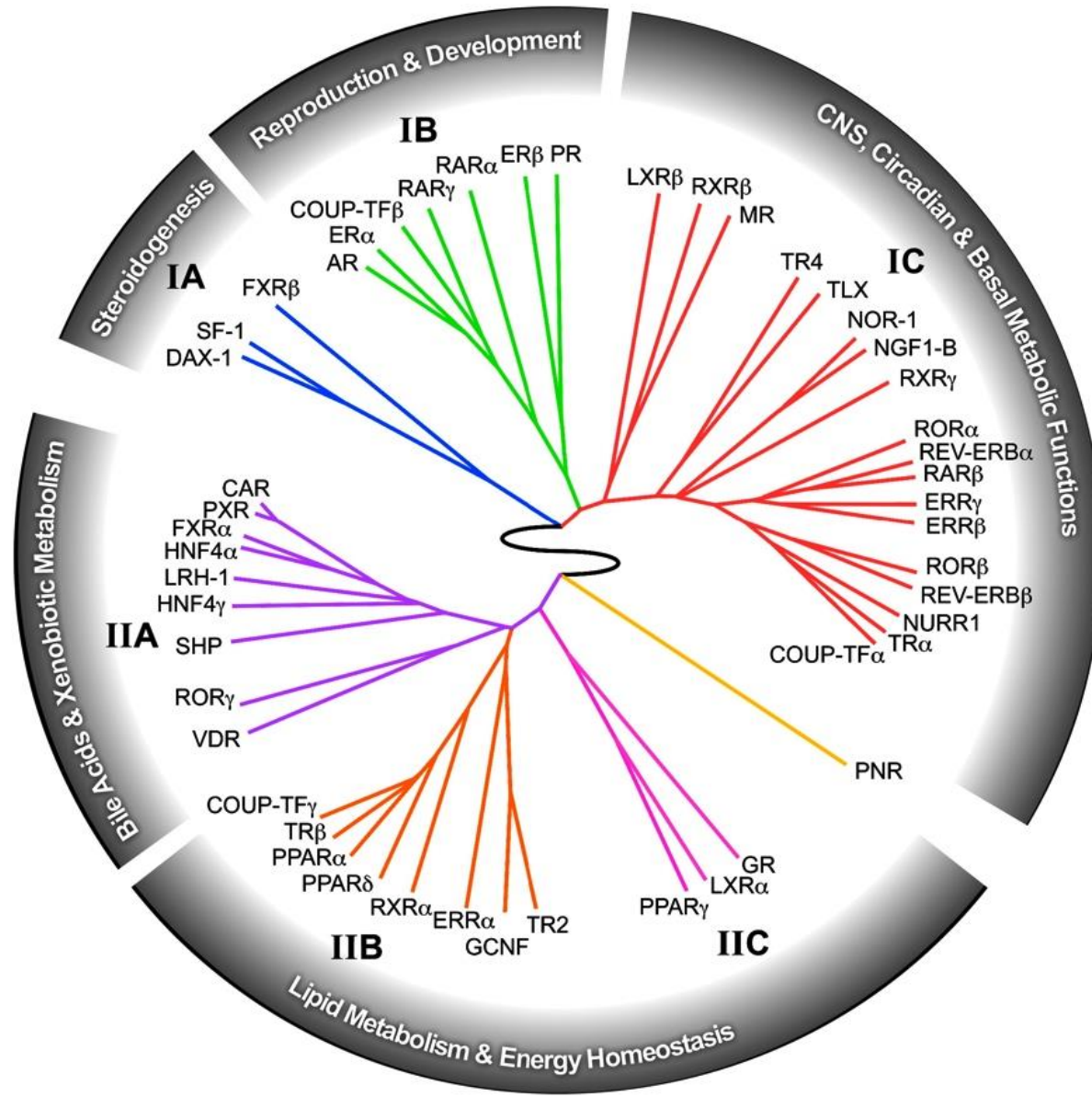
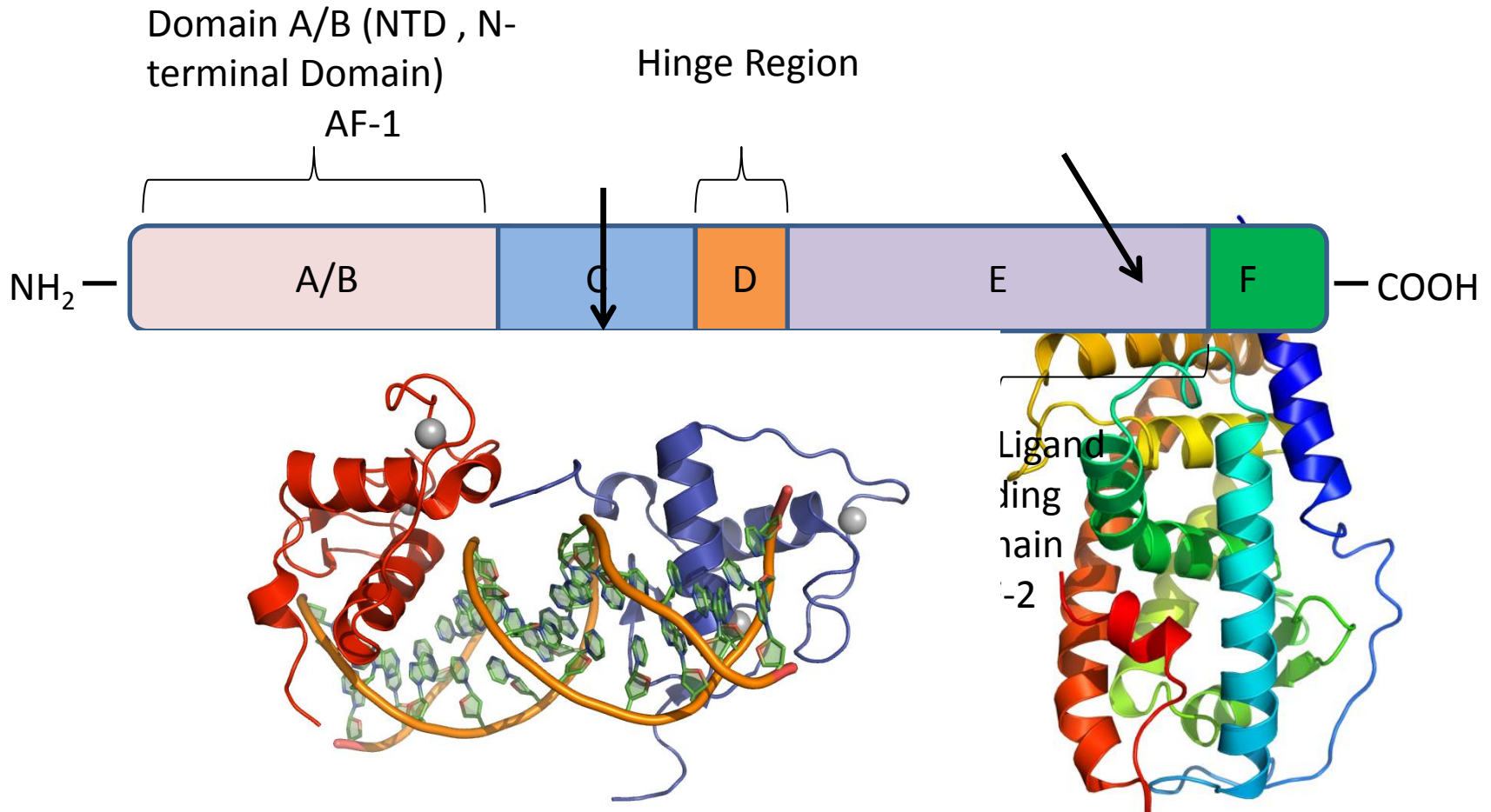


# "Mechanisms of allosteric regulation in nuclear receptor proteins elucidated by molecular dynamics simulations"

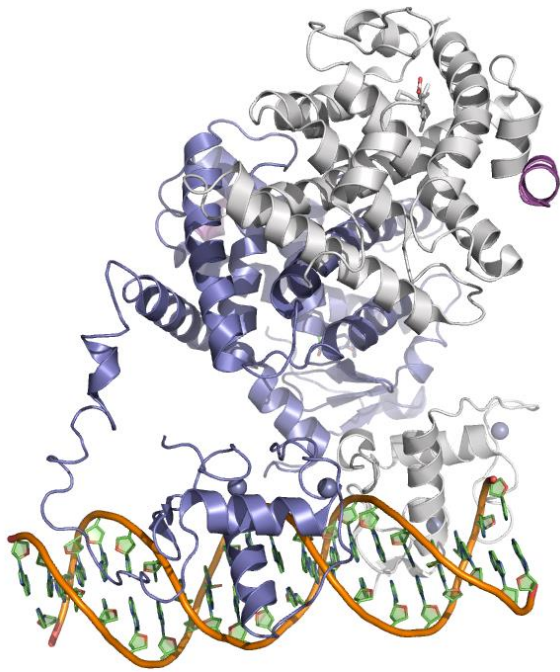
# NRs govern the expression of a broad range of genes



# Structure of the retinoic acid receptor

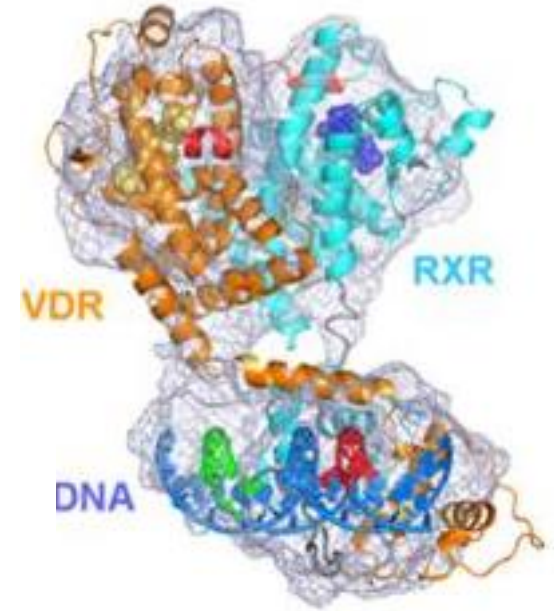
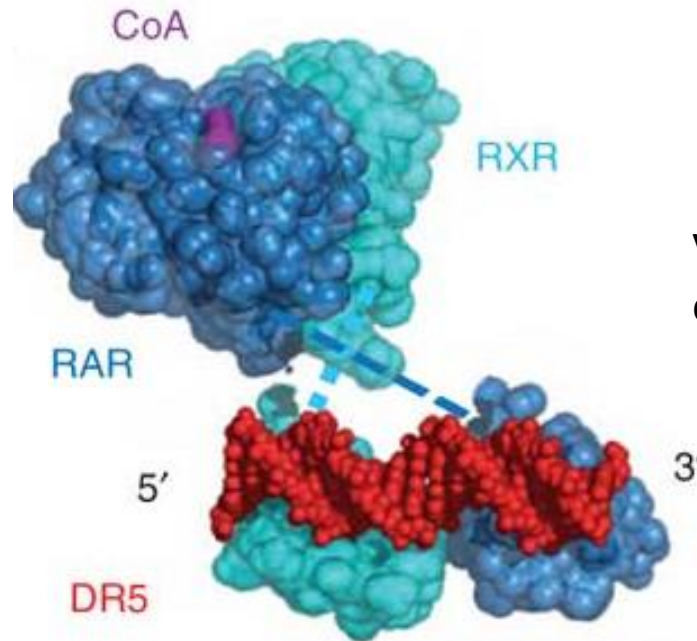


# Structures of full length nuclear receptors



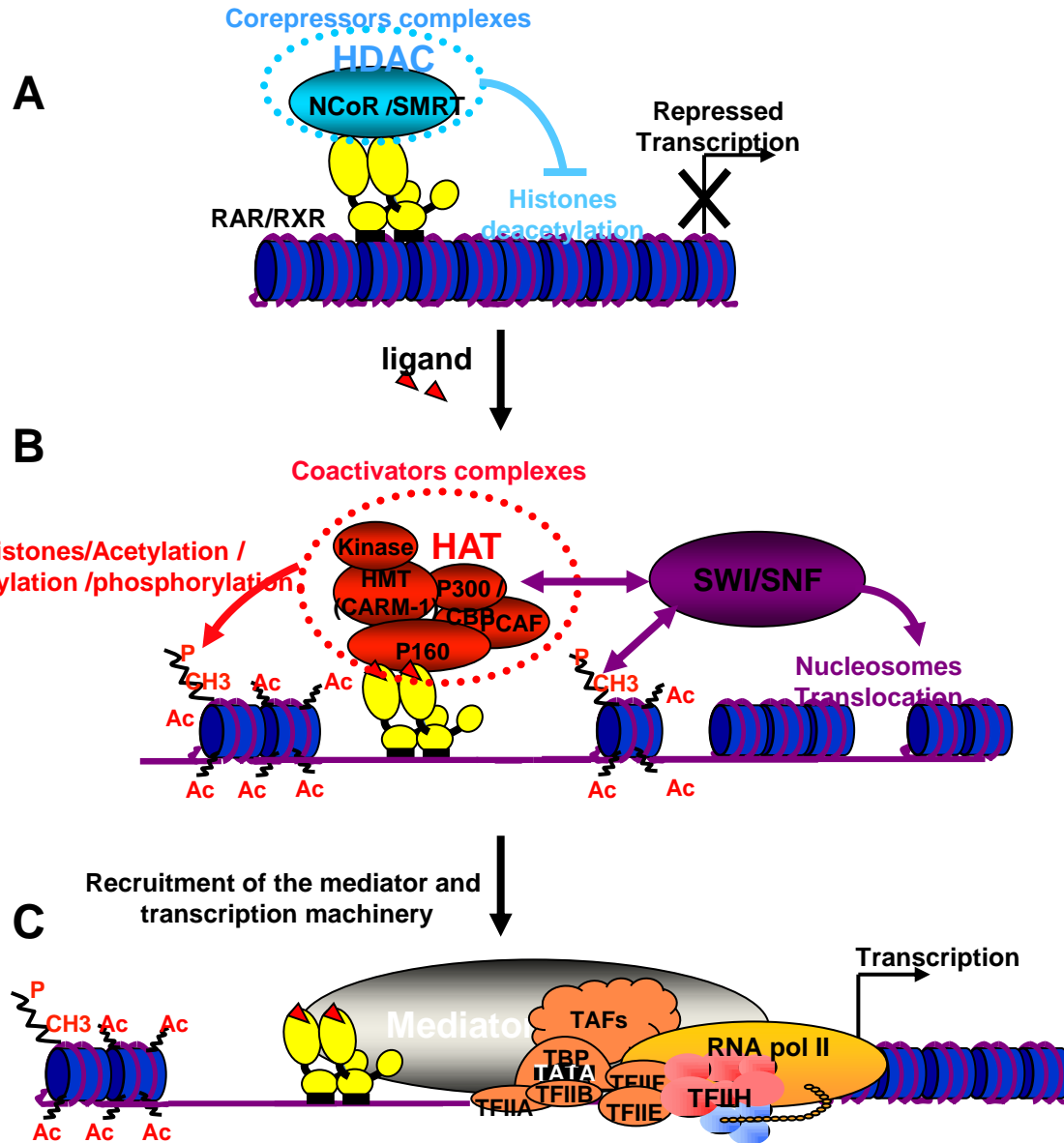
PPAR/RXR (X-ray diffraction) :  
Chandra V et al, 2008

PPAR/RXR, VDR/RXR, RAR/RXR  
(SAXS) : Rochel N et al, 2011



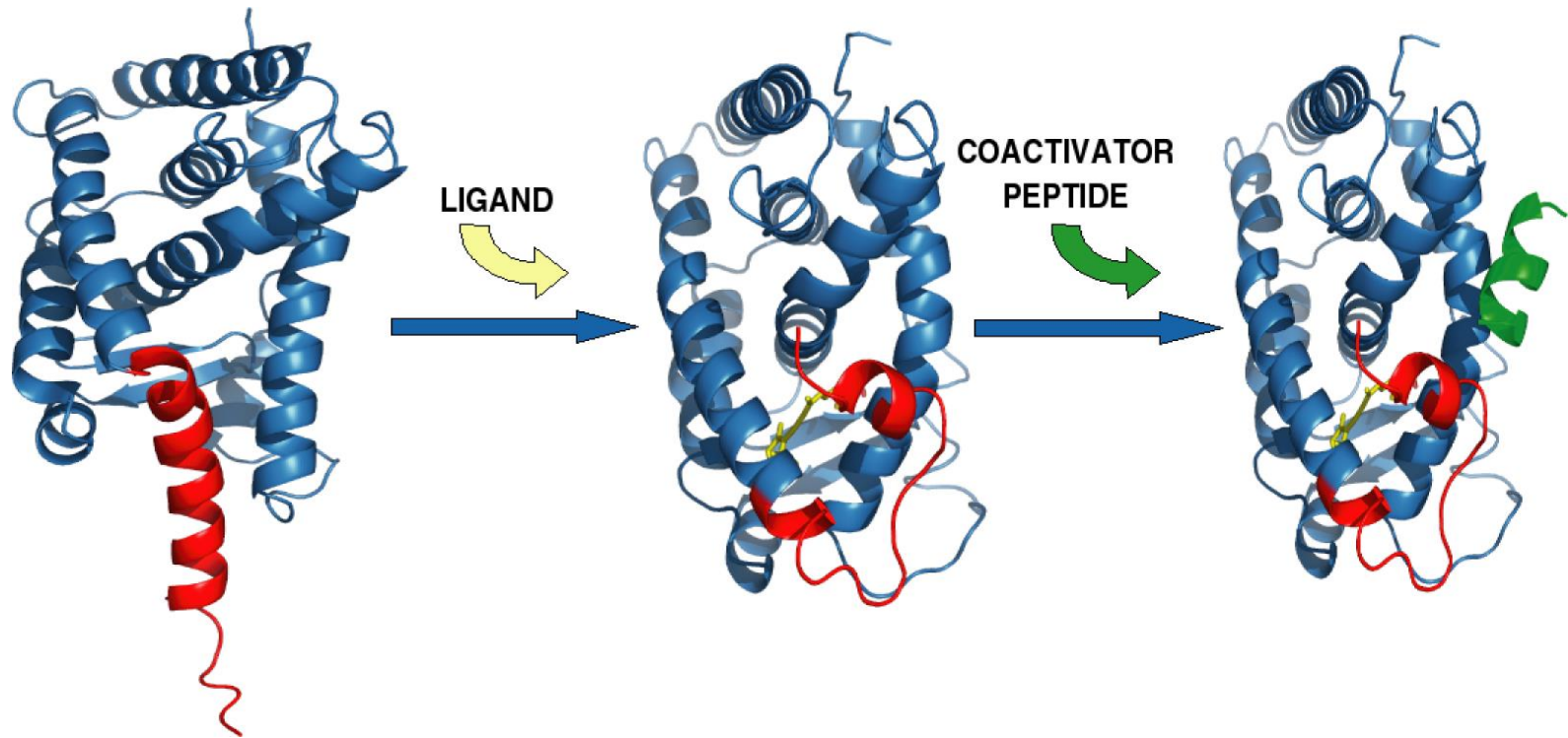
VDR/RXR (cryo-EM) : Orlov I  
et al, 2012

# Activation of RAR



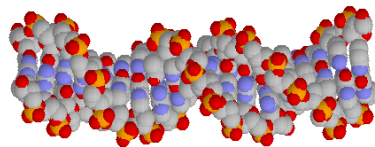
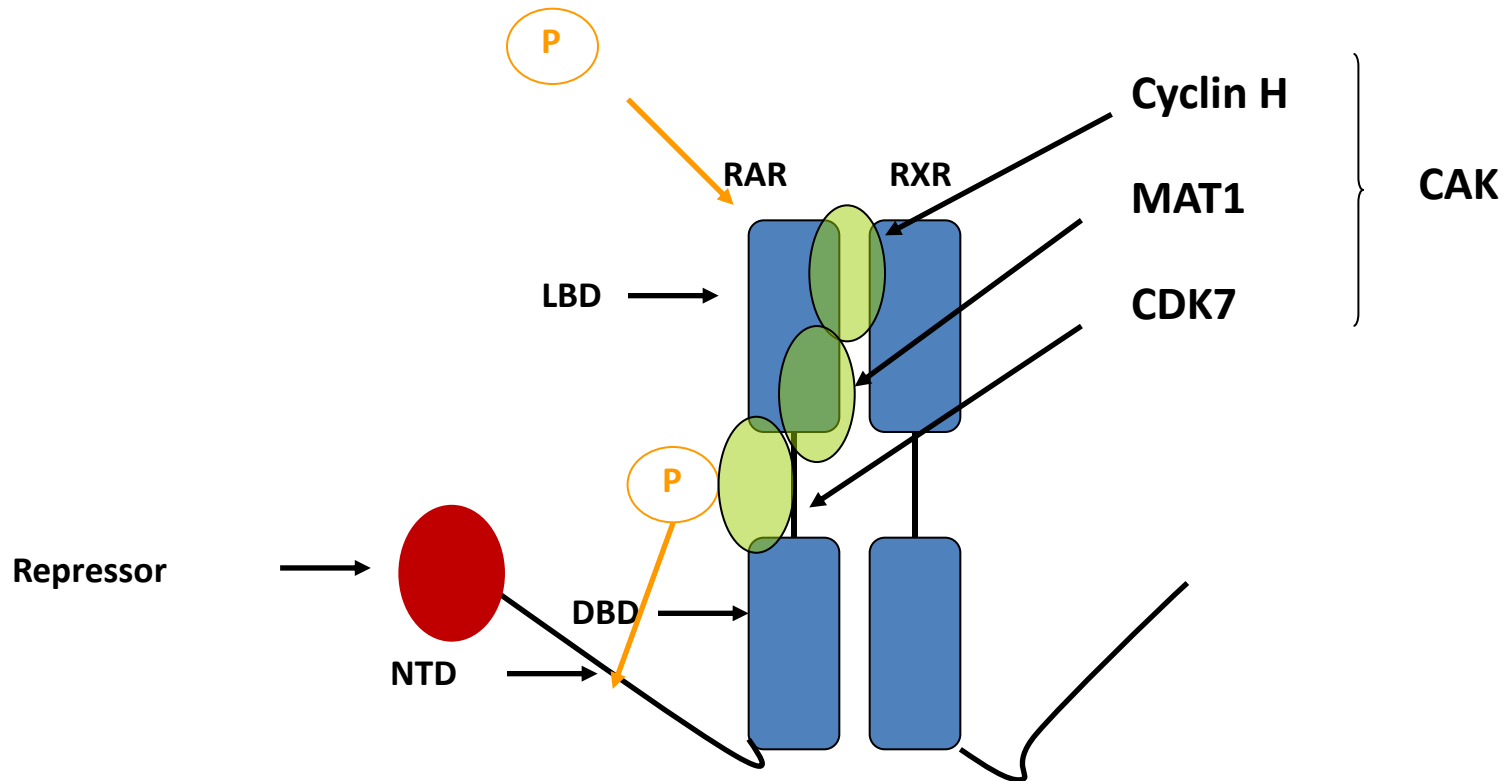
# Ligand dependent activation of NR

Ligand binding by the LBD leads to conformational changes, dissociation of co-repressors and recruitment of co-activators

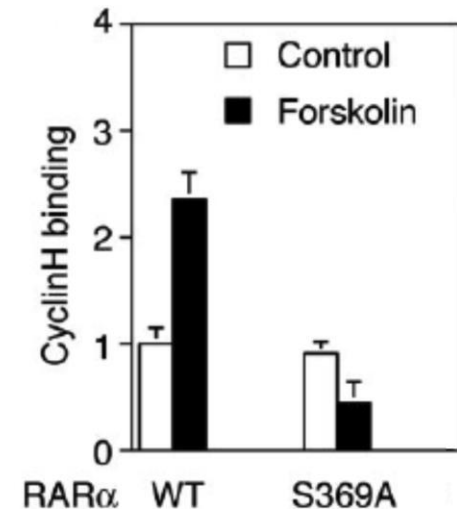
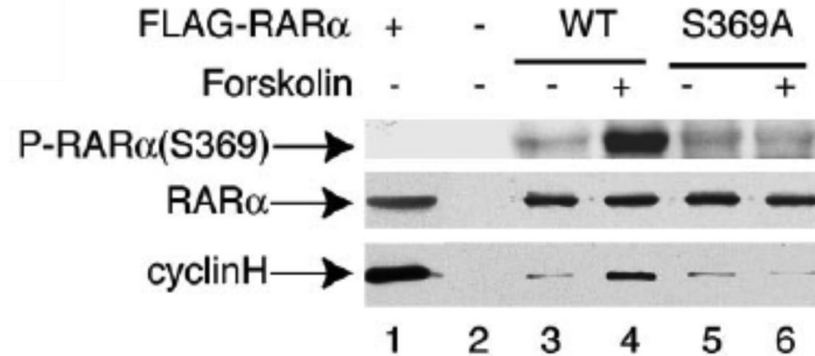
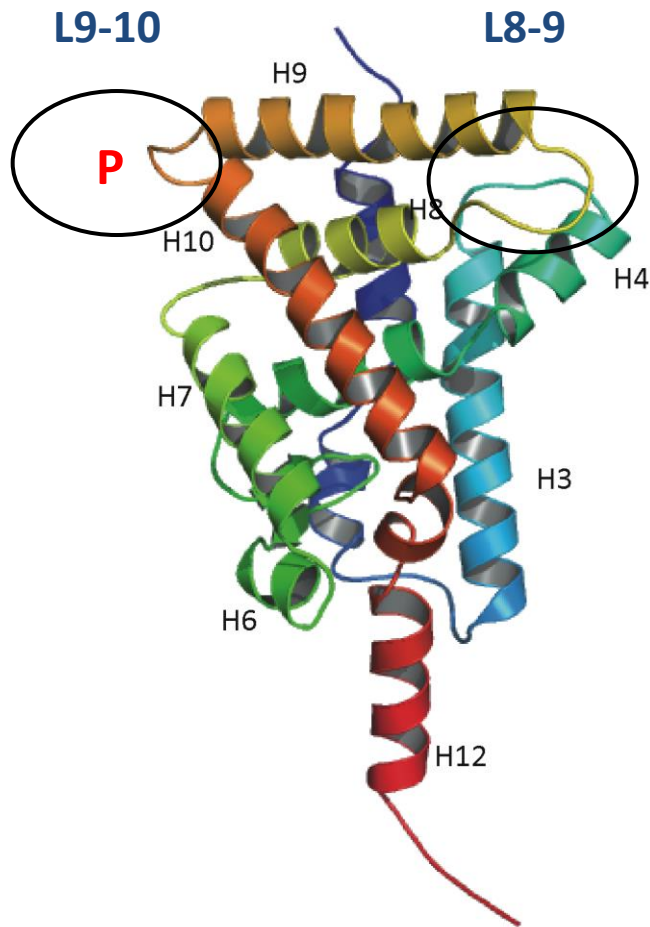


*Renaud, J. P. & Moras, D. (2000). Cell Mol Life Sci 57, 1748-69*

# Regulation by phosphorylation



# Regulation of RAR by phosphorylation

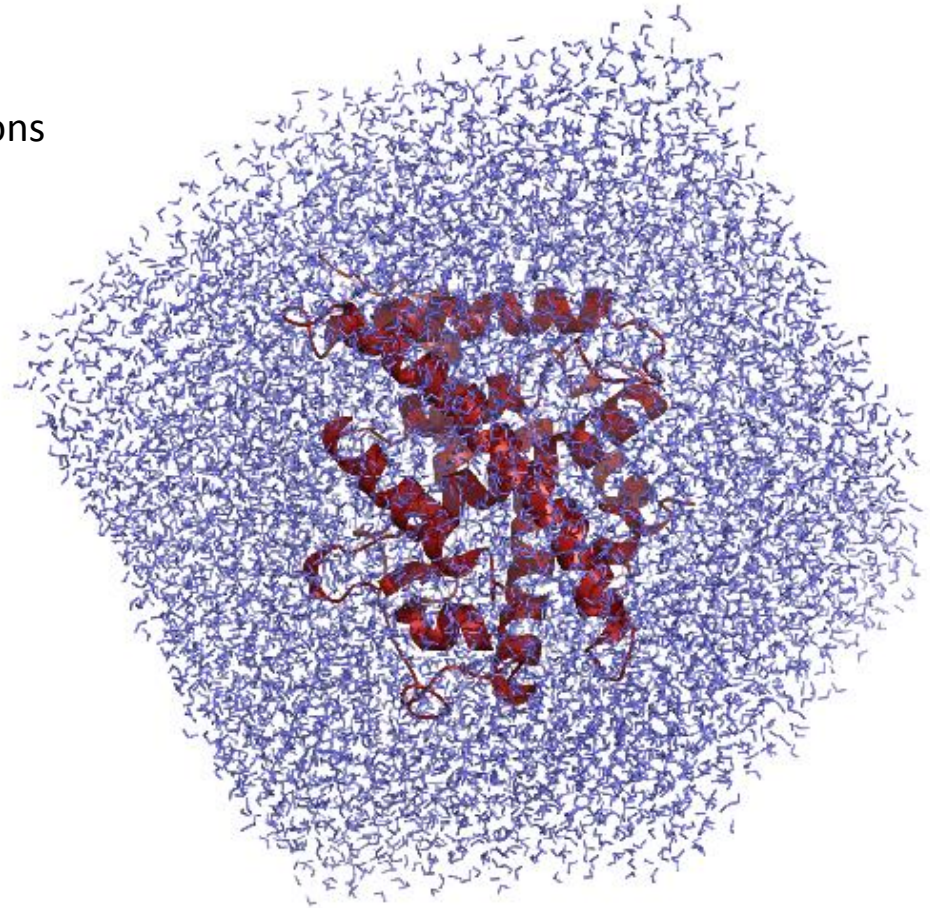


Bour G. ... Rochette-Egly C. PNAS 2005  
 Bruck N. ... Rochette-Egly C. EMBO J 2009

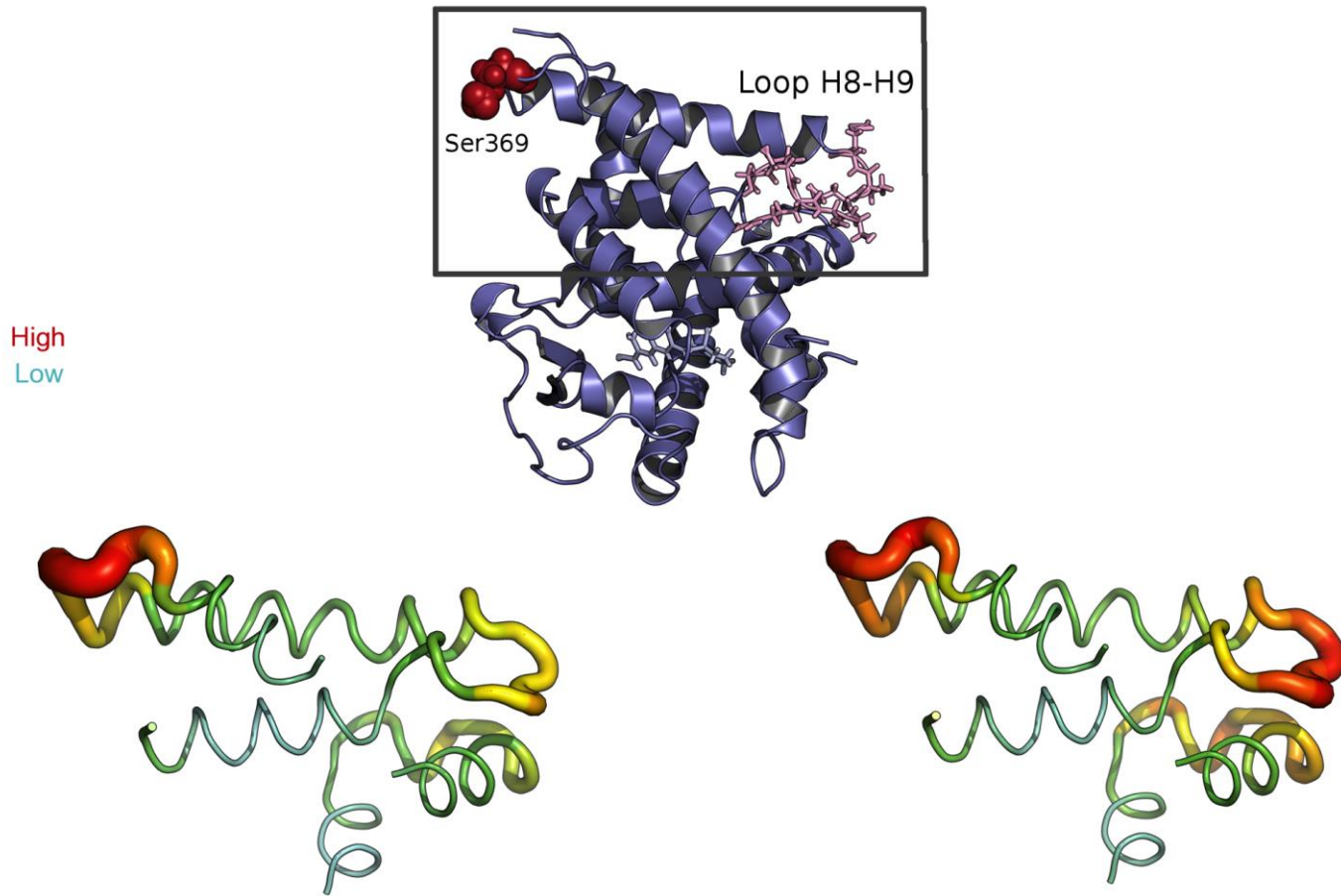


# Investigation by Molecular Dynamics Simulations

- Explicit solvent molecular dynamics simulations
- Simulations of both unphosphorylated and phosphorylated forms of RAR $\alpha$
- 100s ns simulation time for each state
- Explicit ions to neutralize the system
- PME Ewald summation
- NAMD program for running the simulations
- CHARMM program for analysis



# Effect of the phosphorylation on the LBD

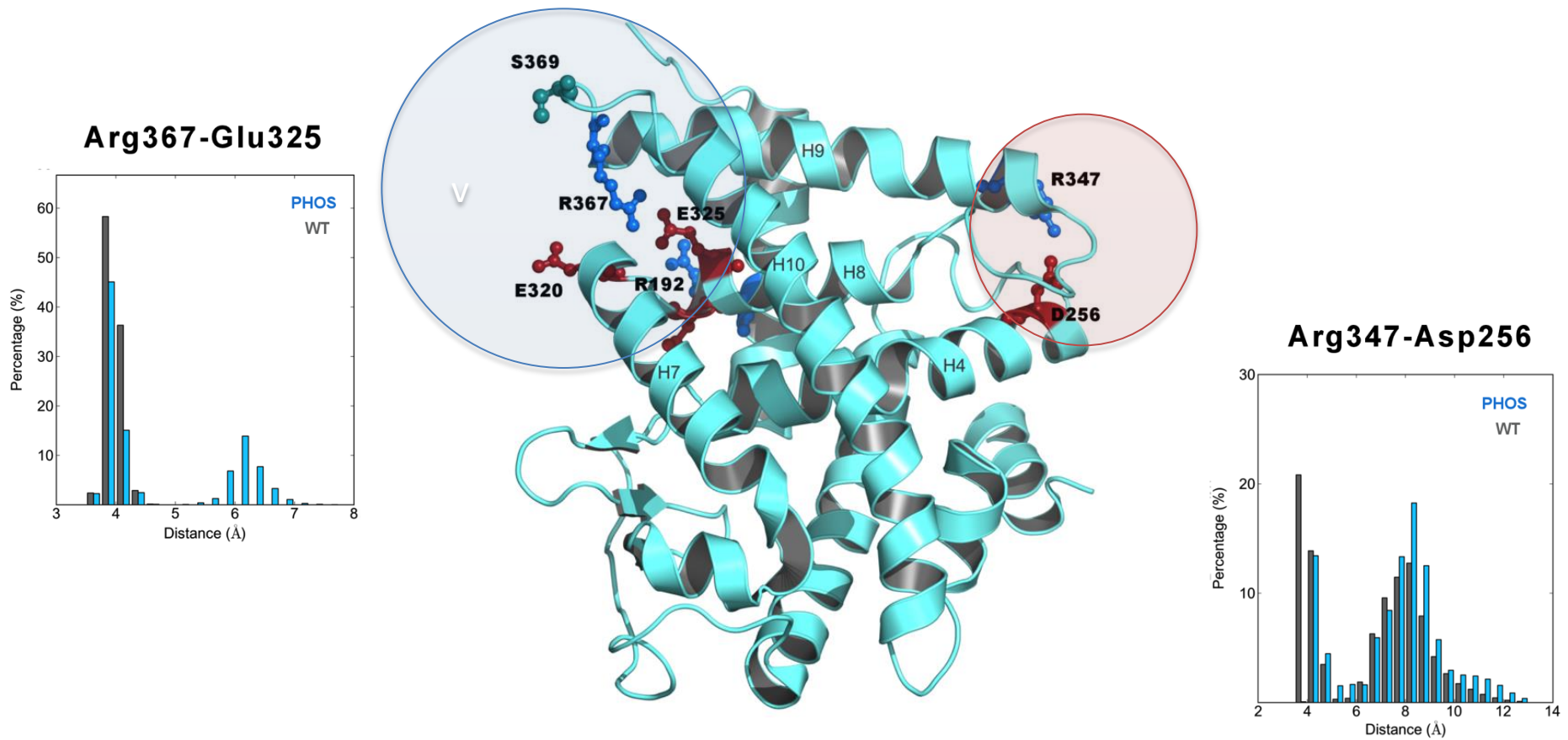


Unphosphorylated RAR $\alpha$

Phosphorylated RAR $\alpha$

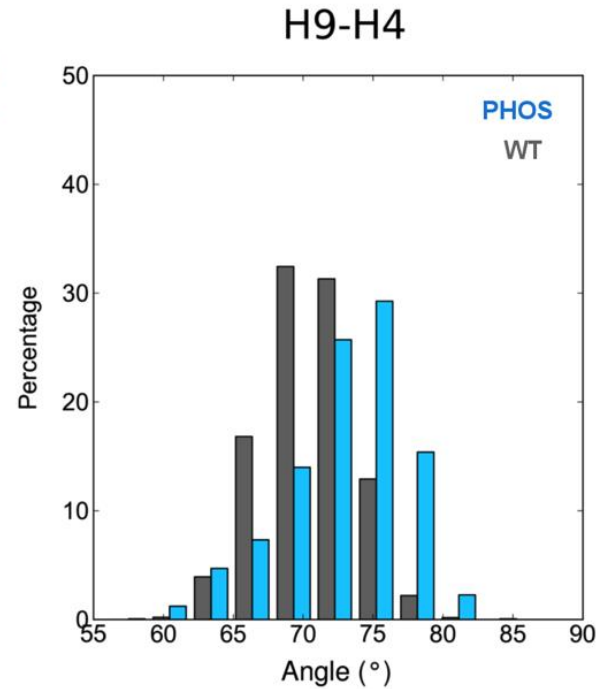
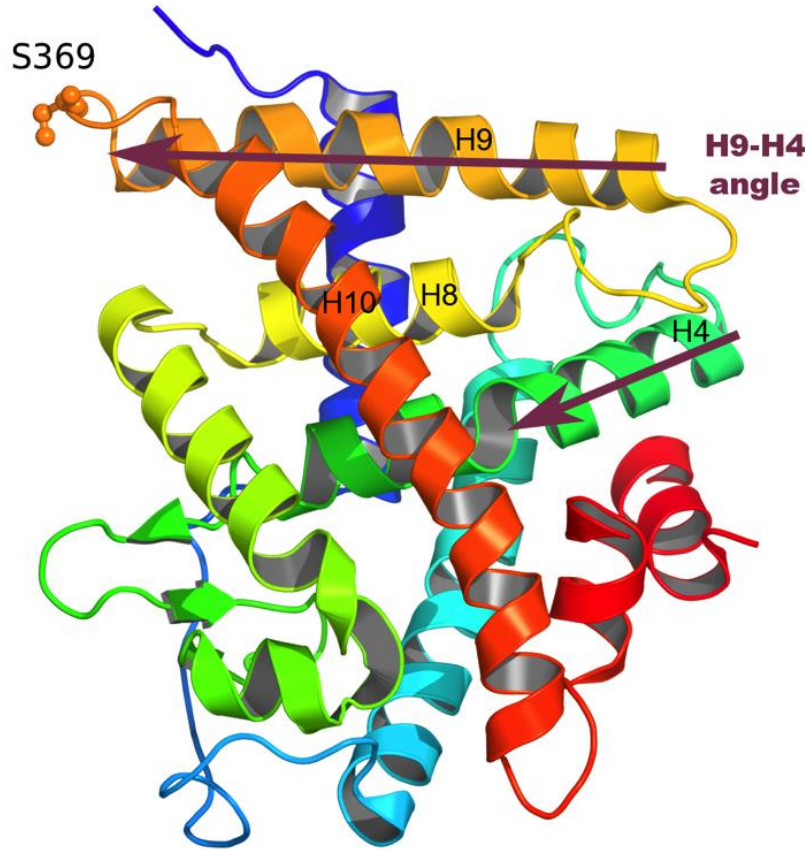
Phosphorylation induces an increase in the atomic fluctuations of the cyclin binding site

# Structure: salt bridges network



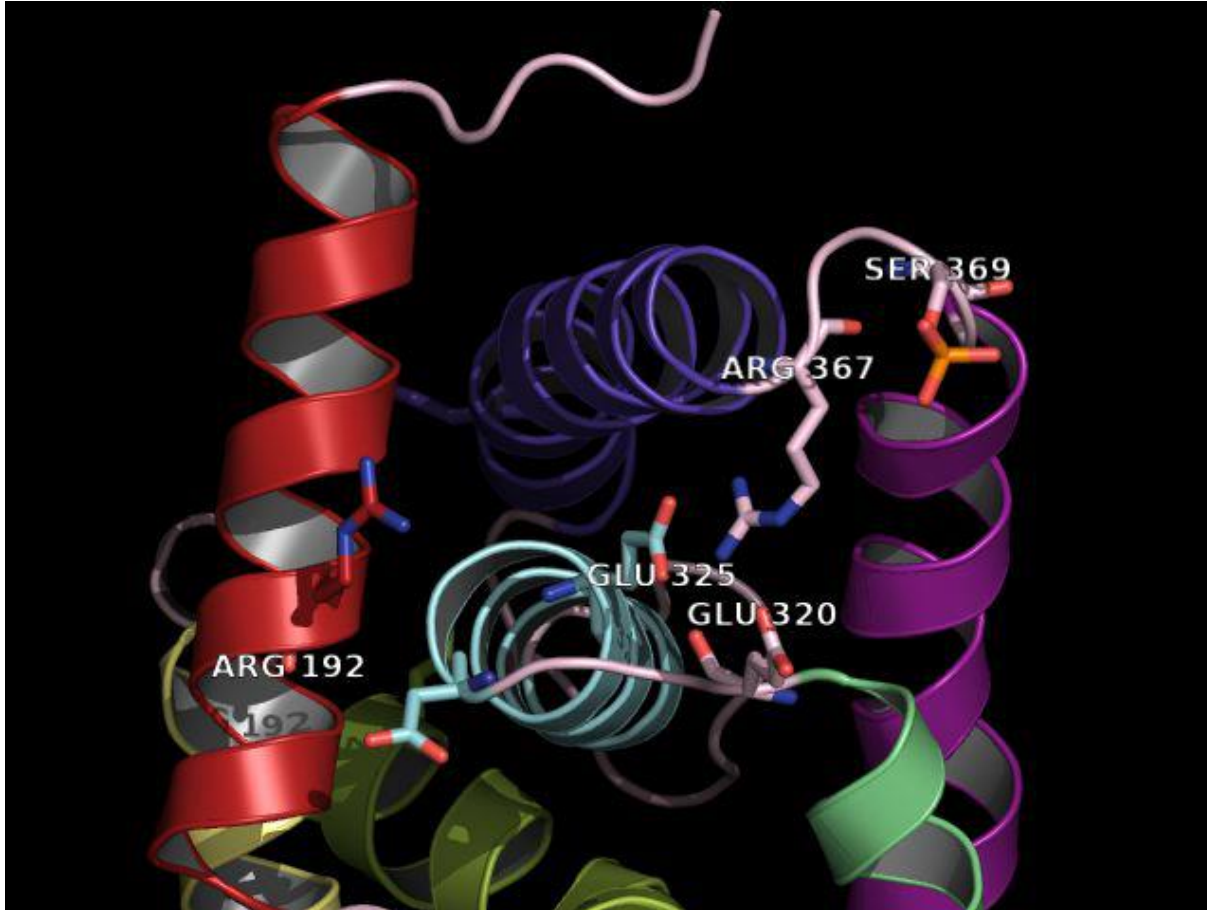
Phosphorylation leads to a reorganization of salt bridge network local to S369

# Structure: helix orientations



Phosphorylation leads to a change in relative orientation of  $\alpha$ -helices

# Structure: local changes

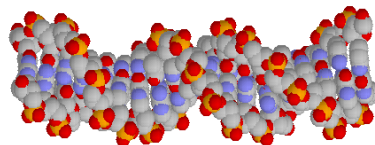
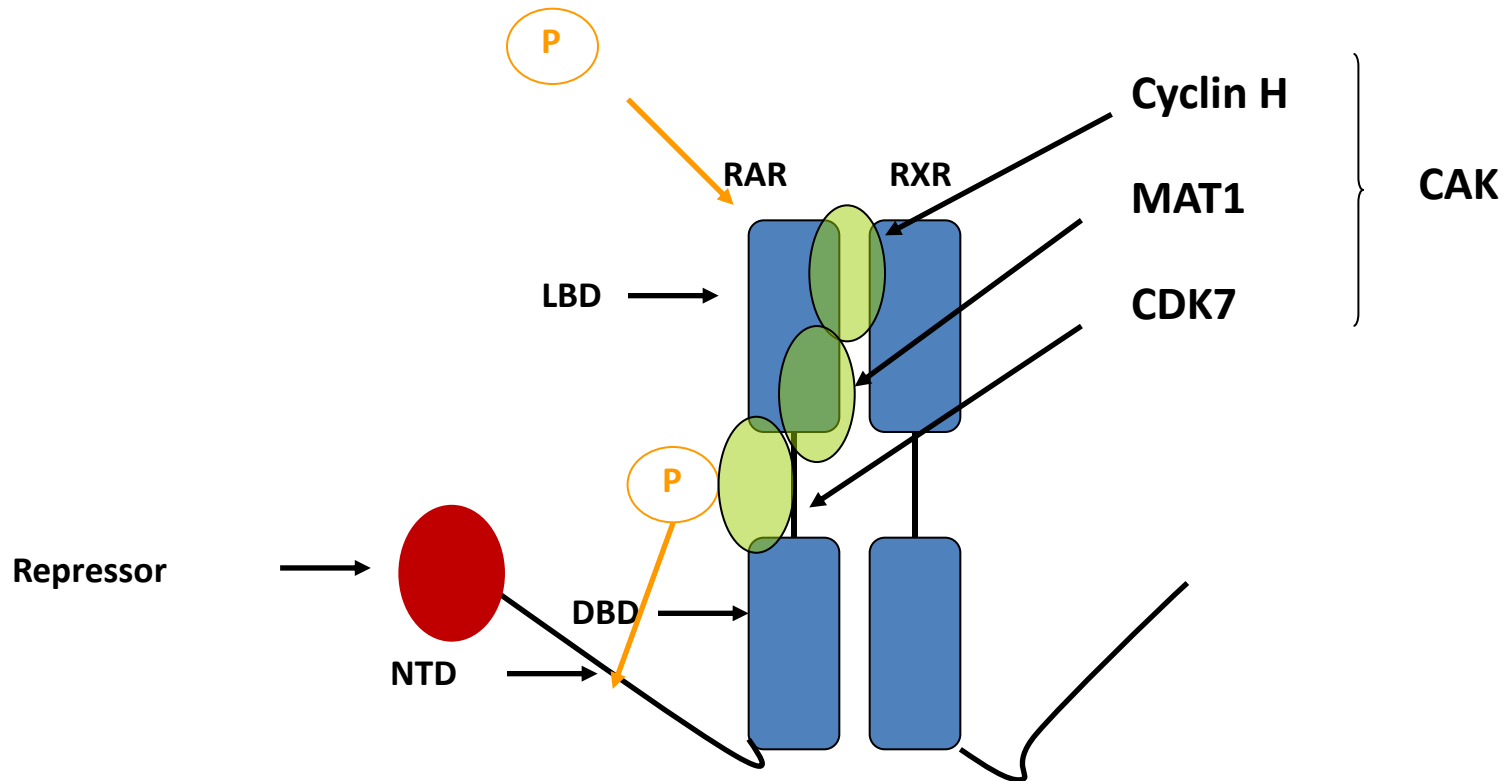


# Conclusions: phosphorylation effects on the LBD

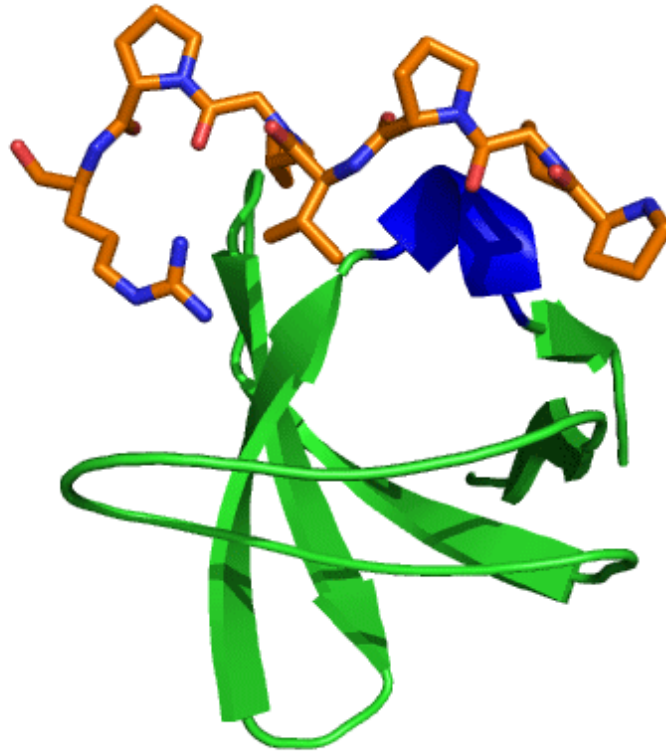
Phosphorylation induces local conformational rearrangements of salt bridge network with **structural consequences for cyclin binding site**

Next: build a model of the RAR $\alpha$ -cyclin H complex

# Regulation by phosphorylation



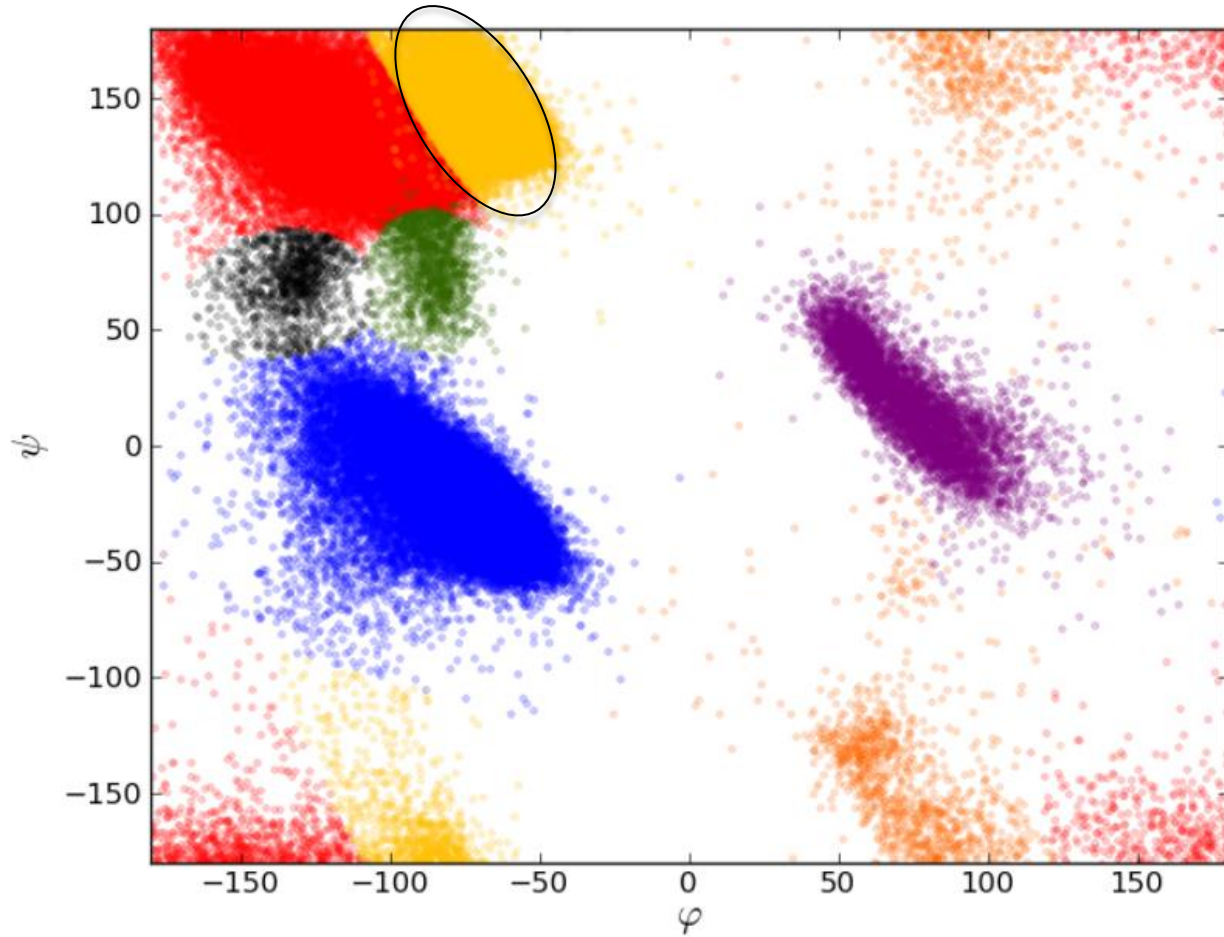
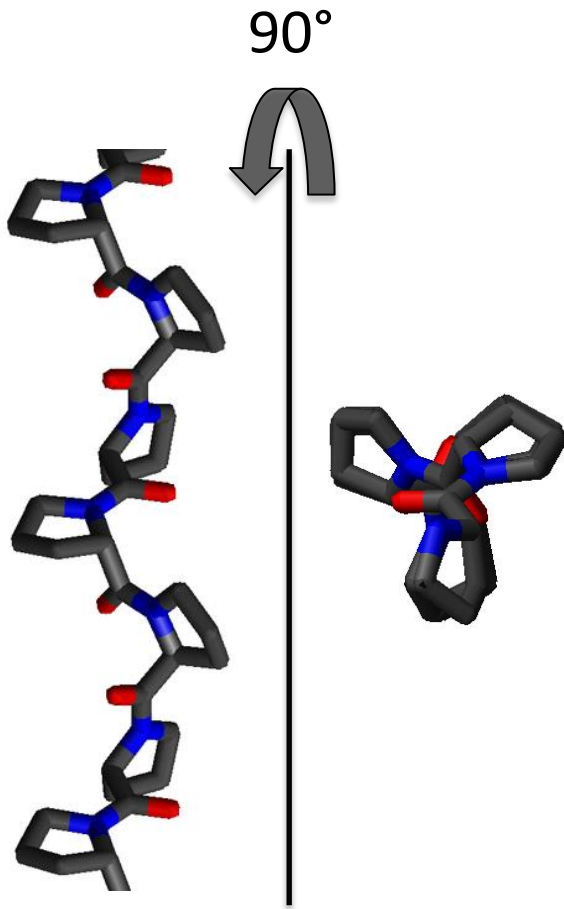
# SH3 domain peptide interactions



- Generally bind to Pro-rich peptides with the minimal consensus Pro-X-X-Pro
- Peptide forms a left-handed polyPro type II helix (PPII)
- Two classes of SH3 domains have been defined (Class I and Class 2) which recognize RKXXPXXP and PXXPXR motifs, respectively



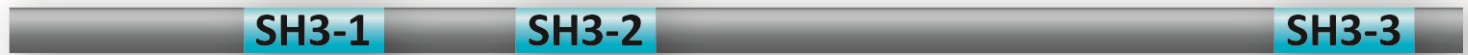
# PPII Conformation



**PPII conformation of the peptide is important for the interaction with the SH3 domain**

# The NTD of RAR $\gamma$ interacts with the SH3.3 domain of vinexin $\beta$

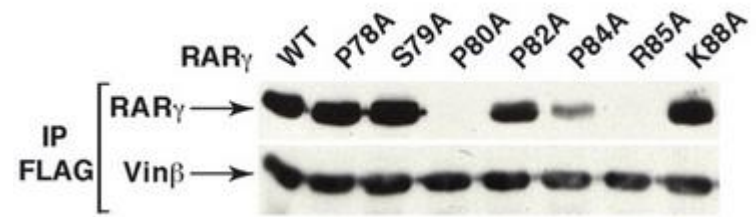
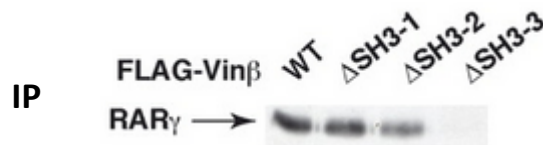
Vinexin $\beta$



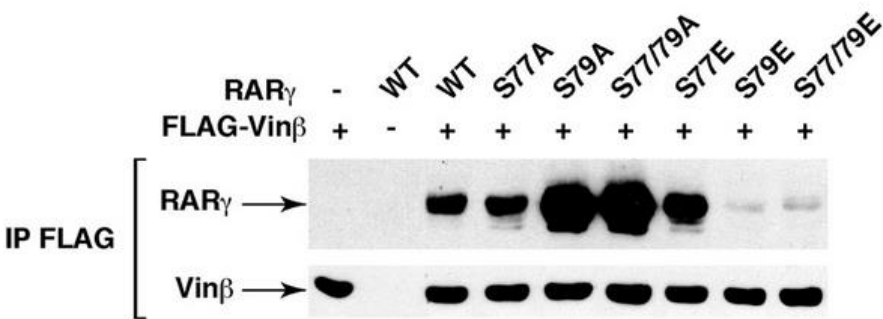
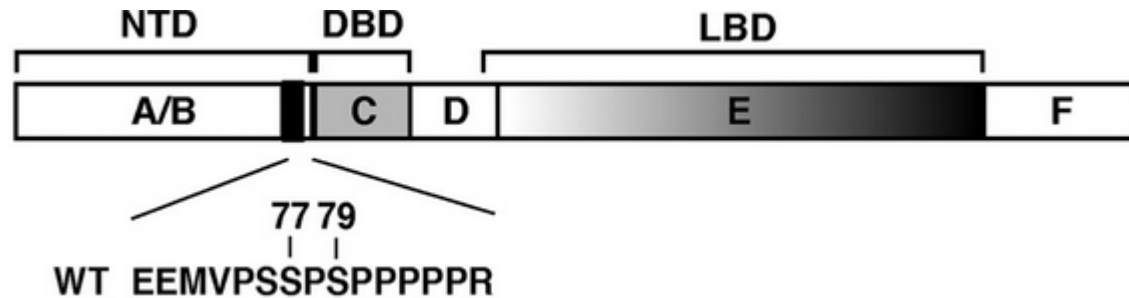
NTD peptide

E<sub>71</sub>EMVPSSPSPPPPPRVYKP<sub>89</sub>

**PXXPXR** → class II ligand for SH3 domain



# Two serines of the NTD play an important role in the association



PI118 EEMVPSSSPSPPPPPRVYK Kd=544  $\mu$ M  
 PI119 EEMVPSSSPSPPPPPRVYK Kd=280  $\mu$ M  
 PI120 EEMVPSSSPSPPPPPRVYK Kd=134  $\mu$ M  
 PI121 EEMVPSSPSPPPPPRVYK Kd= 37  $\mu$ M  
 S. Lalevée et al. 2010



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- Dr. Marc-Andre Delsuc
- Dr. Marc Quiternet
- Dr. Christian Koehler
- Dr. Denise Martinez
- Dr. Mathieu Tanty

## Nuclear retinoic acid receptors phosphorylation and cross-talk with signaling pathways

Dr. Cecile Rochette-Egly

Mr. Eric Samarut

## Expression of genetic information

Dr. Natacha Rochel



*pour la vie*

