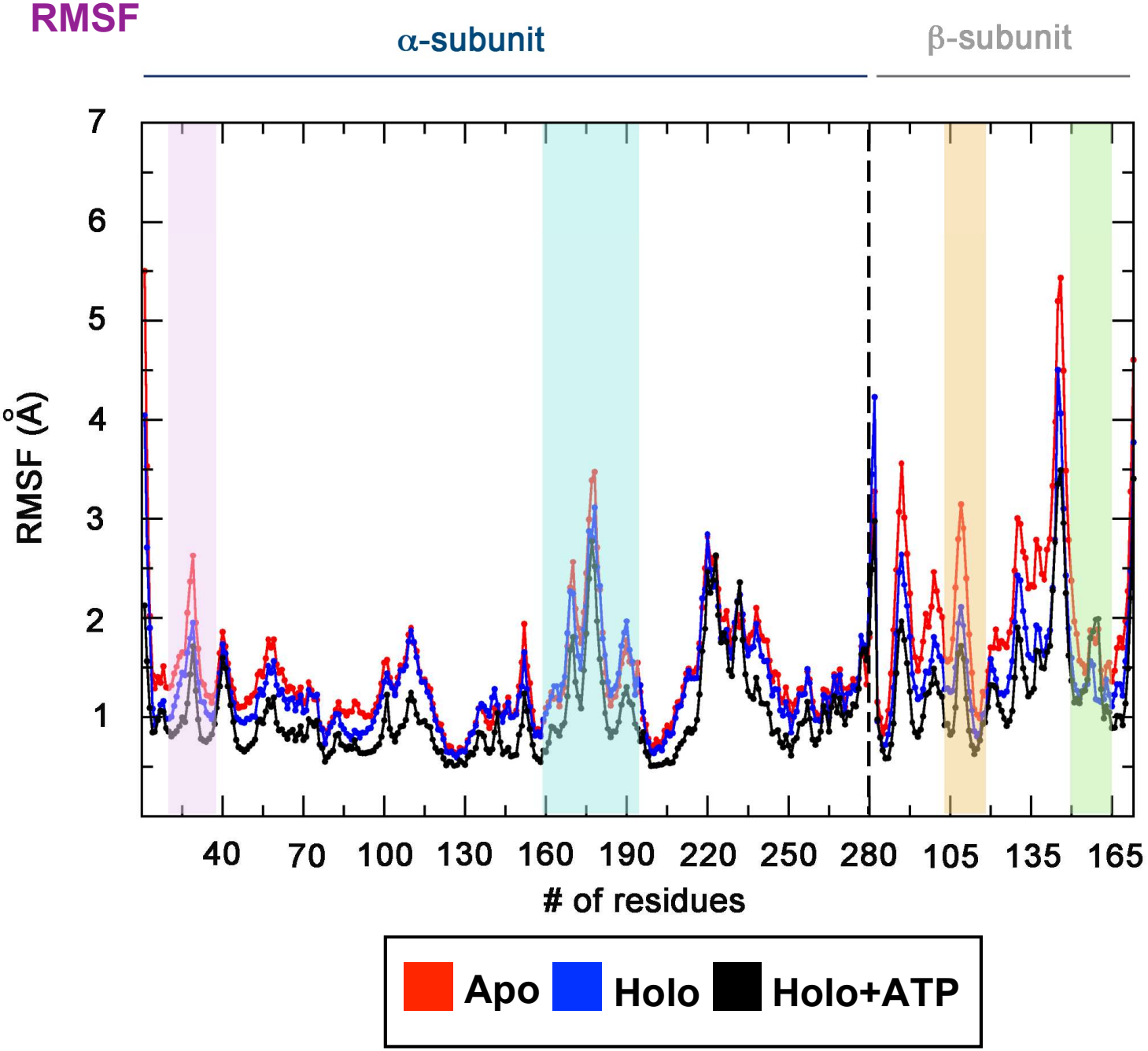


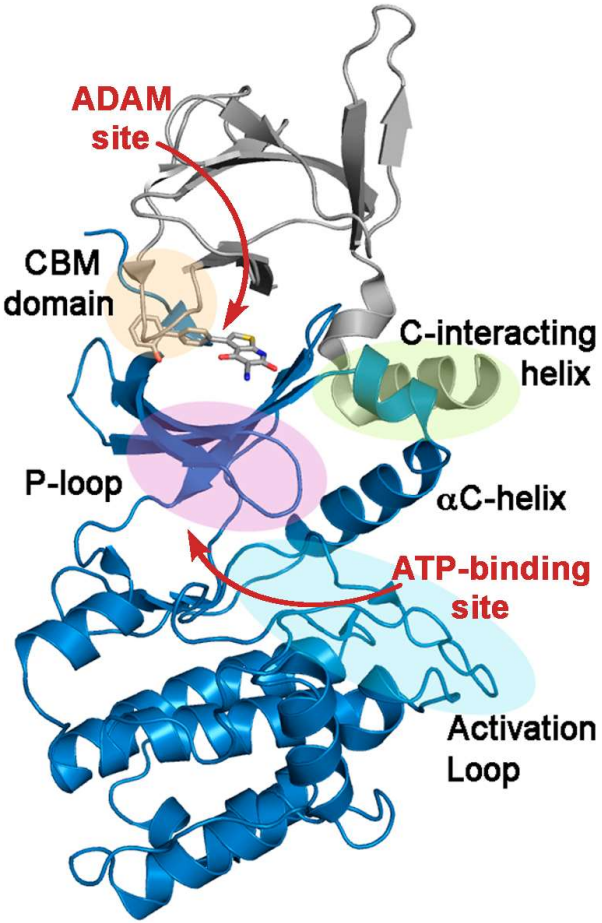
System	Replica 1 (black)	Replica 2 (blue)	Replica 3 (red)
Apo	2.5 ± 0.5	3.0 ± 0.6	2.6 ± 0.3
Holo	2.3 ± 0.4	1.9 ± 0.3	2.2 ± 0.3
Holo + ATP	1.9 ± 0.2	2.0 ± 0.3	1.9 ± 0.2



RESULTS -I: Structural Stability

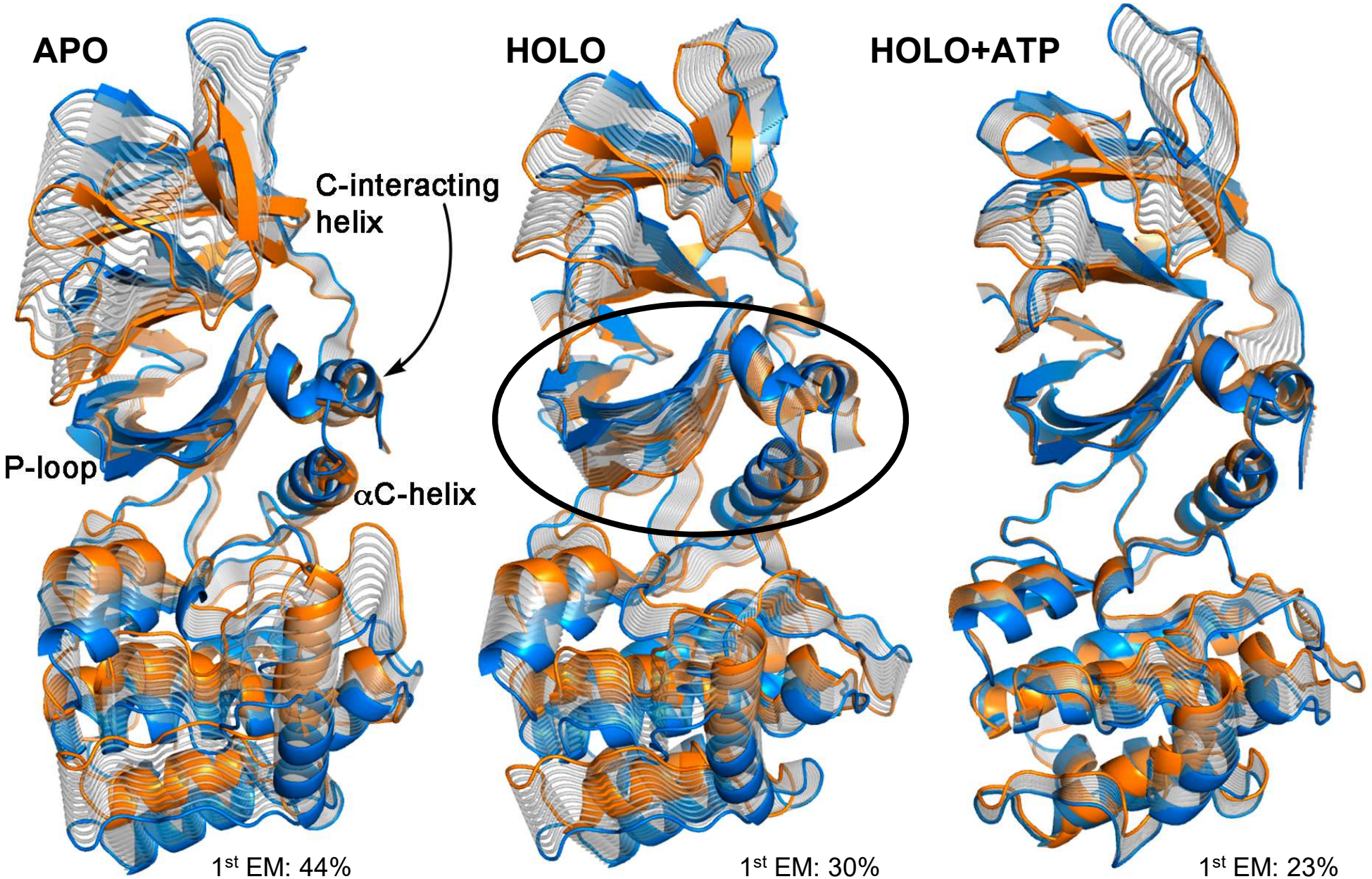
RMSF





Principal Component Analysis (PCA)

Molecular glue

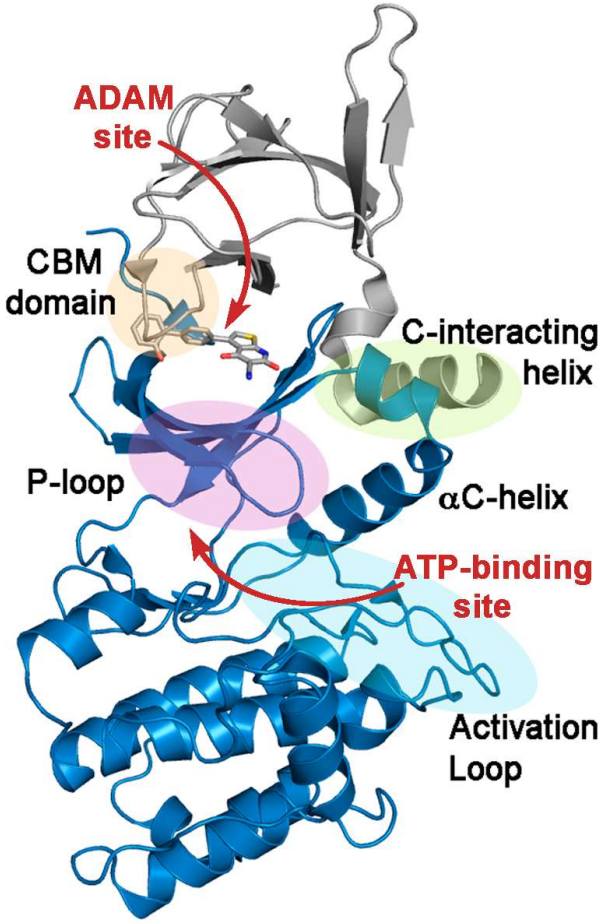


Conformational Entropy

System ($Kcal \cdot K^{-1} \cdot mol^{-1}$)	Replica 1	Replica 2	Replica 3	Mean
S_∞ apo	46.4	44.1	46.8	45.8 (1.4)
S_∞ holo	33.8	33.7	39.2	35.5 (3.1)
S_∞ holo+ATP	32.8	34.5	34.1	33.8 (0.9)
ΔS (holo − apo)	-12.6	-10.4	-7.6	-10.2
ΔS (holo+atp − apo)	-13.6	-9.6	-12.7	-12.8
ΔS (holo+atp − holo)	-1.0	0.8	-5.1	-1.8

- Backbone and Side Chains
- aa 5-360

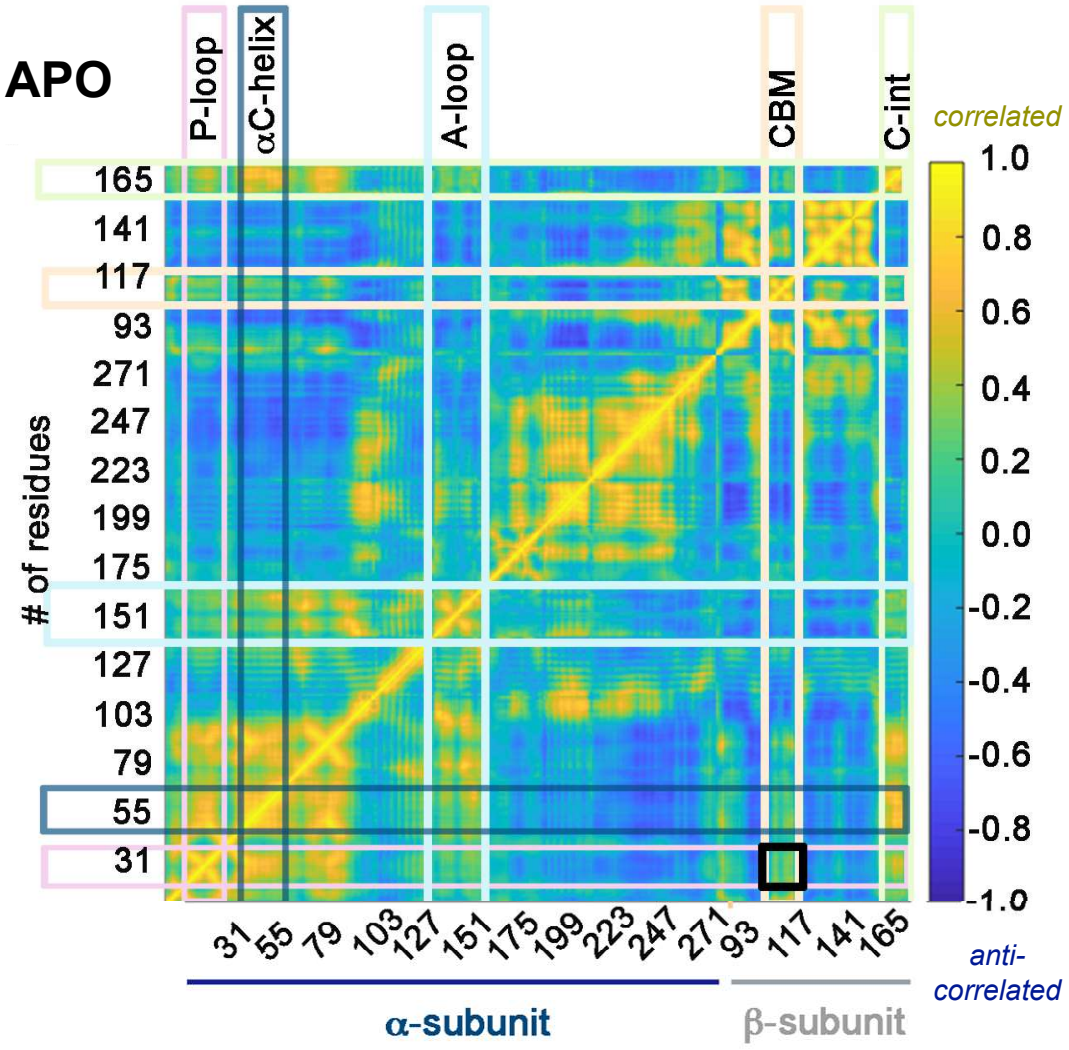
Binding of the activator has a sizable influence on the overall conformational flexibility of the protein domains

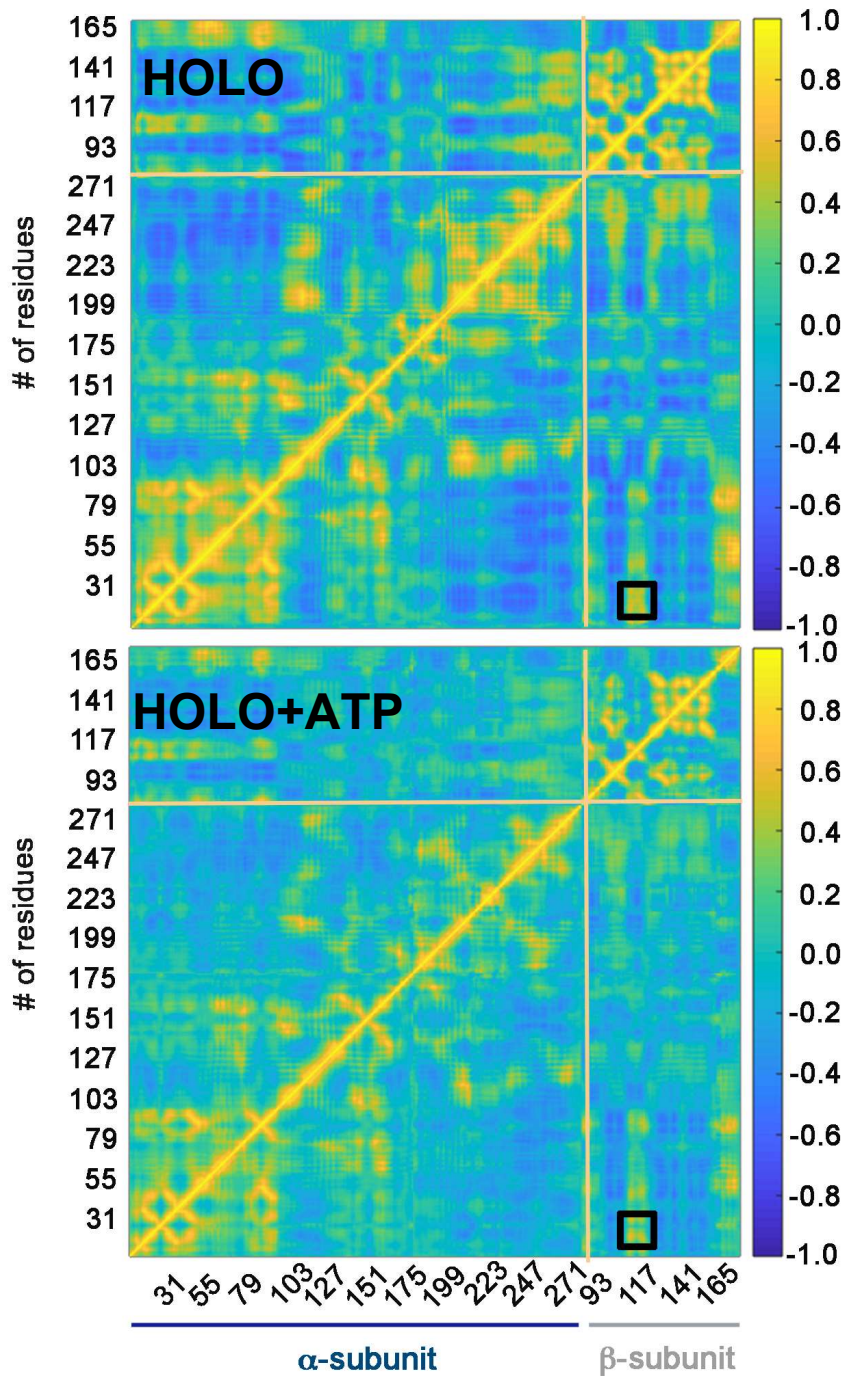


Harris, S. A.; et al. *J. Am. Chem. Soc.* **2001**, *123*, 12658−63.

RESULTS -II: Essential Dynamics

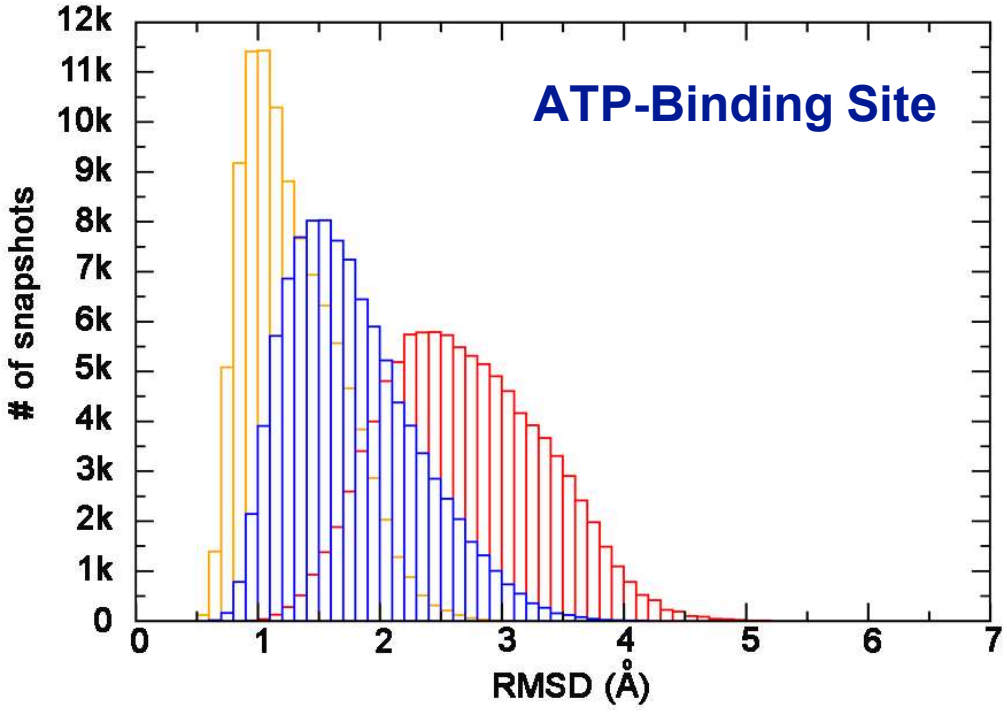
Dynamic Cross-Correlation Matrix (DCCM)

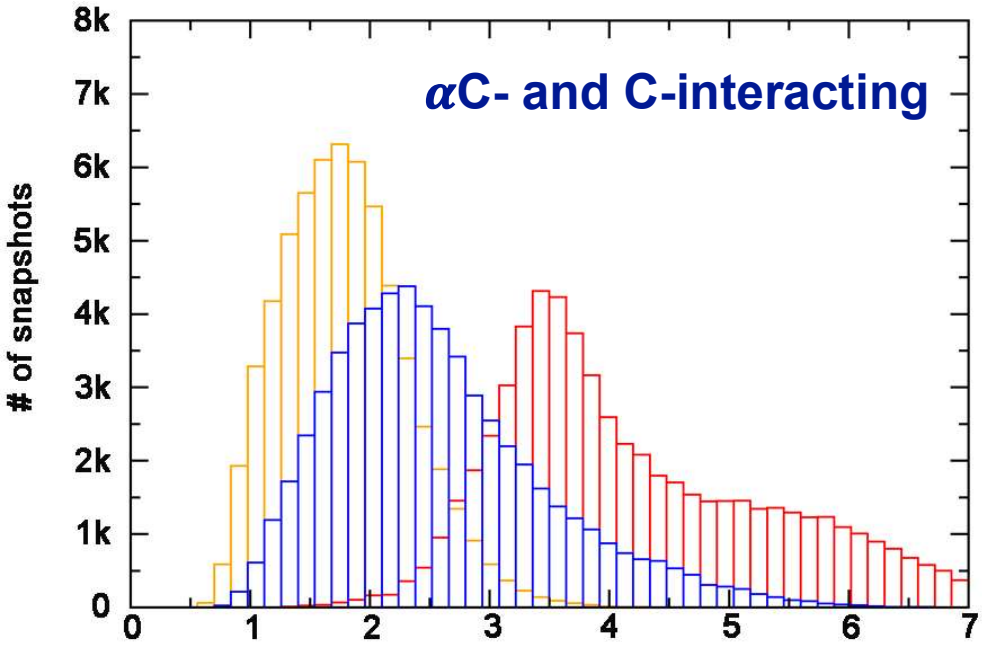


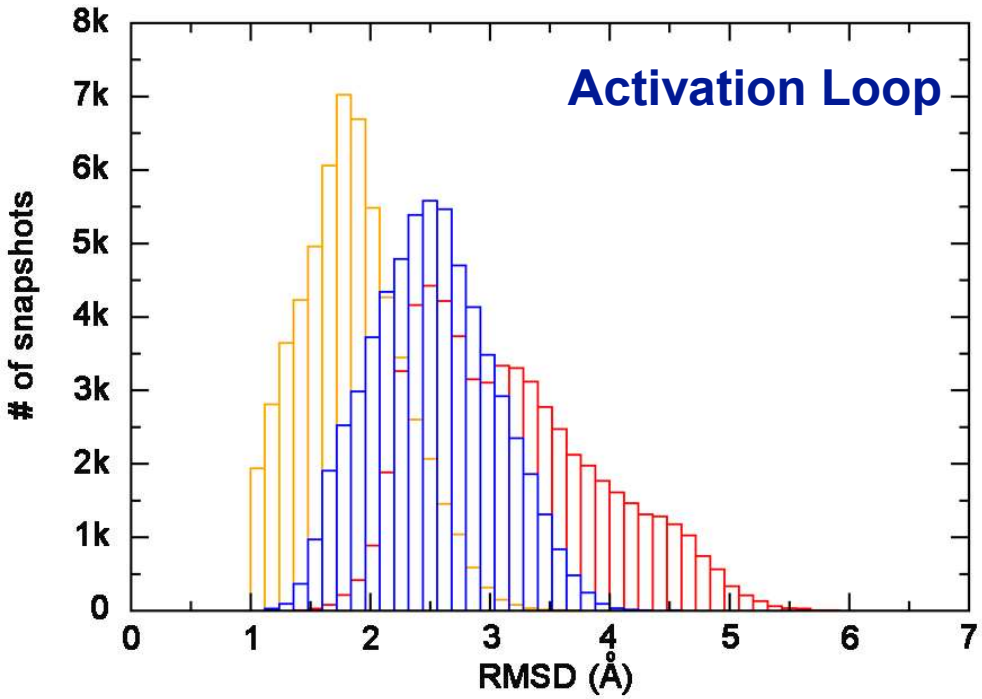


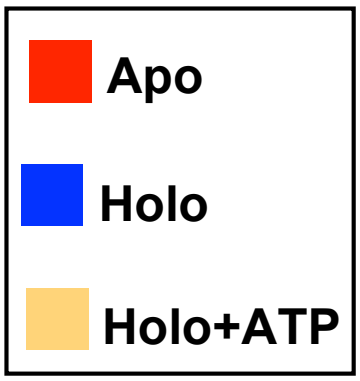
The conformational rearrangement of the P-loop may be relevant for enabling the adoption of a proper conformation well suited for recognition and binding of ATP

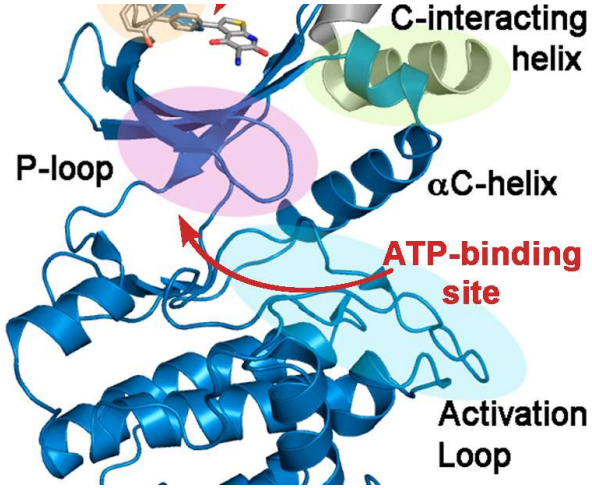
Pre-organization of ATP-binding site





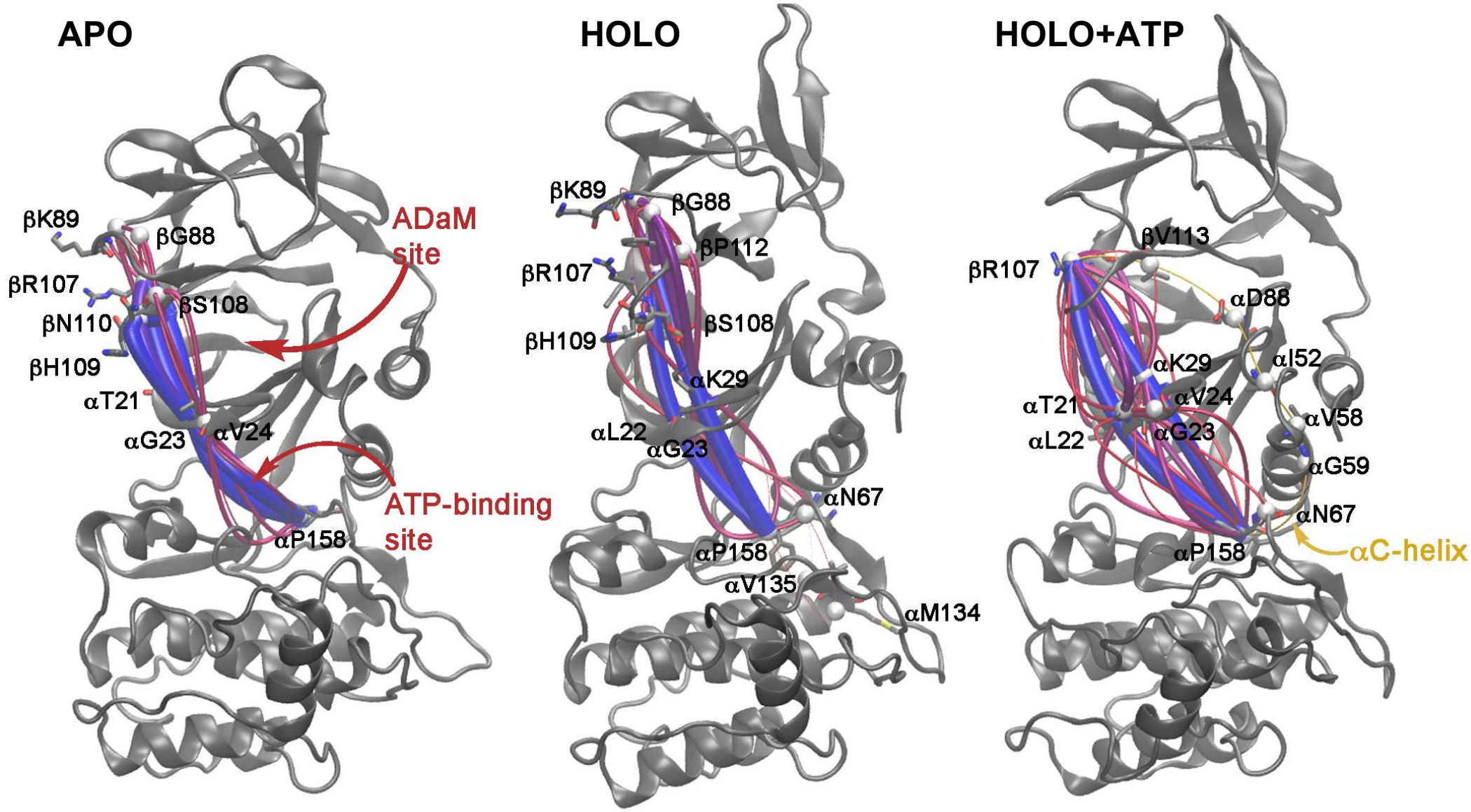






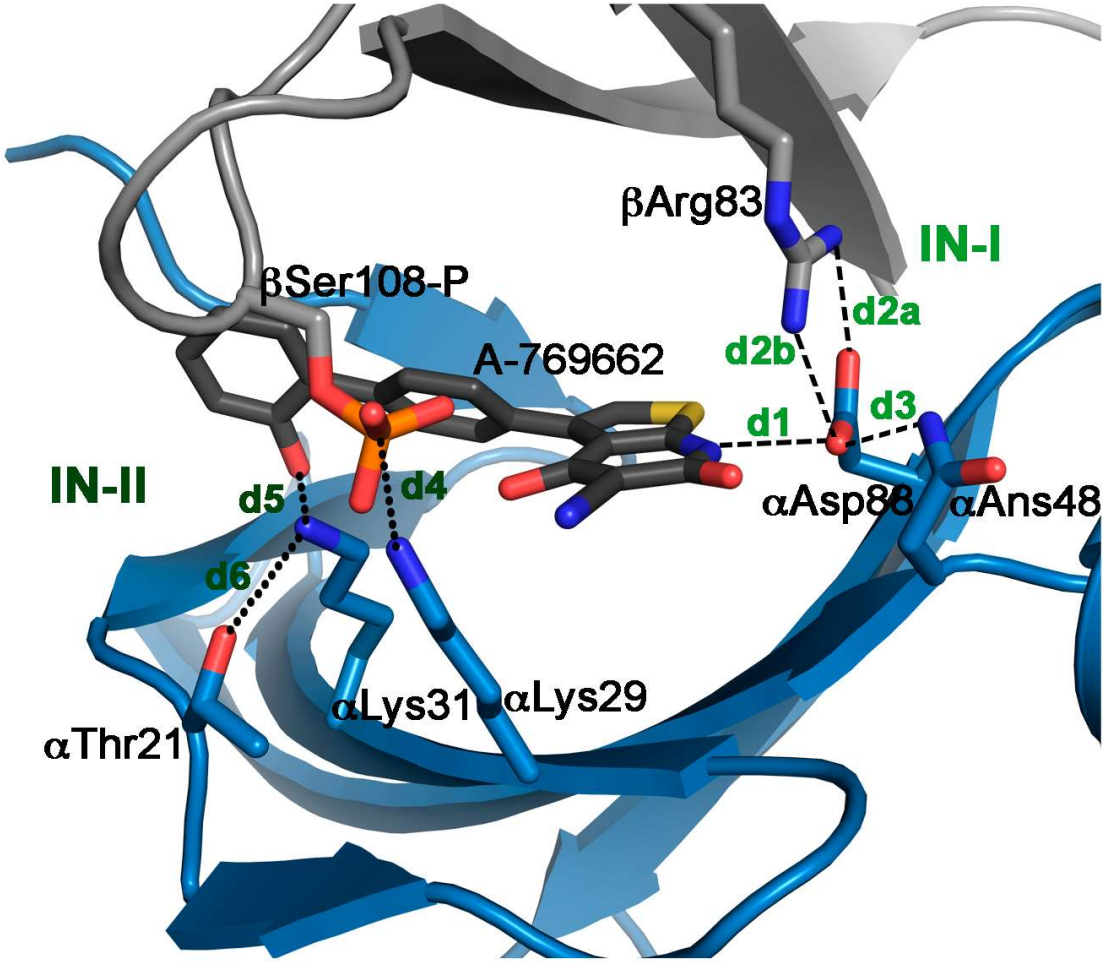
Interaction Network Analysis

How is the pre-organization of the P-loop achieved through binding of A-769662?



Van Wart, A. T.; et al. *J. Chem. Theory Comput.* **2014**, *10*, 511−517.

Interaction Network Analysis



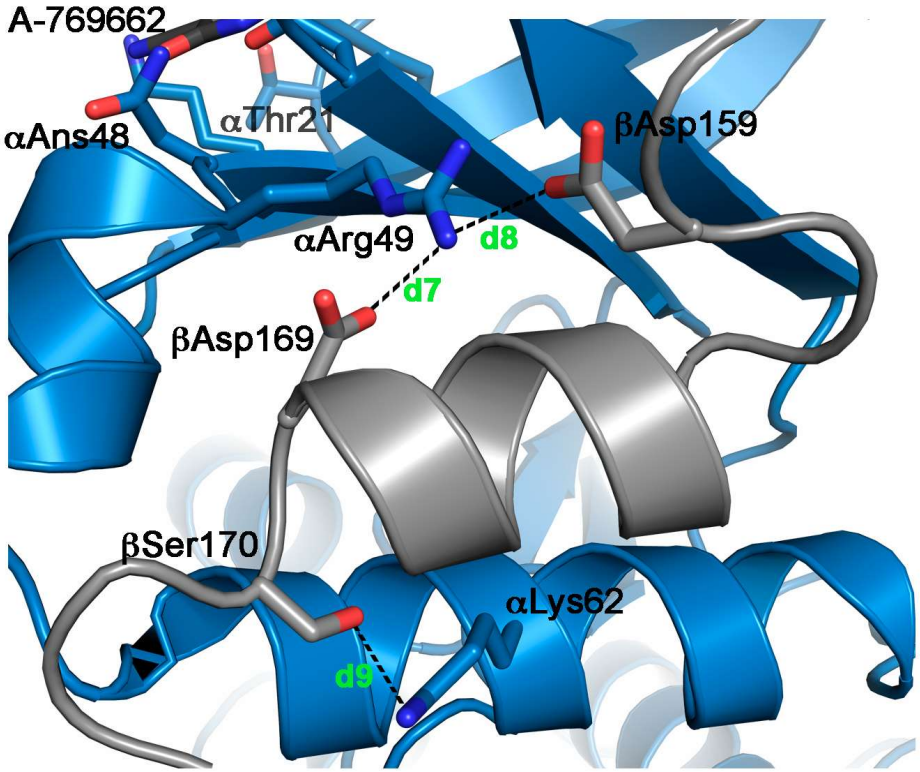
System	IN - I			
	d1	$d2_a$	$d2_b$	d3
apo	--	3.4 (0.8)	3.3 (0.8)	4.3 (1.4)
holo	4.4 (0.2)	2.9 (0.2)	2.8 (0.2)	3.9 (0.4)
holo+ATP	2.8 (0.1)	2.8 (0.1)	2.9 (0.1)	3.0 (0.4)

System	IN - II		
	d4	d5	d6
apo	--	--	3.8 (0.9)
holo	3.8 (0.6)	3.8 (0.5)	3.0 (0.3)
holo+ATP	3.5 (0.3)	4.0 (0.5)	2.9 (0.2)

Xiao, B.; et al. *Nat.Commun.* **2013**, *4*, 3017.

Interaction Network Analysis

Altogether can be viewed as a mechanism to translate the binding energy into changes in the protein dynamics that should facilitate the AMPK enzymatic catalysis



System	IN - III		
	d7	d8	d9
apo	3.6 (0.8)	3.5 (0.7)	9.3 (1.8)
holo	3.7 (0.4)	5.6 (1.2)	5.3 (2.0)
holo+ATP	3.9 (0.2)	5.4 (1.1)	4.6 (1.6)

J|A|C|S JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Cite This: J. Am. Chem. Soc. 2019, 141, 3320−3331
Perspective
pubs.acs.org/JACS

Protein Flexibility and Stiffness Enable Efficient Enzymatic Catalysis

John P. Richard*

"The existence of many enzymes in flexible, entropically rich, and inactive ground states provides a mechanism for utilization of ligand-binding energy to mold these catalysts into stiff and active forms"

CONCLUSIONS - I

1.- The presence of the activator affects the protein flexibility, regulating the shape and the size of the ATP-binding pocket, which tends to adopt a topology well suited for ATP binding.

Thus, it can be hypothesized that the ***activator might act as a glue***

2.- To the best of our knowledge, this **specific binding cavity** is not found in other kinases, which makes the therapeutic value of AMPK to highly attractive.

3.- The structural analysis has disclosed **key residues** required for the formation of the **allosteric network that connects the ADaM and ATP-binding** sites through interactions with the A-769662 ligand.

αAsp88 - βArg83 and **βpSer108 - αLys29**

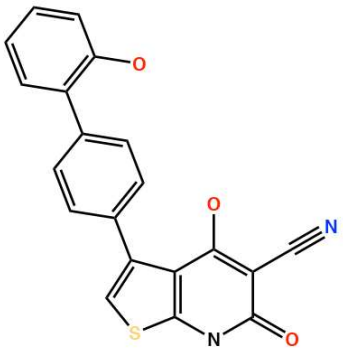
4.- *The understanding of the direct activation mechanism of AMPK opens **new opportunities** not only **for the rational development** of small compounds that might **modulate the activity of specific isoforms** of this cellular energy sensor in different tissues but also for selecting guidelines that enable the **screenings of endogenous metabolites**.*

WORK IN PROGRESS

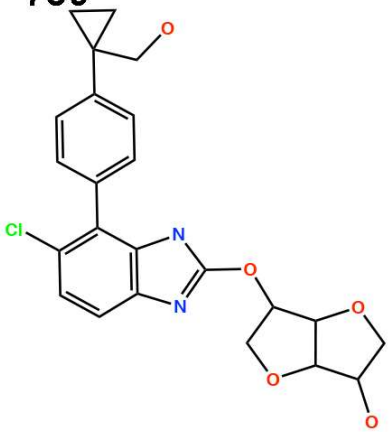
- Why A-769662 is only active in the α2β1γ1 isoform, while compound 991 is also active in β2 AMPK complexes?

- This molecular mechanism may underlie the activity of other direct activators, such as compounds PF-739, 991, and SC4?

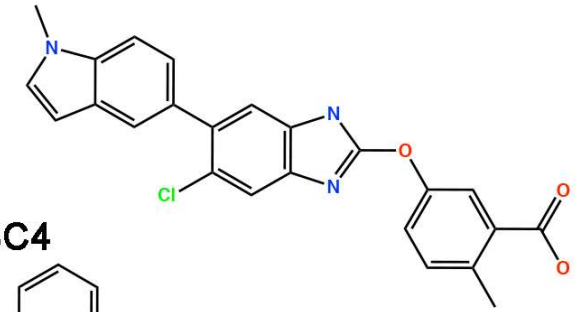
A-769662



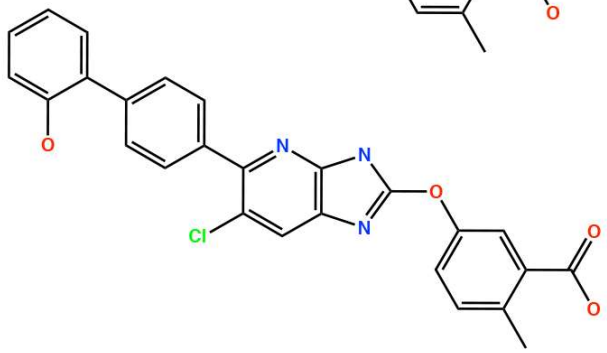
PF-739

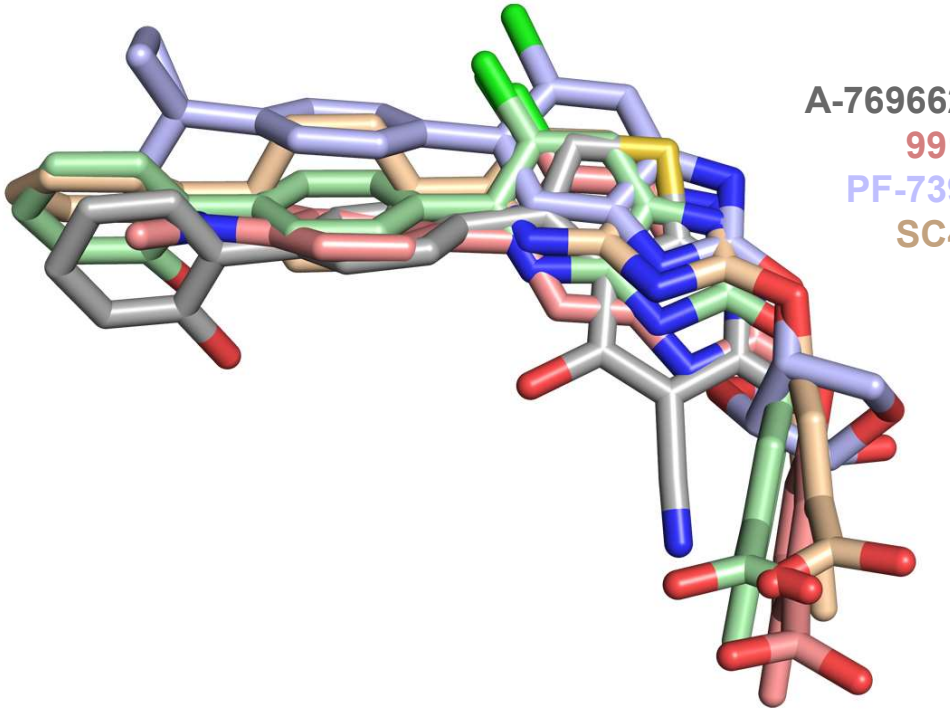


991



SC4

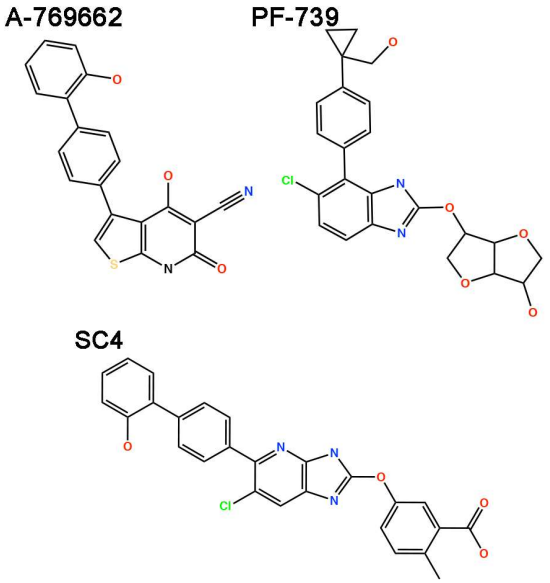




Ligands	A-769662 (2006, Abbot)[1,2]		SC4 (Academia, 2018)[3]		PF-739 (Pfizer, 2017)[4]
Isoforms	K_D (μM)	Activation fold	EC_{50}(nM)	Activation fold	EC_{50}(nM)
α2β1γ1	0.40 (0.15)	14.3 (0.4)	—	—	5.23
α2β2γ1	NI	NA	17.2 (1.6)	2.5 (0.1)	42.4

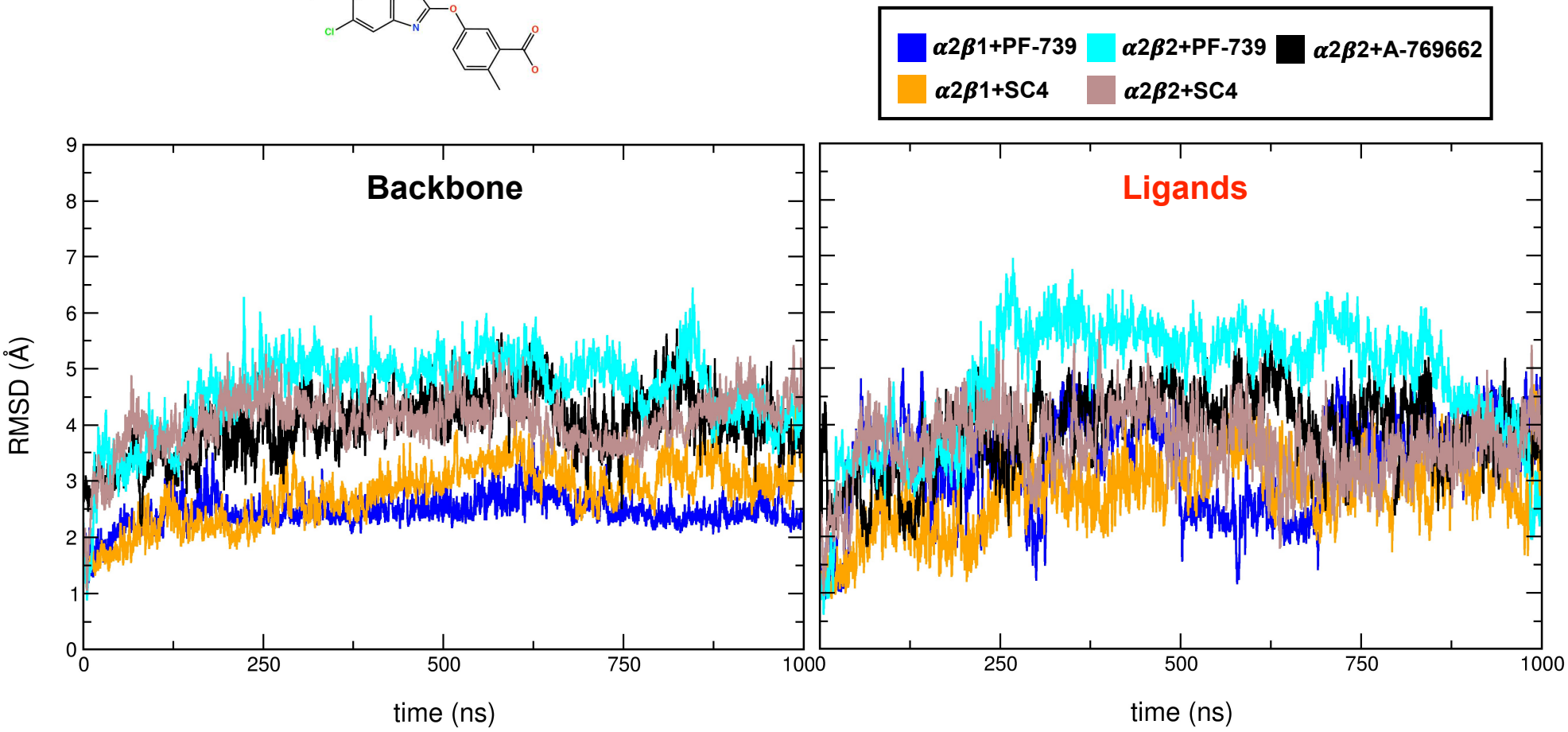
[1] Scott, J.W.; et al. *Chem. Biol.* **2014**, *21*, 619. [2] Xiao, B.; et al. *Nature Commun.* **2013**, *4: 3017.* [3] Ngoei, K.R.W.; et al. *Cell Chem. Biol.* **2018**, *25*, 728. [4] Cokorinos, E.C.; et al. *Cell Metabolism* **2017**, *25*, 1147.

WORK IN PROGRESS



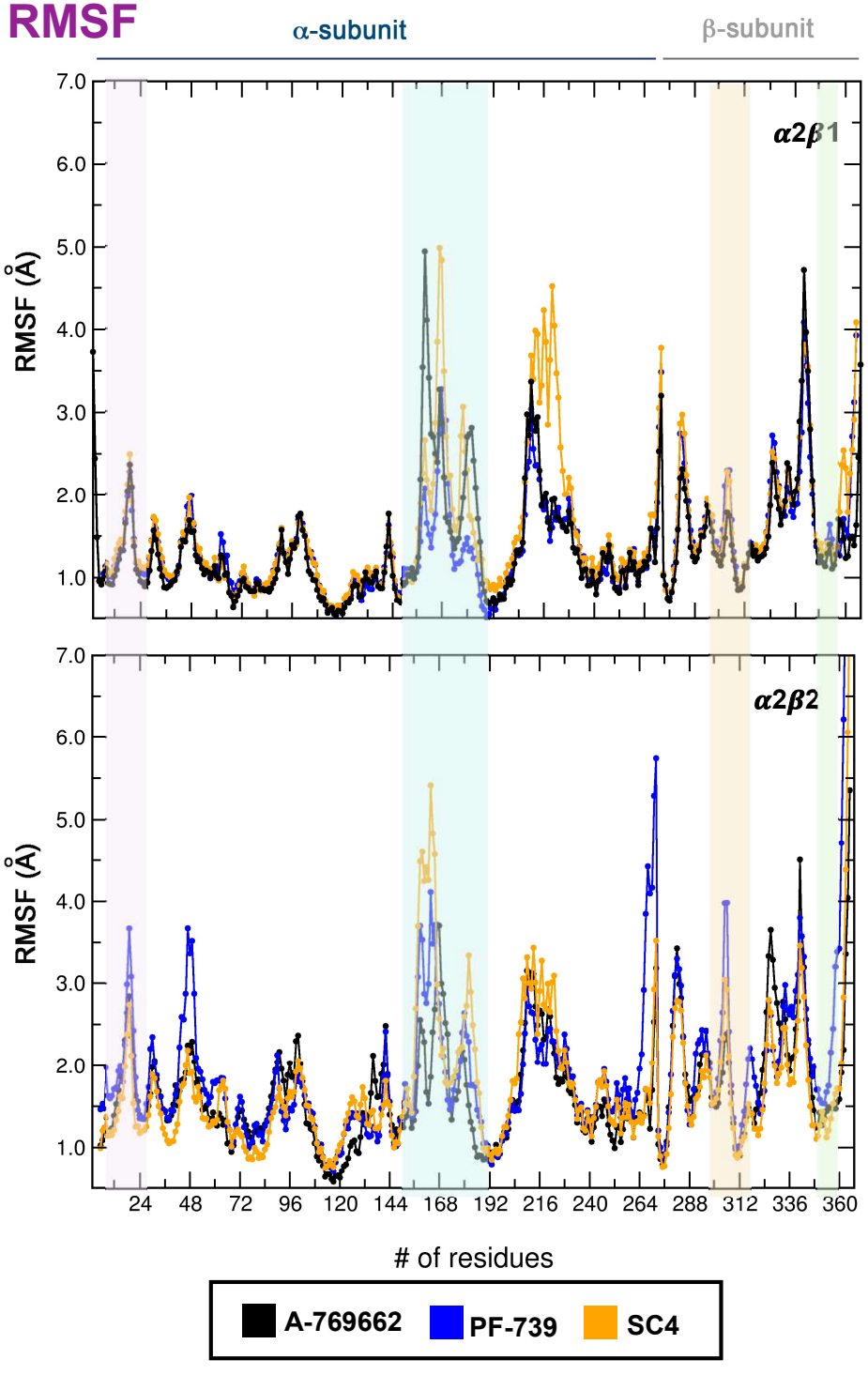
RMSD

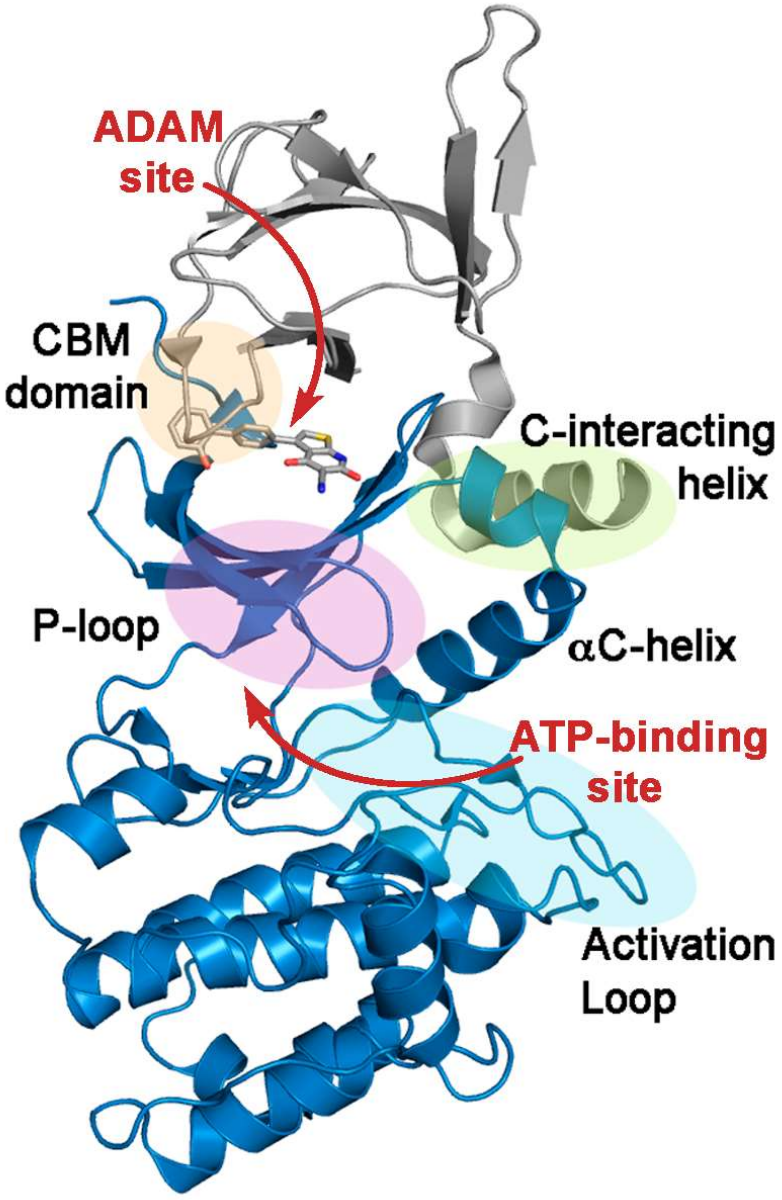
System	A-769662	SC4	PF-739
$\alpha 2\beta 1$	2.3 ± 0.4	2.8 ± 0.6	2.4 ± 0.3
$\alpha 2\beta 2$	4.0 ± 0.6	4.6 ± 0.7	4.1 ± 0.5



WORK IN PROGRESS

RMSF

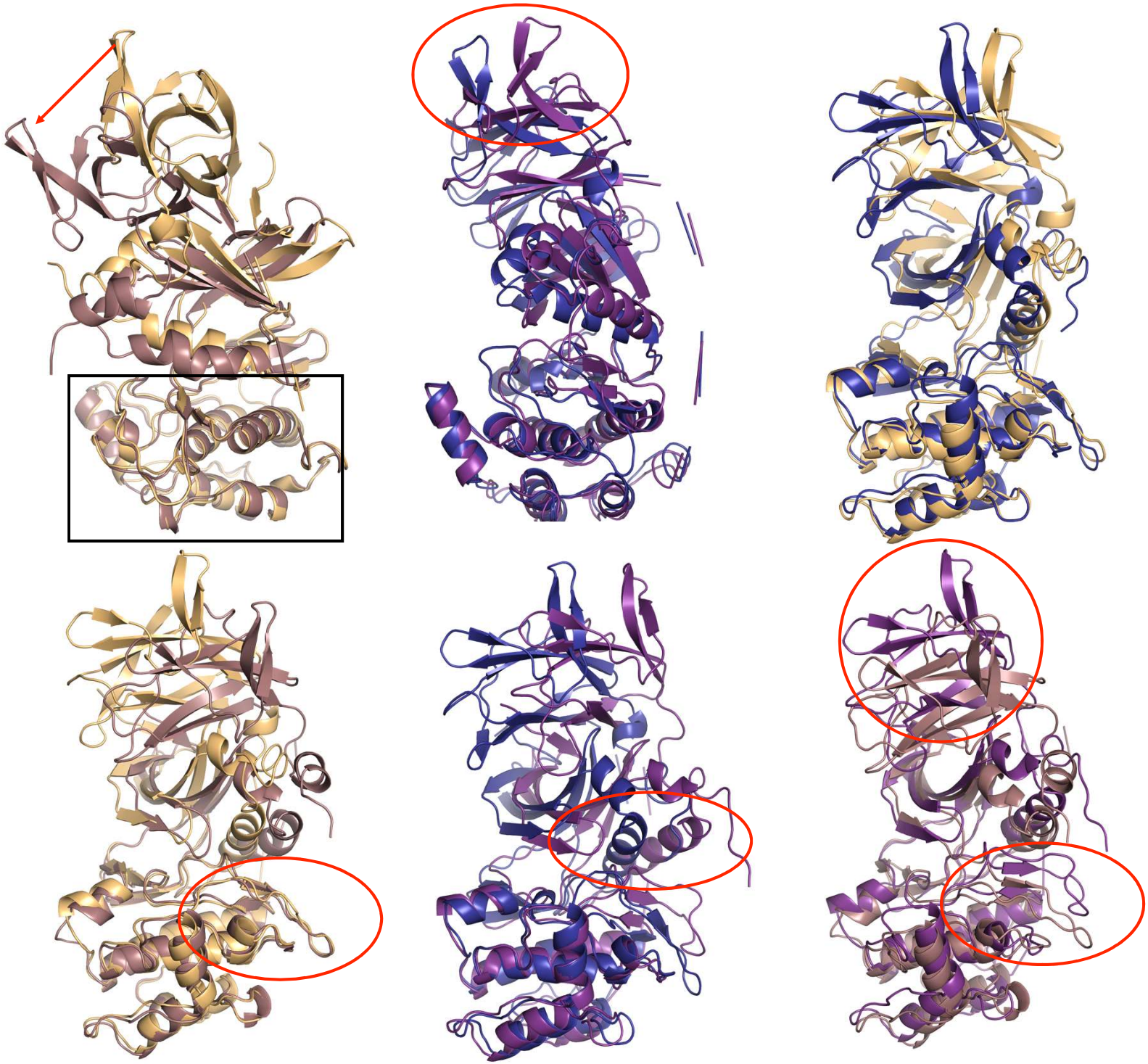




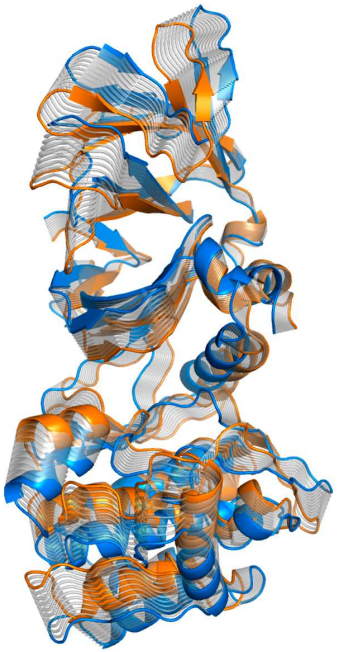
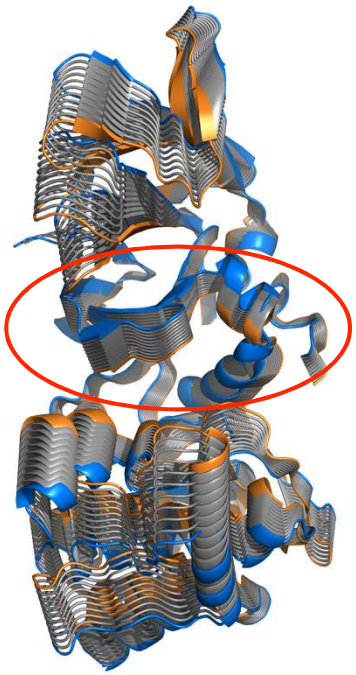
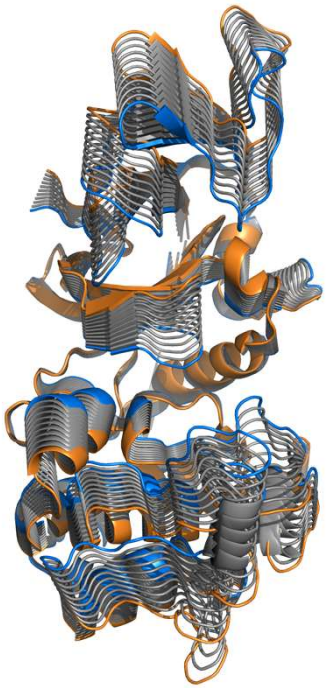
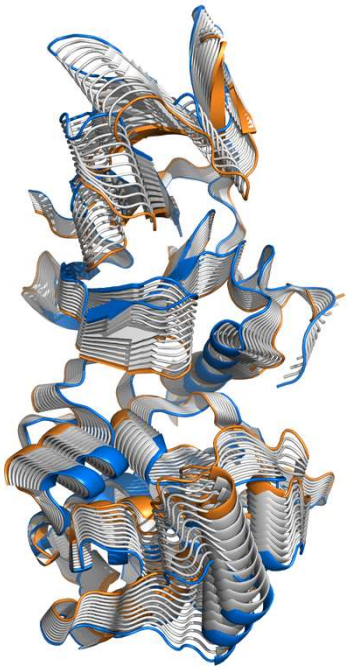
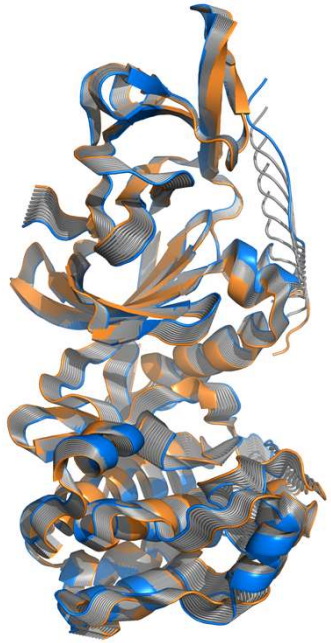
WORK IN PROGRESS

PF-739

- $\alpha 2\beta 1$_INITIAL
- $\alpha 2\beta 1$_after 500ns
- $\alpha 2\beta 2$_INITIAL
- $\alpha 2\beta 2$_after 500ns



Principal Component Analysis (PCA)

	A-769662	PF-739	SC4
$\alpha 2\beta 1$			
$\alpha 2\beta 2$			

CONCLUSIONS - II & FUTURE WORK

1.- The isoform of subunit **β- has higher impact** in the binding **than the isoform of α-** subunits as identified by the collection of available experimental data (data for α1 isoform not shown).

2.- The systems with **isoform β1 are more stable than β2**, as shown in RMSD analysis.

This fact can be also attributed to the high conformational change observed in β2 isoforms which involve changes in main moieties like αC and C-interacting helices, Activation loop or P-loop

3.- The hypothesis that the ***activator might act as a glue***, filling the ADaM binding pocket, making an effective connection between β- and α- subunits, favoring the binding of ATP, and explaining the increase of the AMPK activity, is also supported by **PF-739**.

However, still there exist several factors that we need to consider to fully understand the activation mechanism by the variety of ligands presented and the interaction with the different isoforms, like:

- *Could be the conservative mutations in ADaM the responsible of the difference between α2β1 and α2β2?*
- *Could the A-loop has an important role in the binding of the ligand in α2β2 but not in α2β1 complexes?*

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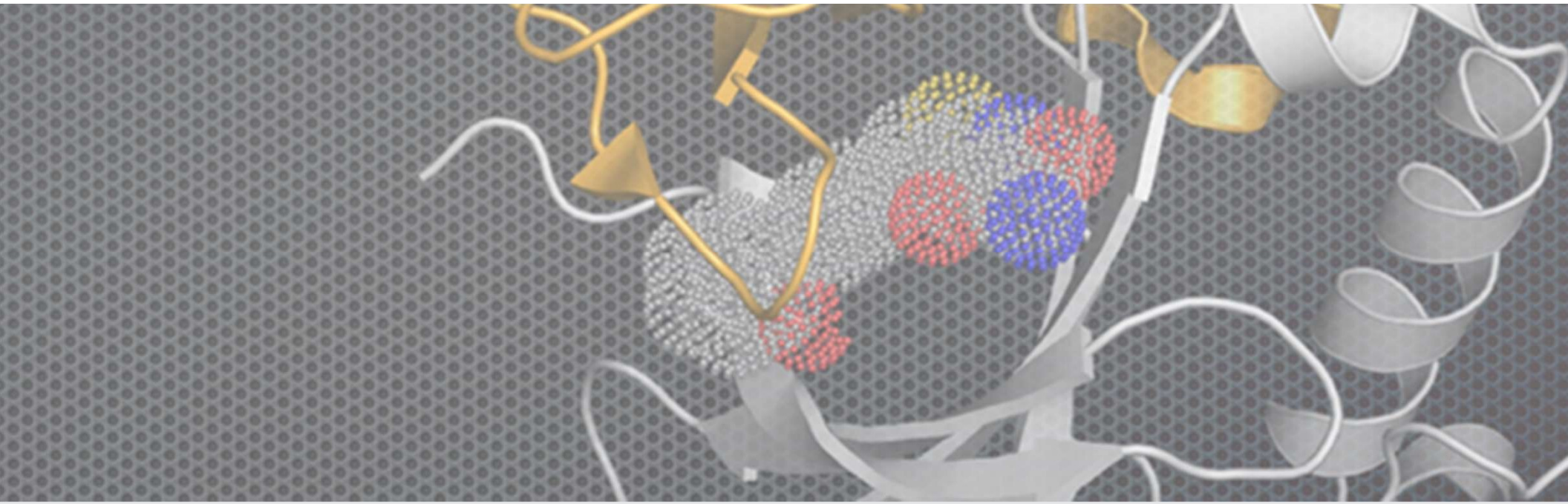

UNDERSTANDING THE MECHANISM OF DIRECT ACTIVATION OF AMPK :

TOWARD A FINE ALLOSTERIC TUNING OF THE KINASE ACTIVITY

THANK YOU FOR YOUR ATTENTION!