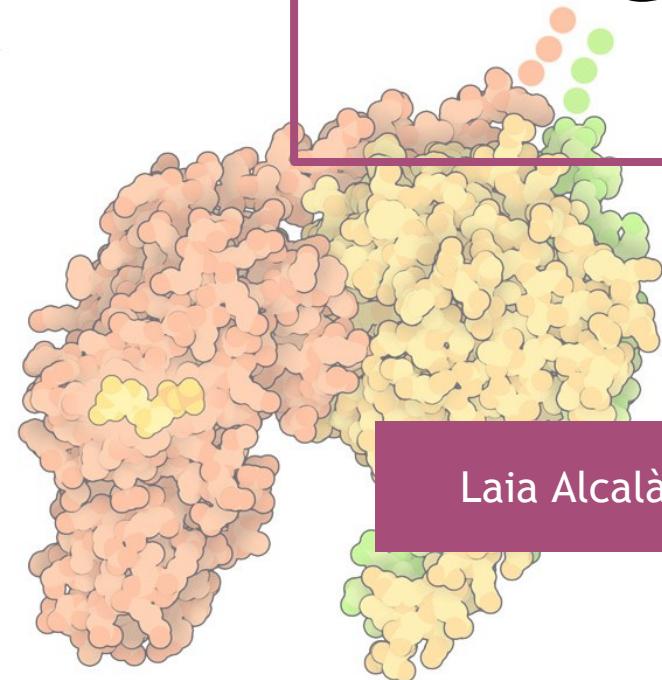


G PROTEINS



Laia Alcalà, Oriol Castellano, Aisha Shah, Júlia Salas

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SIMULATION

Wild Type
Mutant

G-proteins are a family of receptors that play an important role in cell signaling

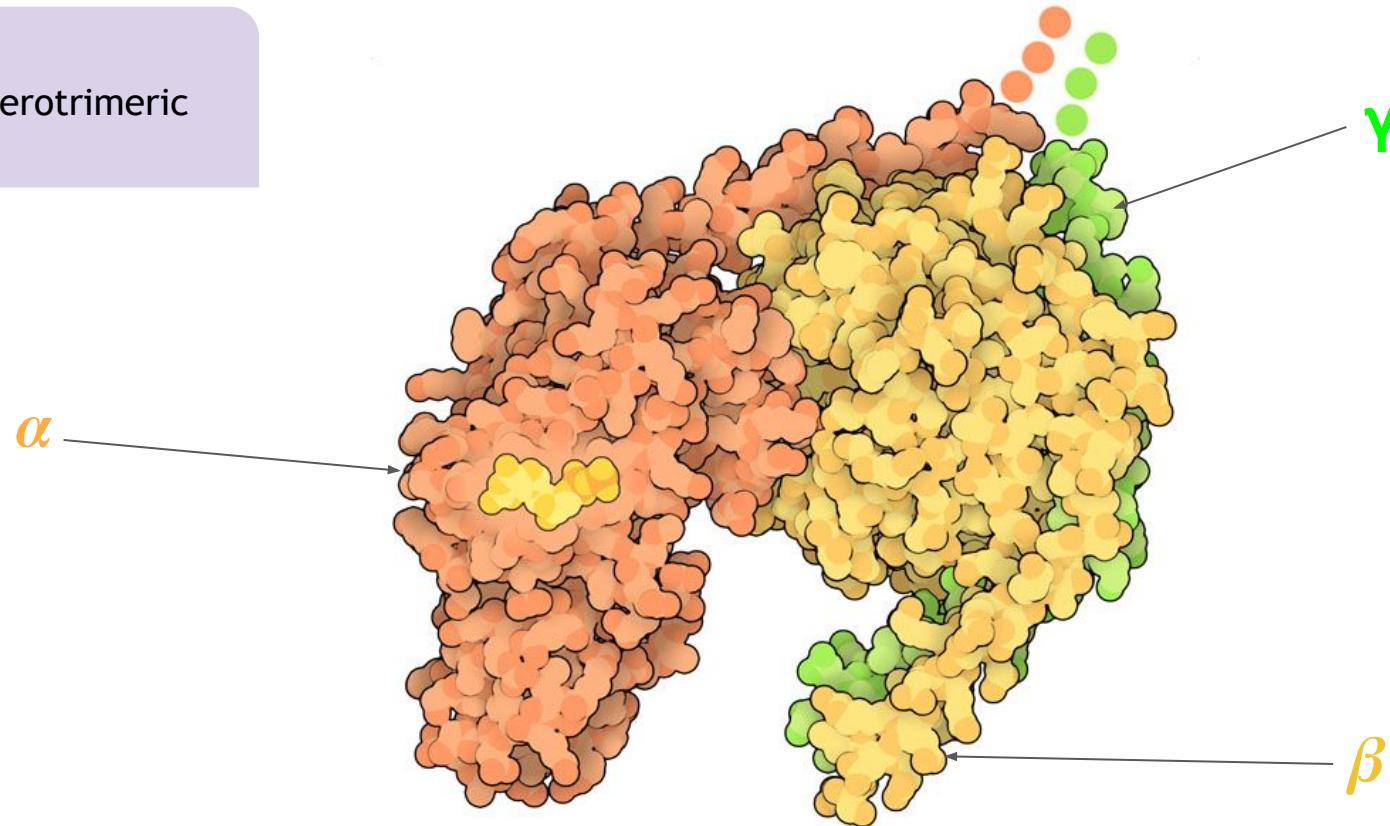
FUNCTIONS:

- Signal transduction
- Membrane vesicle transport
- Cytoskeletal assembly
- Cell growth
- Protein synthesis

G-Proteins structure

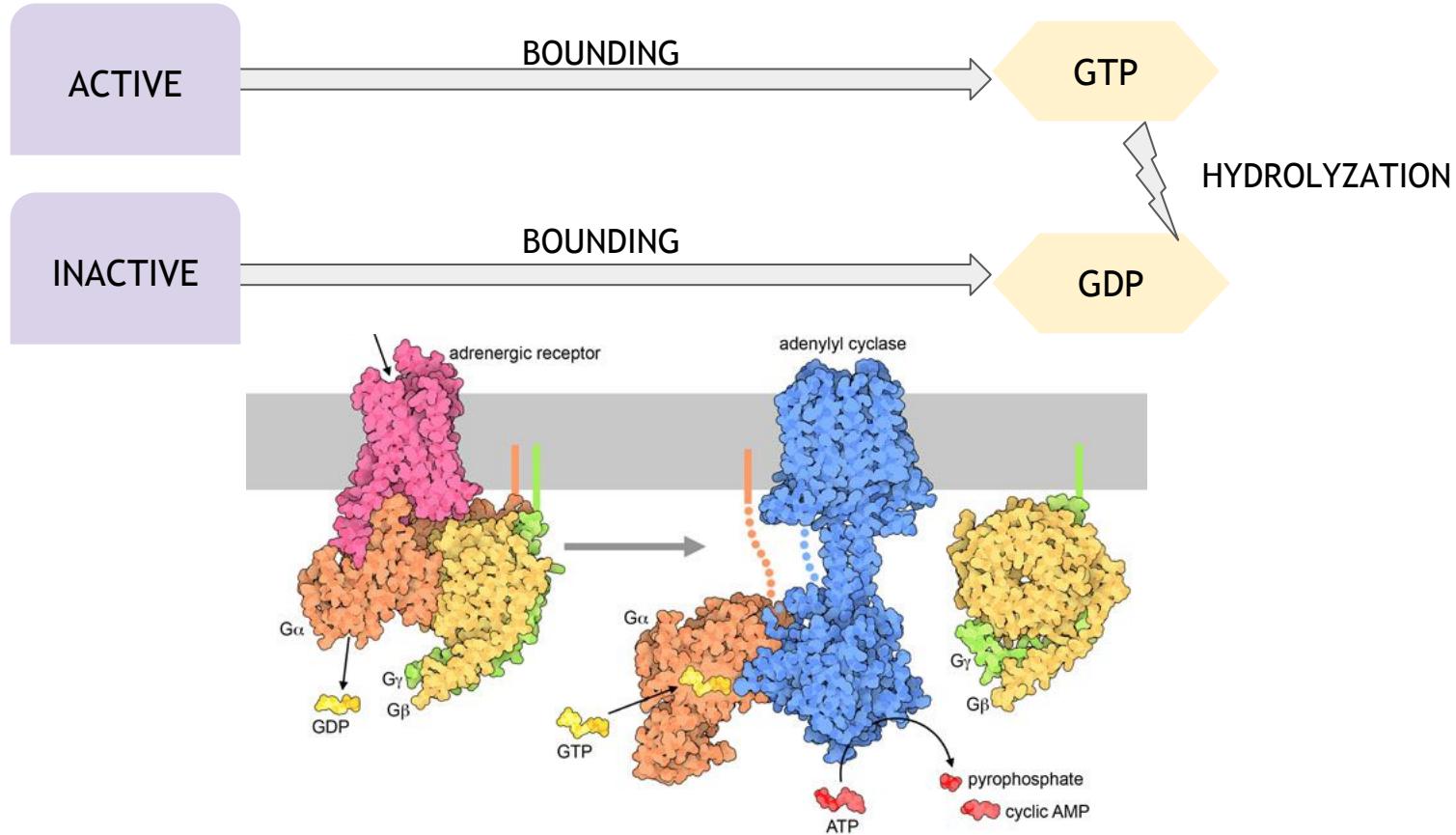
INTRODUCTION

Heterotrimeric



G-Proteins activation

INTRODUCTION

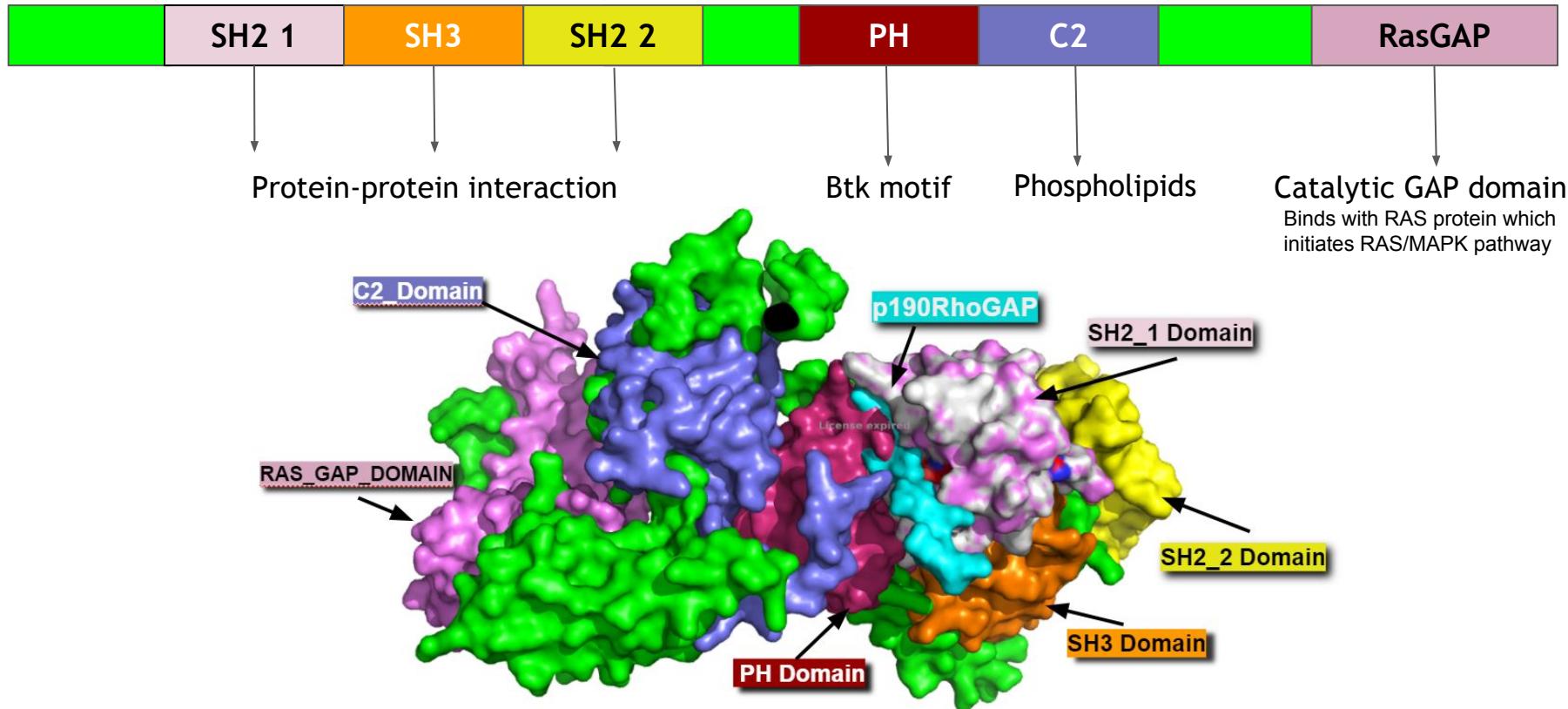


RASA 1

Ras GTPase-activating protein 1(GAP)

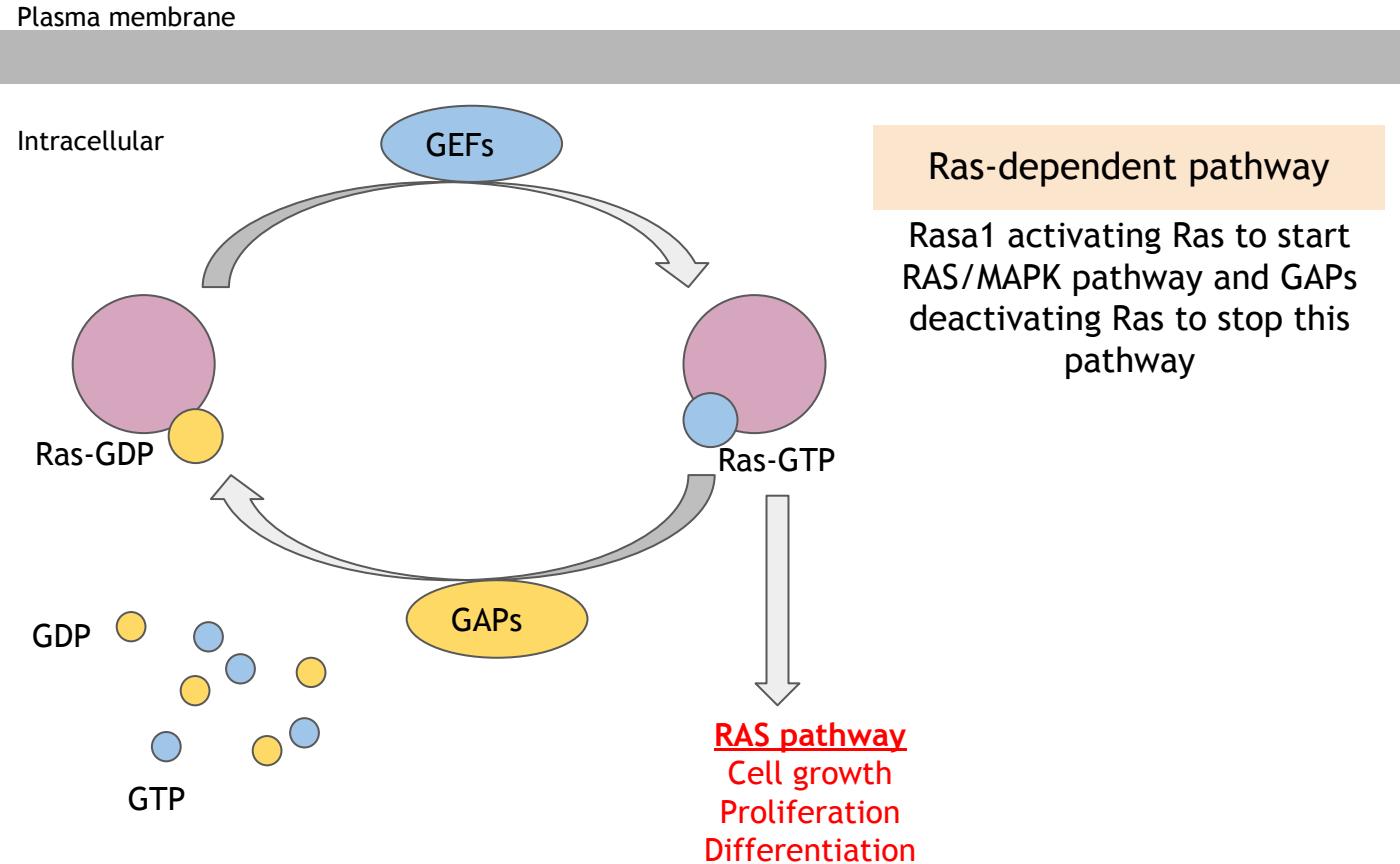
RASA1 Domains

INTRODUCTION



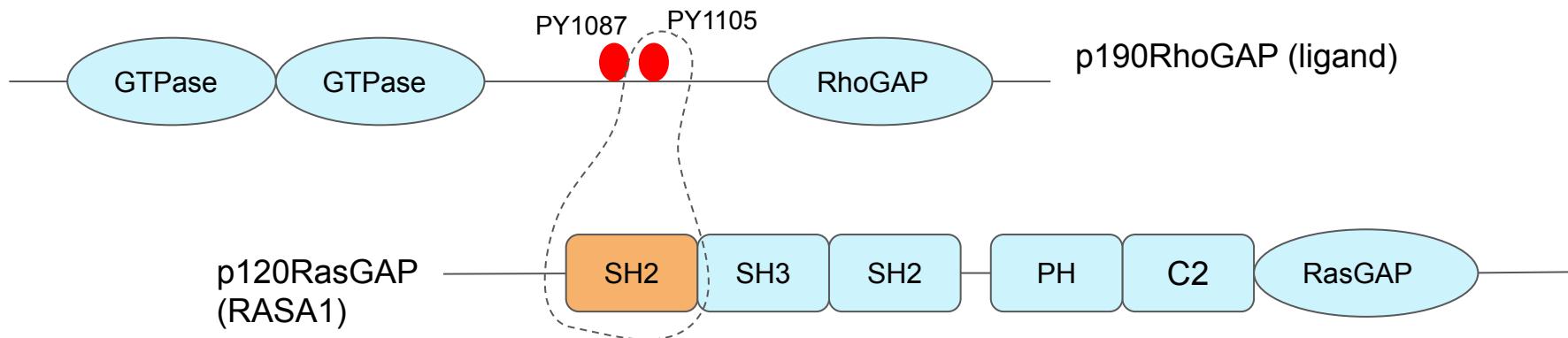
RASA1-SH2 Function : Crosslinking of Rho-Ras Pathways

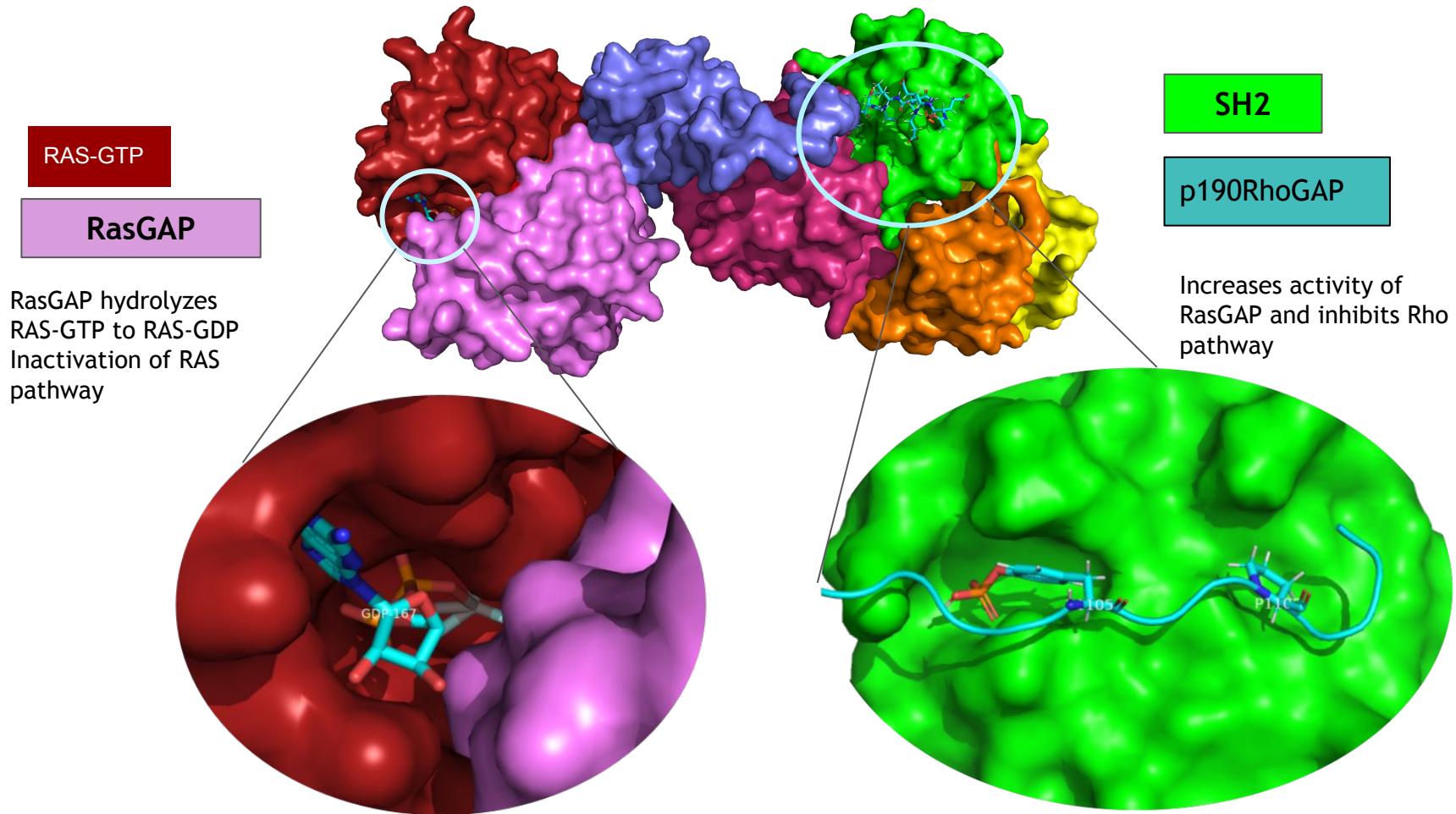
INTRODUCTION



- **Ras -independent pathway**

Inhibits RAS and RHO pathways





Evolutionary history

SEQUENCE
ANALYSIS

SH2 DOMAIN

To study the evolutionary history we have to work with similar sequences containing SH2 domain obtained using hmm search

HUMAN
P20936

RAT
P50904

BOVIN
P09851

CHICKEN
P00523

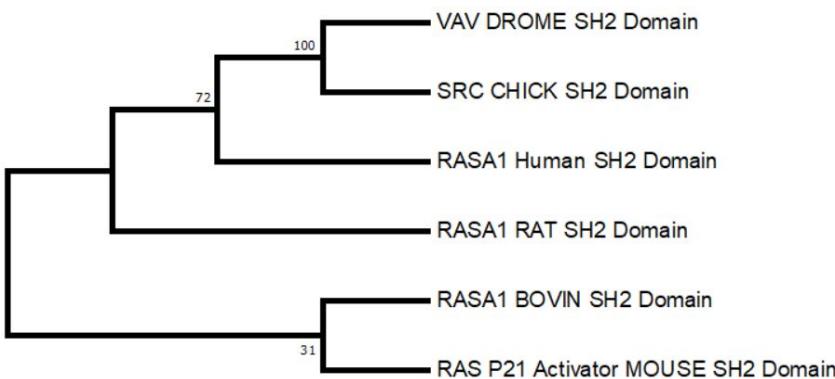
FRUIT FLY
Q9NHV9

MOUSE
E9PYG6

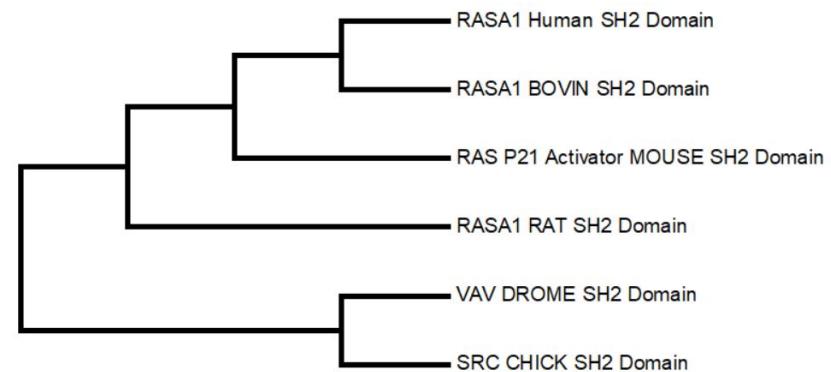
Evolutionary history

SEQUENCE ANALYSIS

Minimum Evolution tree



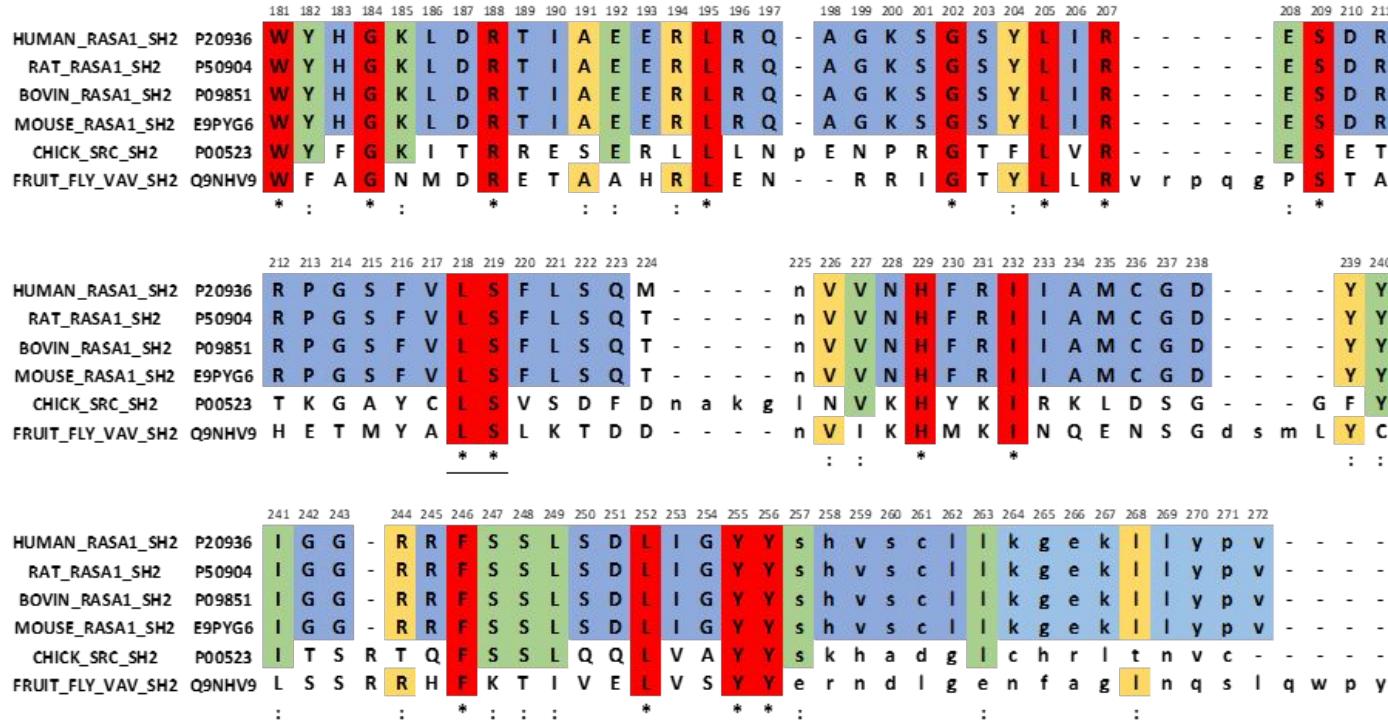
Maximum Parsimony tree



Sequence alignments and conserved regions

SEQUENCE
ANALYSIS

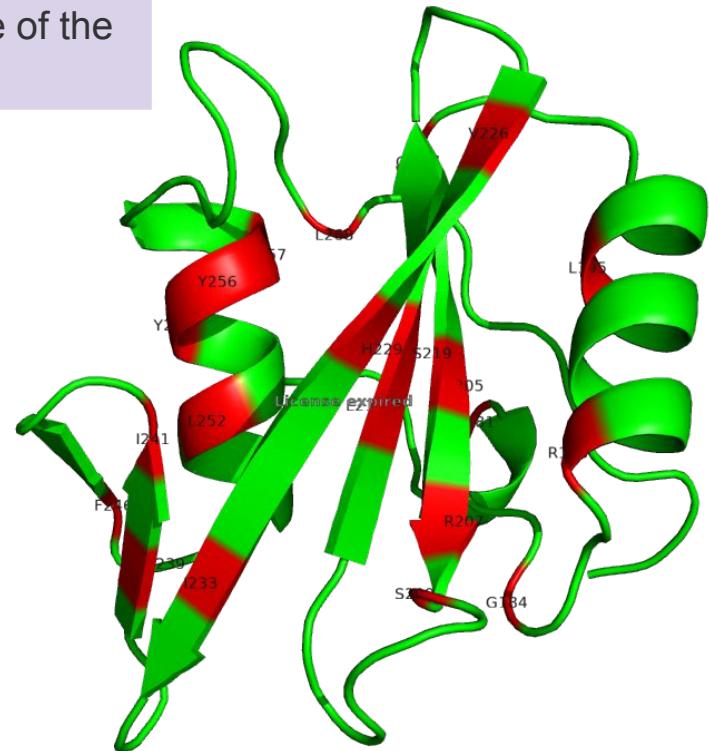
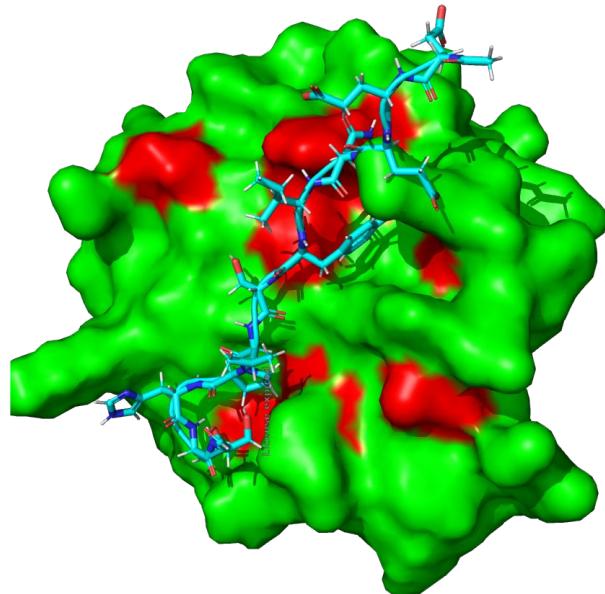
MSA by
Clustaw



Conserved Regions

SEQUENCE ANALYSIS

- Interacting with phosphotyrosine peptide
- Stabilizing alpha helices and beta sheets
- Mutations in these regions can affect the structure of the domain and binding of phosphotyrosine peptide



Structural Analysis

Secondary Structure

STRUCTURE
ANALYSIS

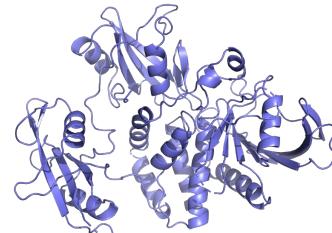
PDB
STRUCTURES

6pxc

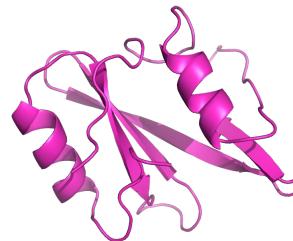
To study the differences in secondary structure we have to obtain a set of structures in PDB. These structures belong to SH2 domains of different proteins not only RASA1.



2c9w_A



2shp_A



1d1z_A



1a07_A

Clustalw alignment and Conservation

STRUCTURE ANALYSIS

Secondary Structure

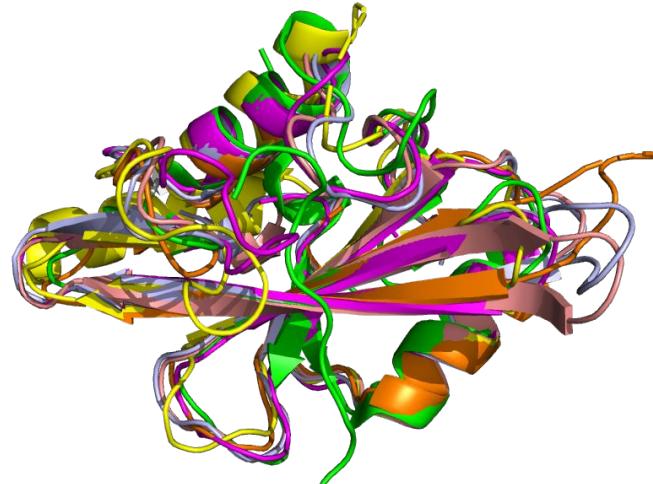
STRUCTURE
ANALYSIS

Secondary
structure
alignment

6pxcA	174 175 176 177 178 179 180	181 182 183 184 185 186 187	188 189 190 191 192 193 194 195	196 197 198 199 200	201 202	203	204 205 206	207 208	209 210	211 212	213	214 215
6pxcA	T A P P T N Q	W Y H G K L D	R T I A E E R L	R Q A G K S G S	S Y L I R E	S D R R P G S						
6pxcA	- - - - - G G G	- T E E E E E	- - - - -	H H H H H H H H H	- H H H -	T T E	E E E E E	E S S S T T	-			
1a07A	- - - - - G G G	- T T B - T T	- - - - -	H H H H H H H H H	- T T S -	T T E	E E E E E	- S S S T T	-			
1d1zA	- - - - - - T T E E T T	- - - - -	H H H H H H H H H	- H H H -	T T E	E E E E E	- S S S T T	-				
2c9wA	- H H H H H H H H H H H H H H H T S	- B - T T -	H H H H H H H H H	- T T S -	T T -	E E E E E	- S T T - S	*	:	*		
			*	*	*	*	*	*	*	*		
6pxcA	F V L S F L	S	Q M N V	V N H F R I I A M	S G	D Y Y	I G G R R	F S S	L S D L I G			
6pxcA	E E E E E E	- - - - -	T T S	- E E E E E E E E	E - -	T T -	E E E	- E T T E E -	- E S S H H H H H			
1a07A	E E E E E E	E E E T	T T E	E E E E E E E E	E - T	T S -	E E E	- E T T E E -	- E S S H H H H H			
1d1zA	E E E E E E	E - - -	T T E	- E E E E E E E E	- T	T S -	E E E	- S T T S -	- B S S H H H H H			
2c9wA	E E E E E E	E - - -	T T E	- E E E E E E E E	E - -	T T E	E E E	- T T T T T G G G -	- B S - H H H H H	*	*	*
	*	*	*	*	*	*	*	*	*	*	*	*
6pxcA	Y Y S H V S S							L L K G	E K L L Y P	V A P P E P		
1a07A	I I I I I S	- - - - -						S B T T B	- - - E E	- - - S - -		
1d1zA	H H H H -	- - - - -						T T S S S	- - - E E	- - - - -		
2c9wA	H T T S S S	- - - - -						S S S S S	- - - E E	- - - - -		
	H H H H H T	- - - - -	B - - S S - - -	H H H H H H H H H H H H H H H S - G G G S S S - H H H H H H H H H T								
								:	:	.		
	255 256 257 258 259 260 261							262 263 264 265	266 267 268 269	270 271	272 273 274 275 276 277	
	Y Y S H V S S							L L K G	E K L L Y P	V A P P E P		

Superimposition

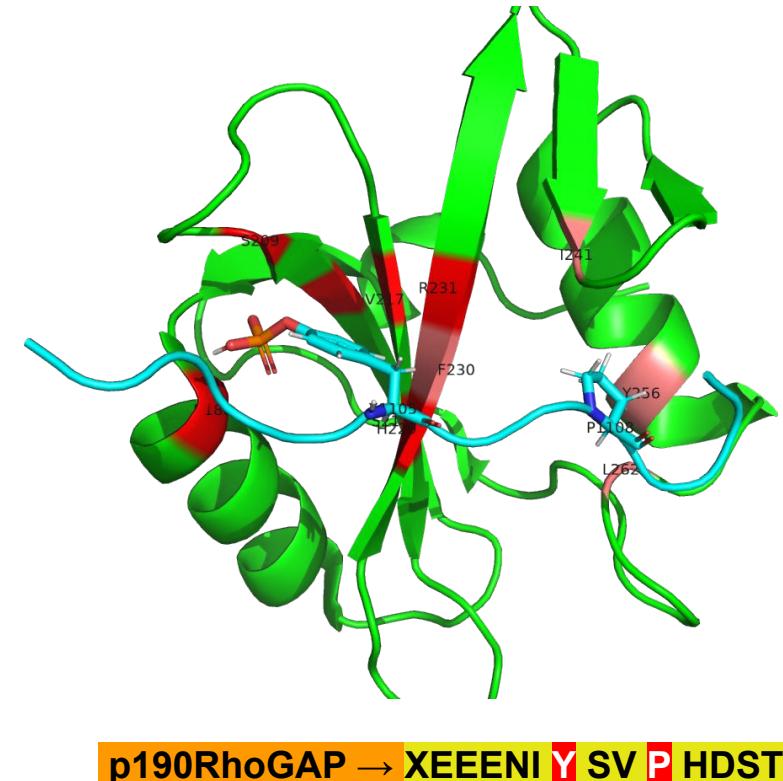
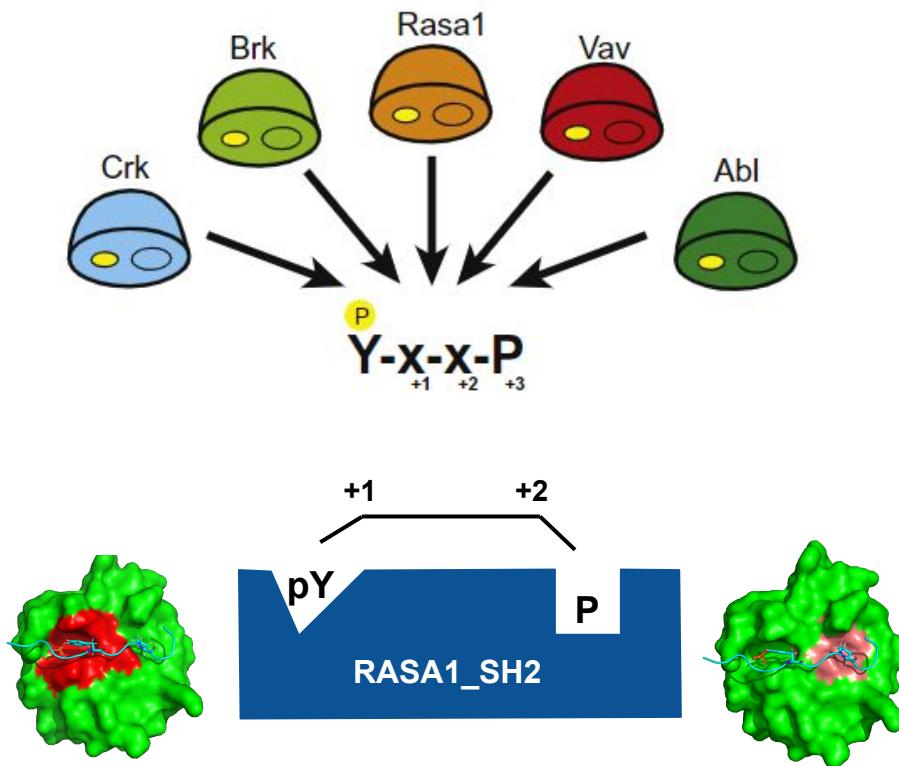
STRUCTURE
ANALYSIS



SEQ 1	SEQ 2 (template)	RMSD
2c9wA	6pxc_RASA1_sh2	2.083
2shpA	6pxc_RASA1_sh2	1.670
1d1zA	6pxc_RASA1_sh2	2.059
1a07A	6pxc_RASA1_sh2	1.675
1o41A	6pxc_RASA1_sh2	2.195

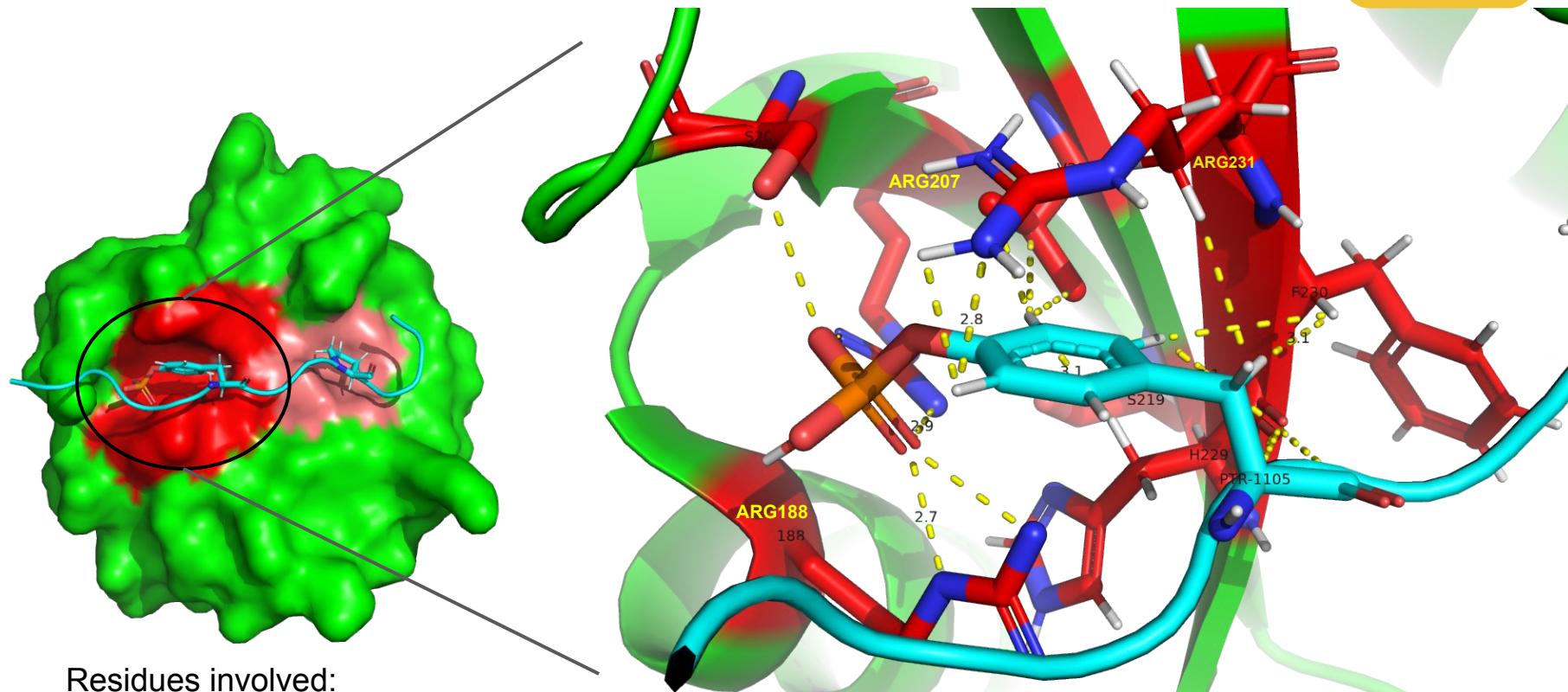
Active site for p190RhoGAP phosphotyrosine peptide binding

STRUCTURE ANALYSIS



Active site: Binding Pocket for pY

STRUCTURE ANALYSIS

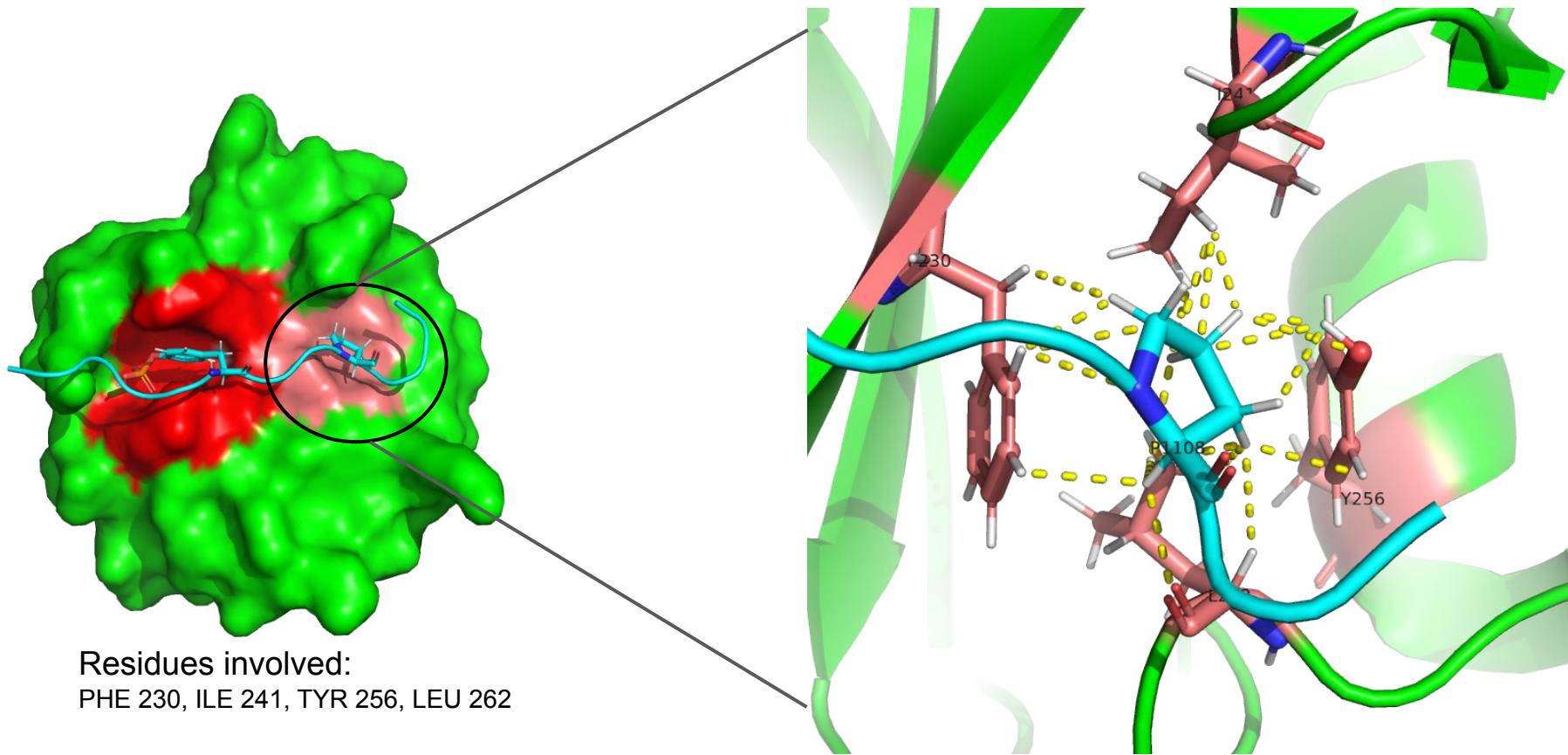


Residues involved:

ARG 188, ARG 207, SER 209, HIS
229, ARG 231

Active site : Binding Pocket for Proline

STRUCTURE
ANALYSIS



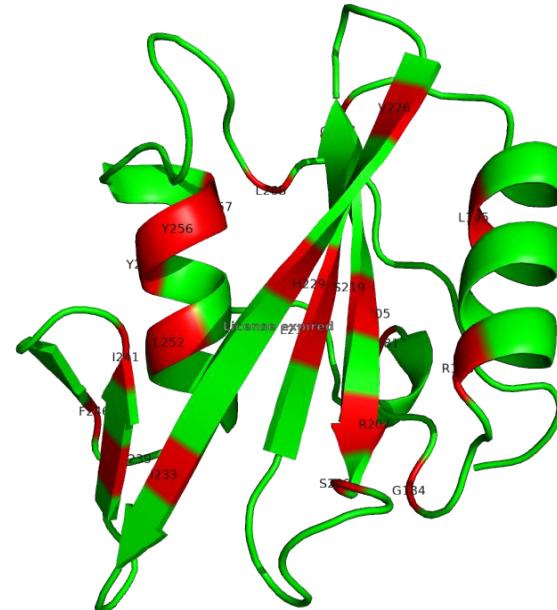
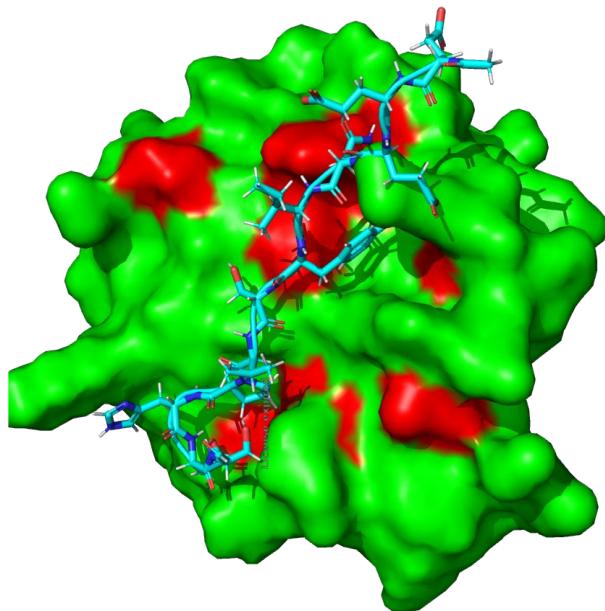
Modelling Mutations

Mutations found in Conserved regions of the sequences

Mutations found in Conserved regions of the sequences

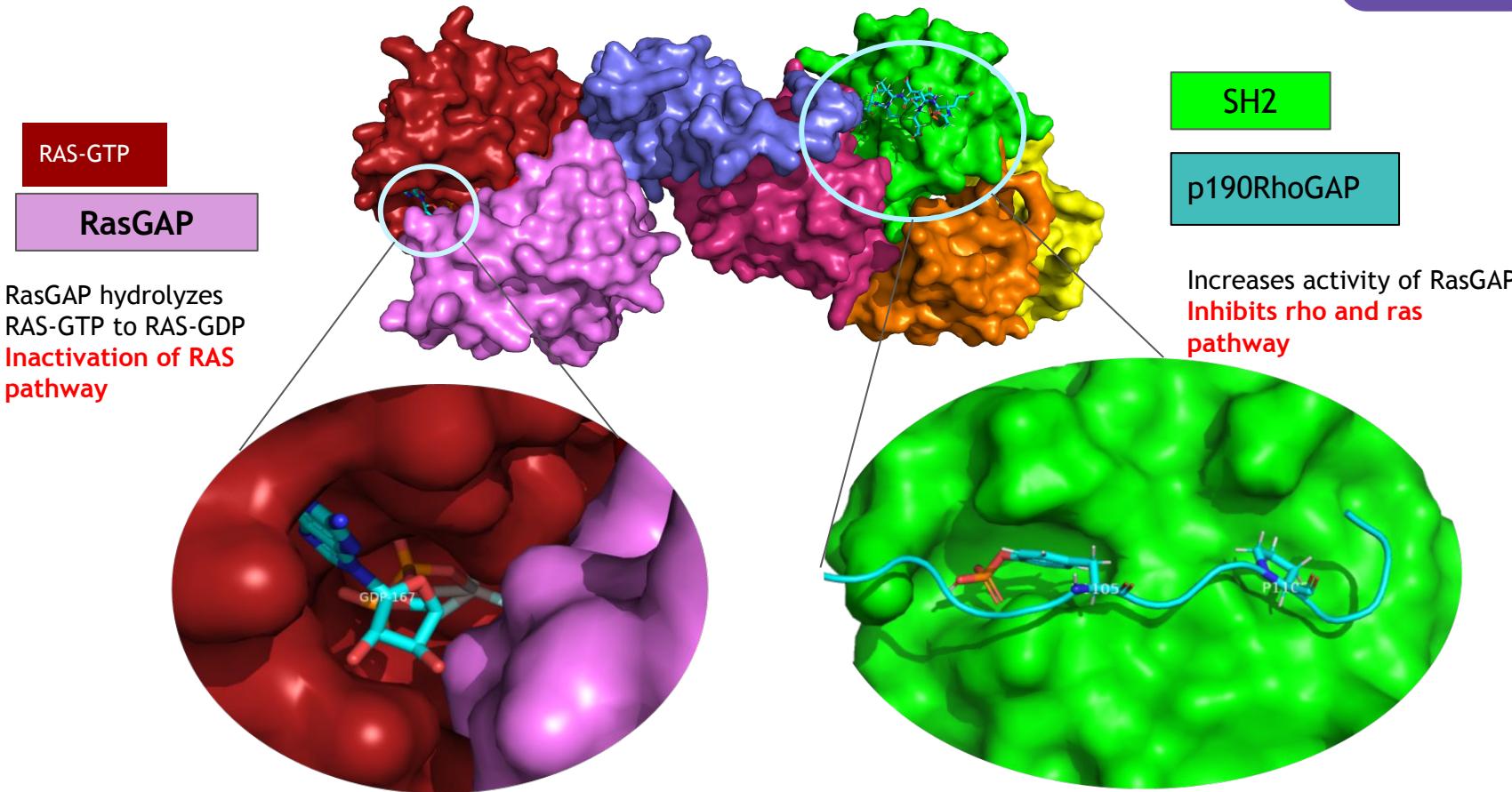
MUTATIONS

Mutations are highlighted in red



Effect of Mutations in RAS_GAP and SH2 domain

MUTATIONS



SH2 domain : Wild type vs mutant type

MUTATIONS

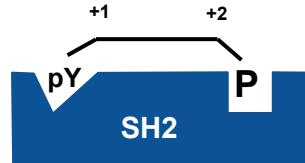
Position 229

Reference residue: H
Altered residue: Y

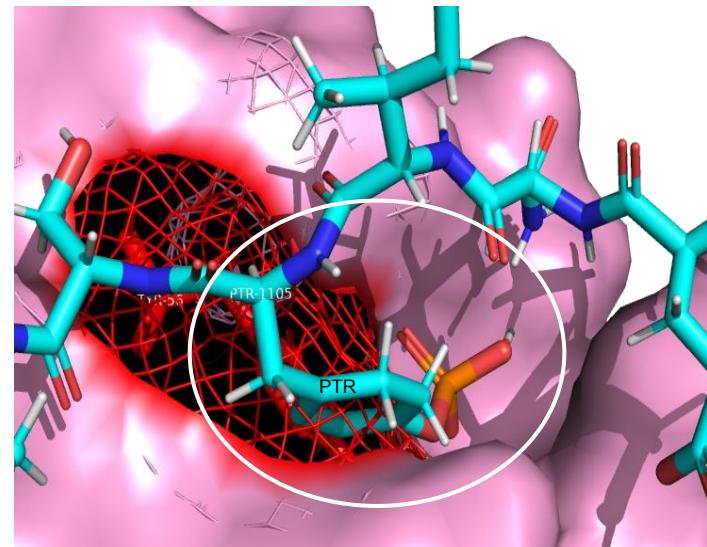
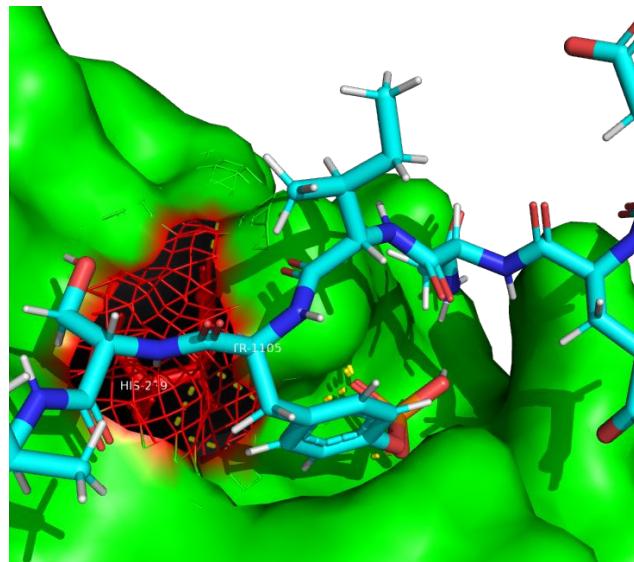
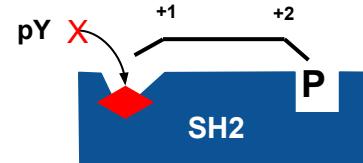
Nature	
HIS	+
TYR	Polar Neutral
Prosa Z-scores	
Template	-6.31
Pos 229	-6.23

p190rhoGAP cannot bind
- Binding pocket for PTR of rhoGAP which is one of the two main residues that SH2 can recognize gets distorted.
- RAS pathway will not be inhibited from over activation
- uncontrolled cell proliferation

Wild Type



Mutant



Wild type vs mutant type mutations

MUTATIONS

Position 229

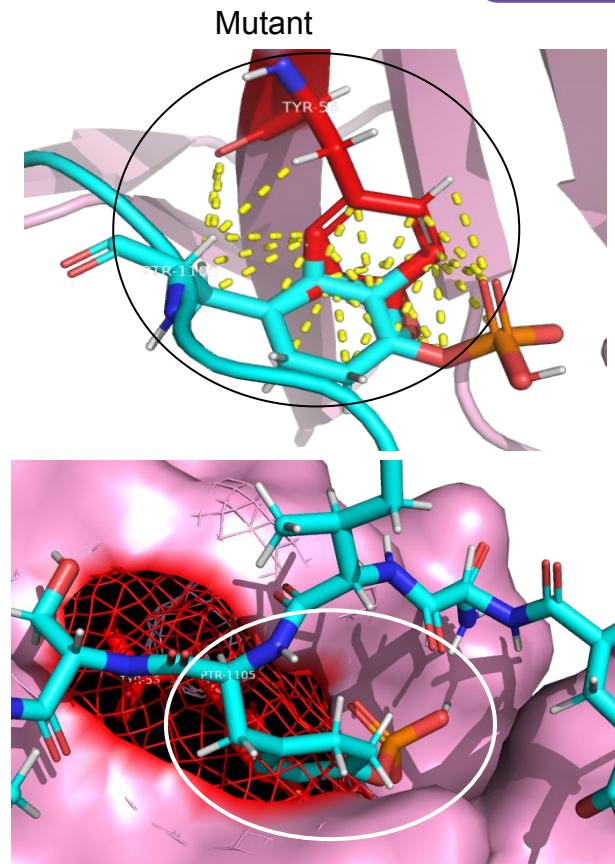
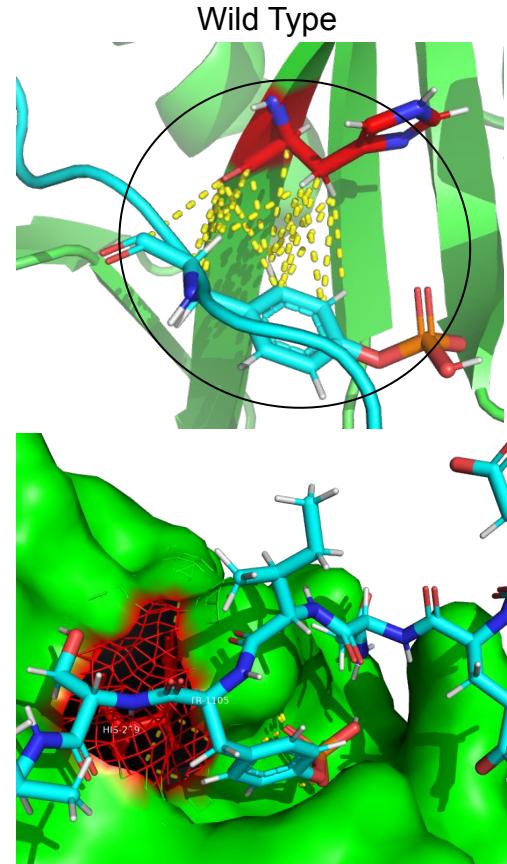
Reference residue: H
Altered residue: Y

Nature	
HIS	+
TYR	Polar Neutral

Prosa Z-scores	
Template	-6.31
Pos 229	-6.23

p190rhoGAP cannot bind

- RAS pathway will not be inhibited from over activation
- uncontrolled cell proliferation



SH2 domain : Wild type vs mutant type

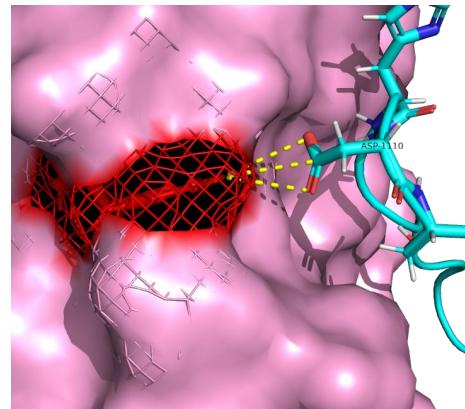
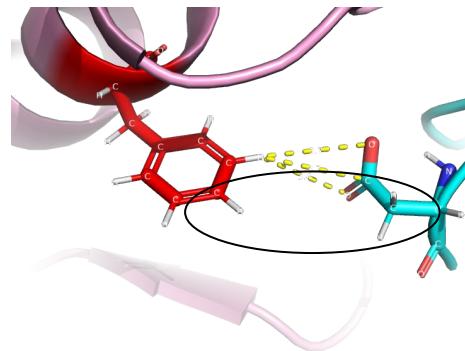
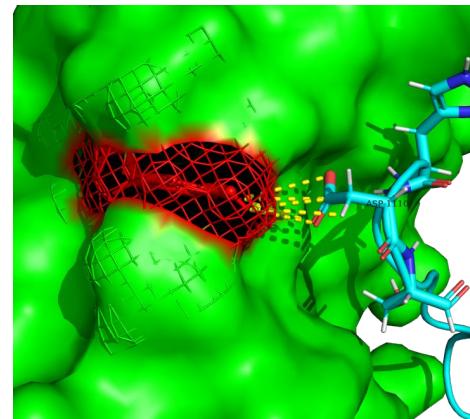
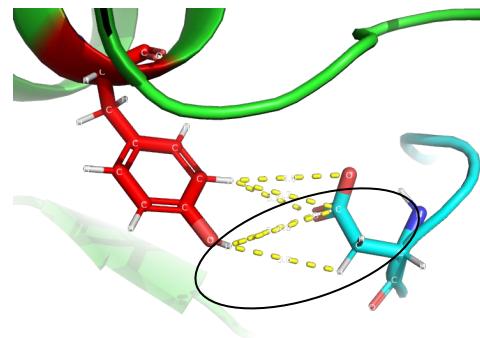
MUTATIONS

Position 225

Reference residue: Tyr
Altered residue: Phe

Nature	
TYR	Polar
PHE	Non-Polar
Prosa Z-scores	
Template	-6.31
Pos 225	-6.23

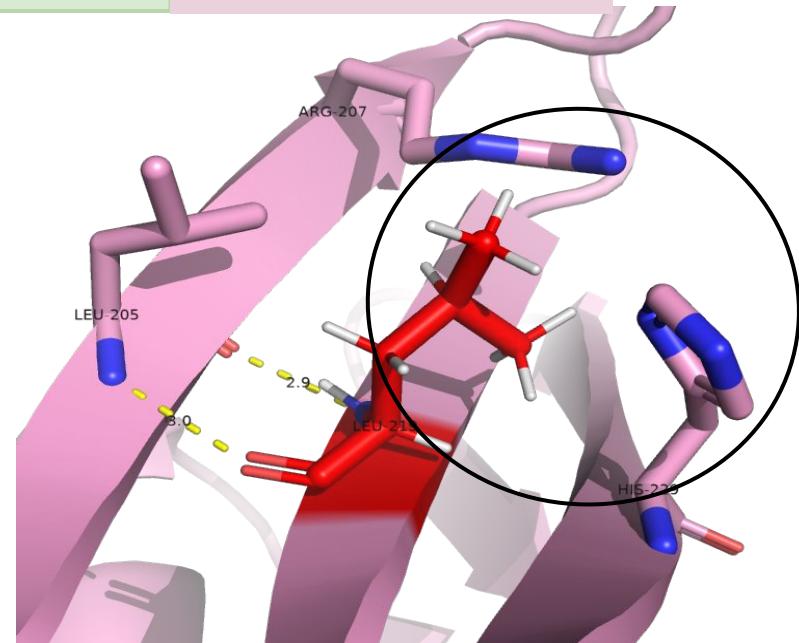
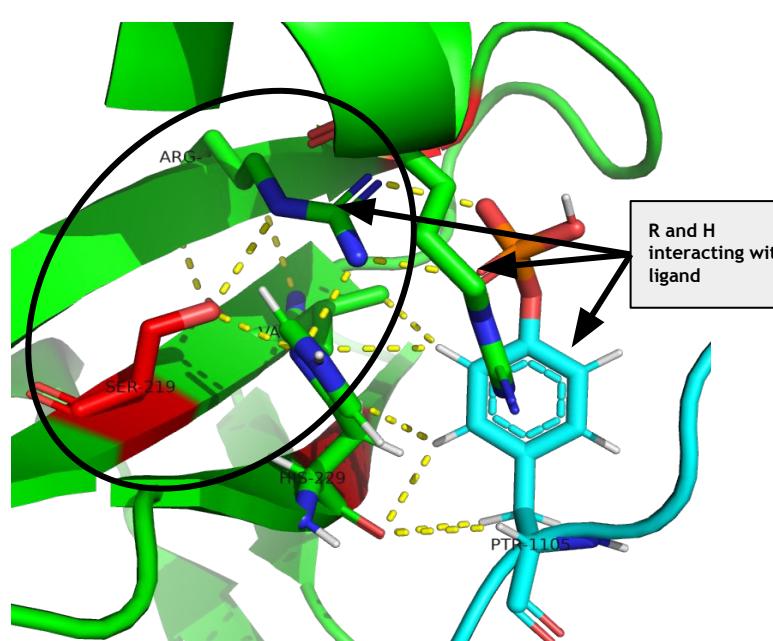
Loss of polar contacts with ligand at this position



SH2 domain : Wild type vs Mutant

MUTATIONS

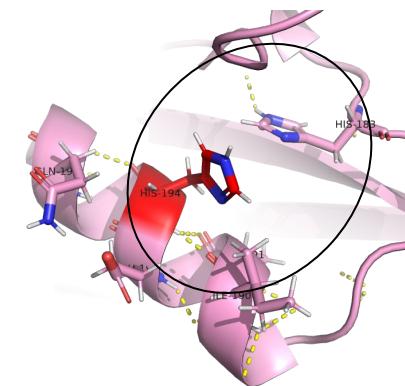
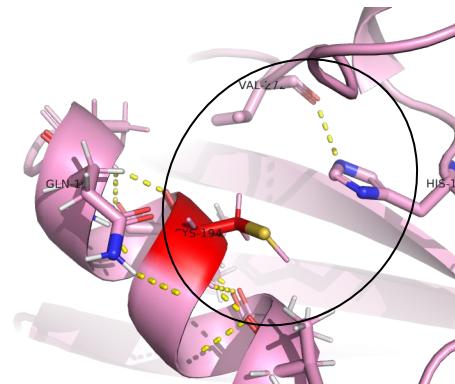
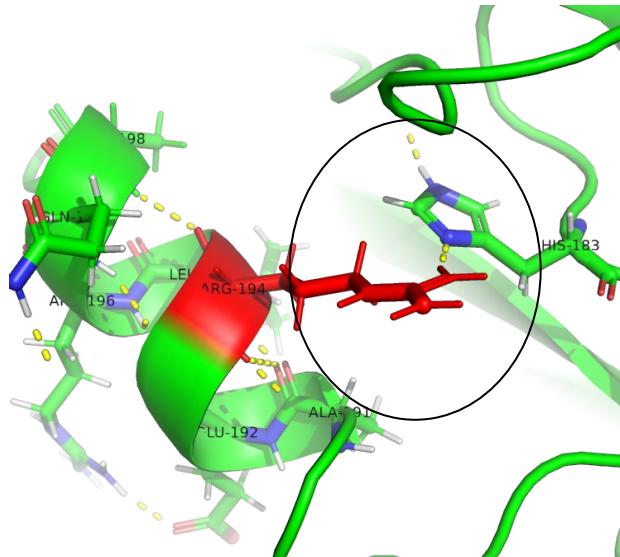
Position 219	Nature		Prosa Z-scores		Indirectly involved in Ligand binding. Loss of polar interactions with nearby ARG(+) and HIS(+)
Reference residue: S	SER	Polar	Template	-6.31	
Altered residue: L	LEU	Non-Polar	Pos 219	-6.27	



Wild type vs mutant type mutations

MUTATIONS

Position 194	
Reference residue: R	
Altered residue: H/C	
Nature	
ARG	+
CYS	Non-Polar
HIS	+
Prosa Z-scores	
Template	-6.31
H / C	-6.33 / -6.2
Loss of H-bond with ligand	



Wild Type VS Mutant Type Mutations → Causing Melanoma

MUTATIONS

**Position 194, 218,
219, 222, 253 267**

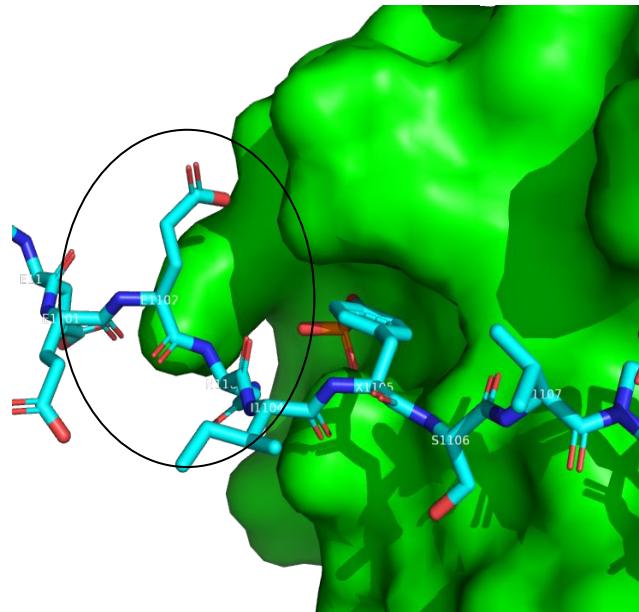
Cancer type: Melanoma

Prosa Z-scores

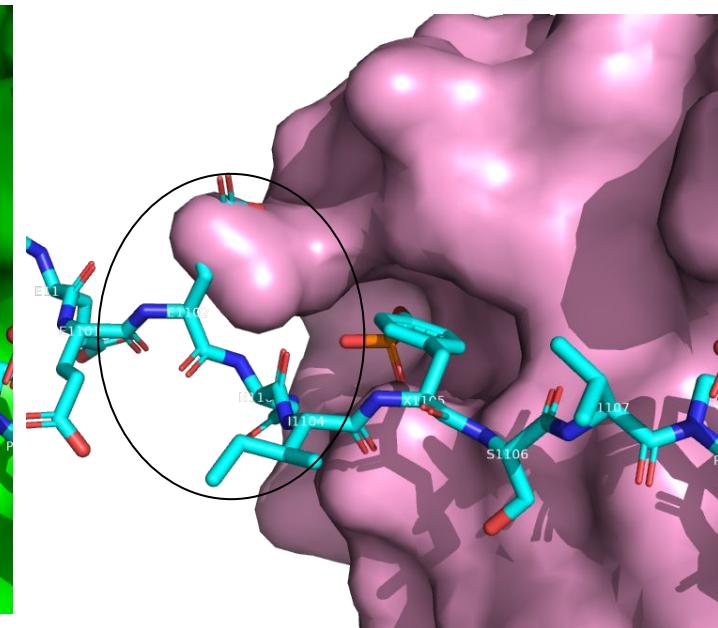
template	-6.31
Melanoma	-6.16

**Distortion of Binding
Pocket**
p190RhoGAP cannot bind
Overactivation of RAS
pathway

Wild Type



Mutant



SH2 domain : Wild type vs mutant → Causing Breast Cancer

MUTATIONS

Position 245, 251, 255

Cancer type: Breast cancer

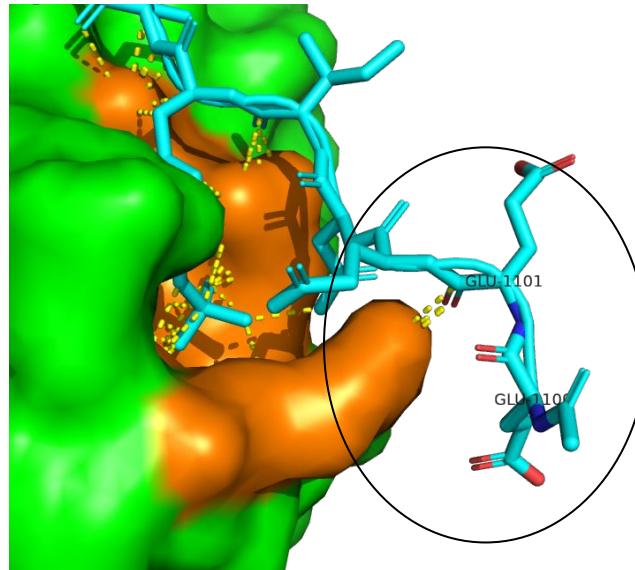
	Z-scores
Template	-6.31
Breast Cancer	-6.02

Distortion of Binding Pocket

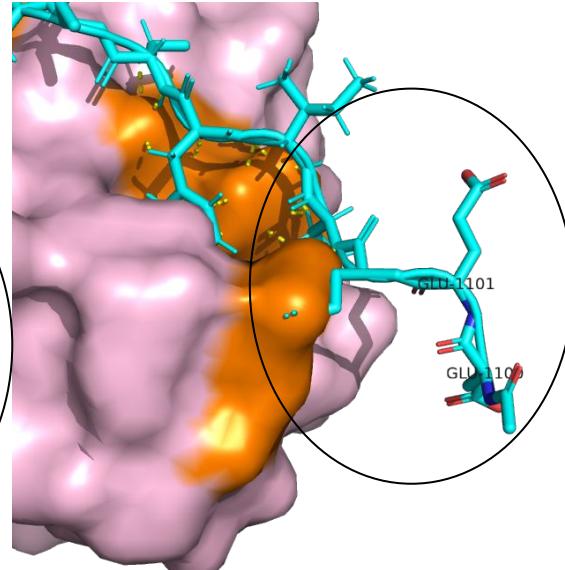
p190RhoGAp cannot bind.

Overactivation of RAS pathway

Wild Type



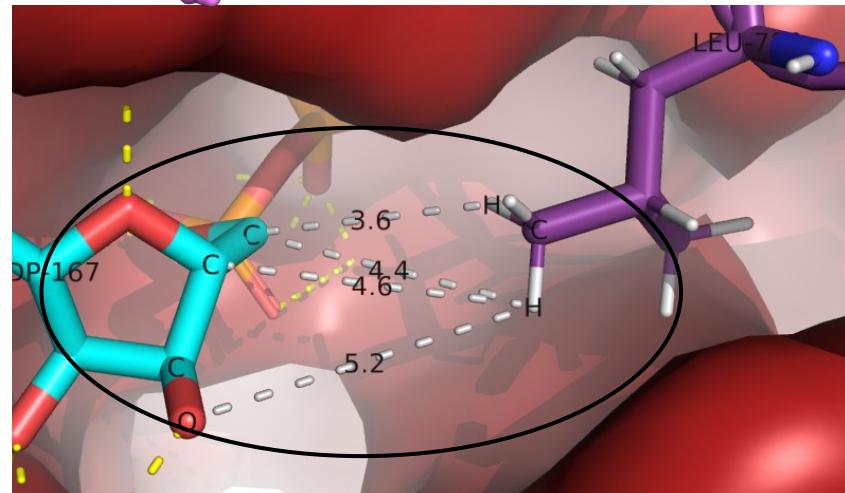
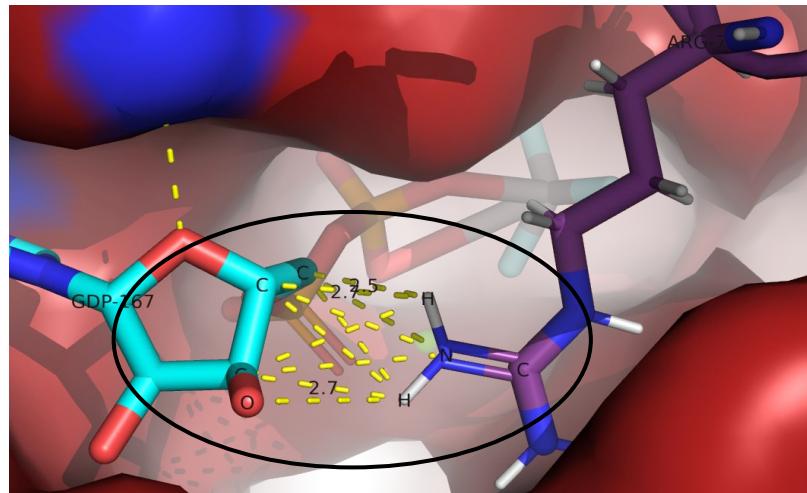
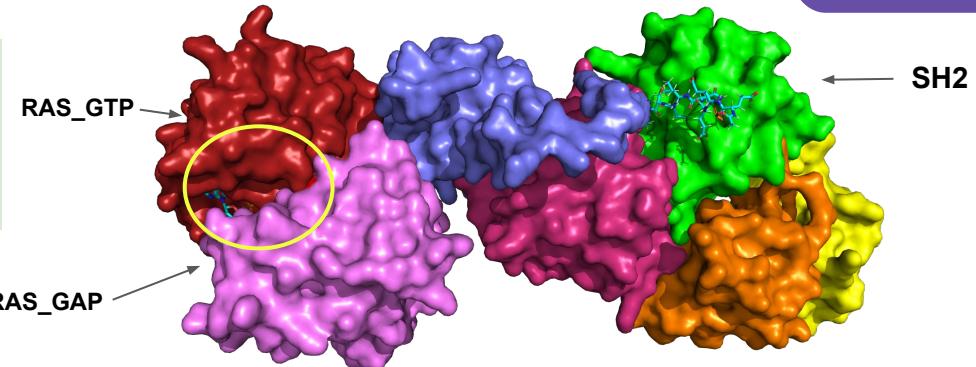
Mutant



Mutation in the Ras_GAP domain causing overactivation of RAS pathway

MUTATIONS

Position 789	
Reference residue: Arg	Loss of GTP binding
Altered residue: Leu	Loss of RAS deactivation



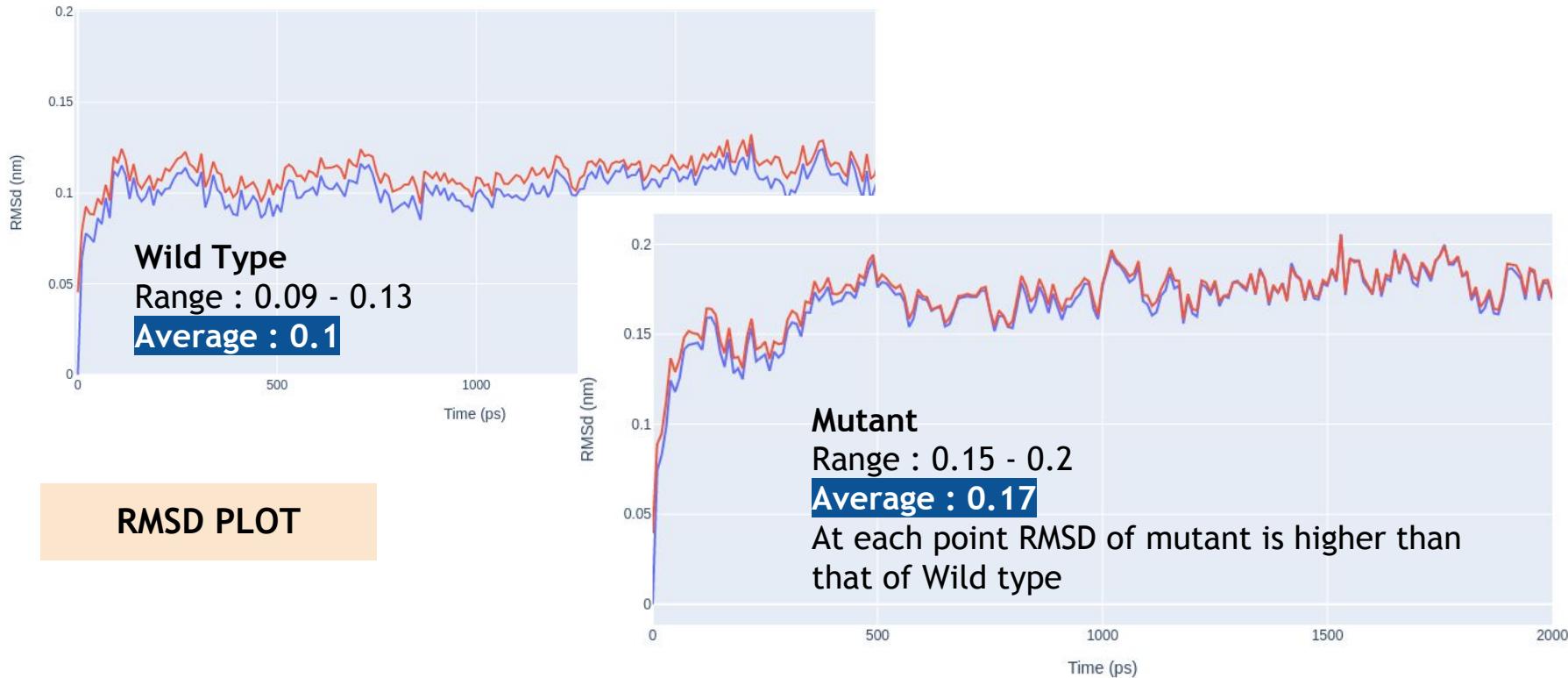
Molecular Dynamic Simulation

Energy Minimization , NVT and NPT equilibration

Simulations

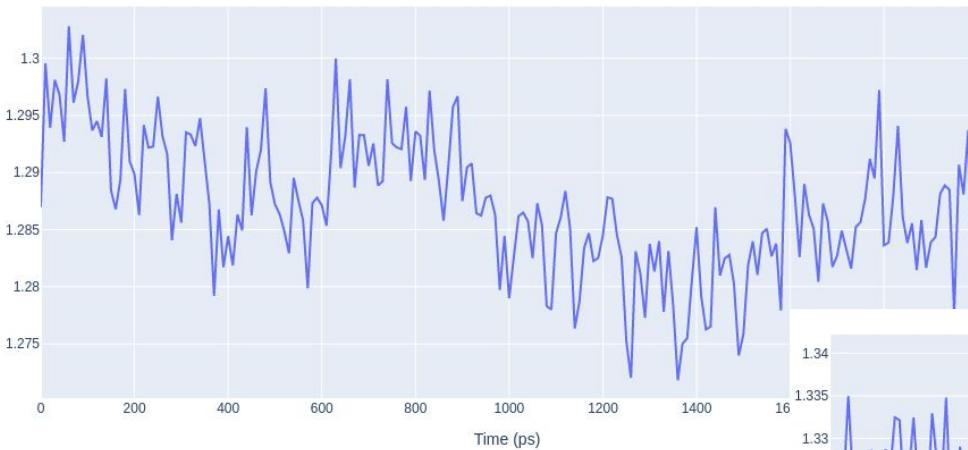
Simulation results of wild type and melanoma mutant

Simulations

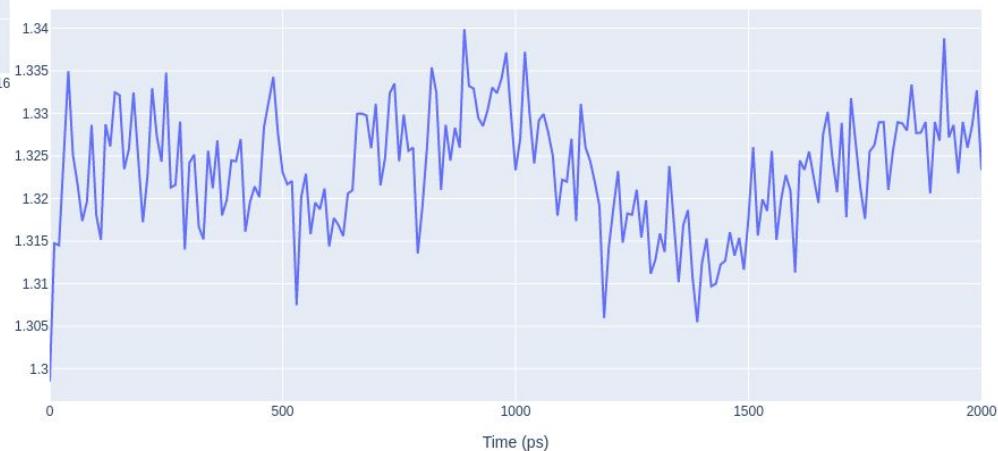


Simulation results of wild type and melanoma mutant

Simulations



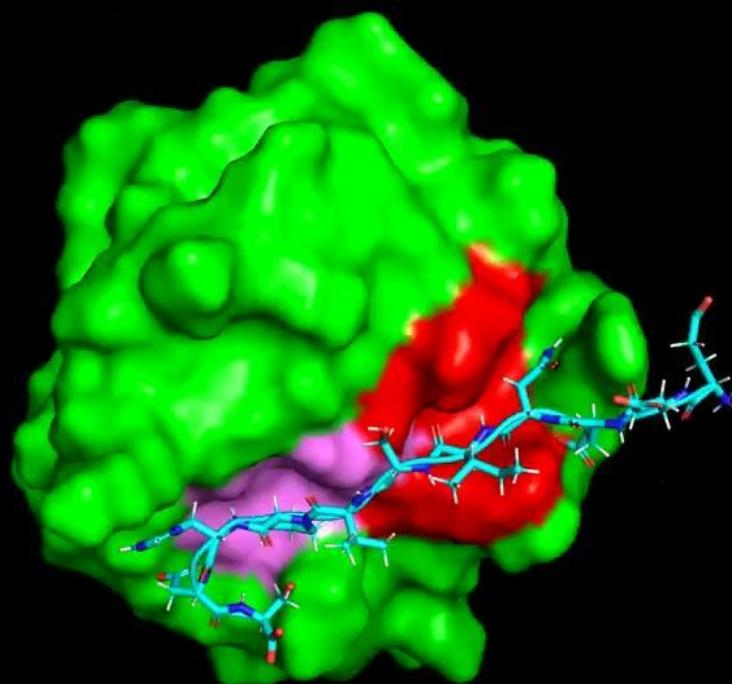
Mutant
Range : 1.32 - 1.335



Radius of Gyration

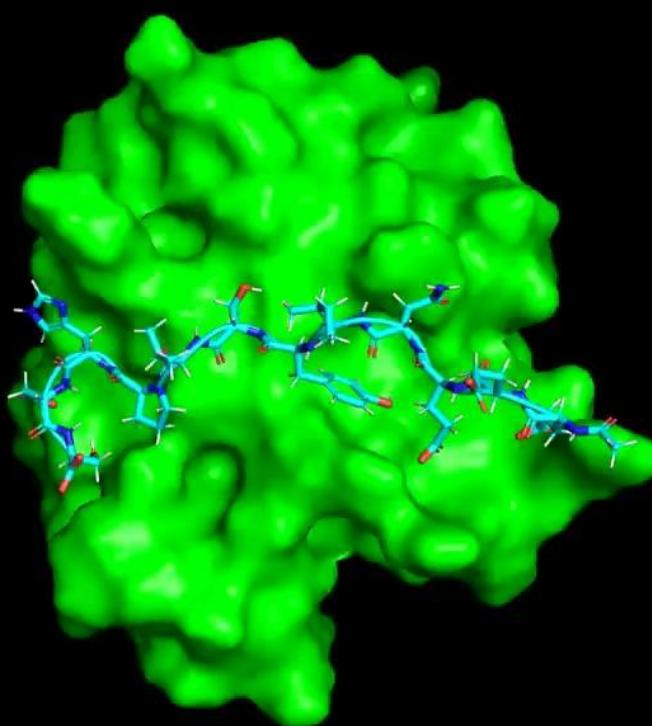
Simulation of SH2 domain bound to a p190RhoGAP phosphotyrosine peptide (Wild Type)

Simulations



Simulation of SH2 domain bound to a p190RhoGAP phosphotyrosine peptide (Mutant Type)

Simulations



Alanine Scanning

Analysis

Statistical Analysis on geo dataset of gene expression

Analysis

Differential Gene expression

Analysis

Extra

G-Proteins interaction

INTRODUCTION

Ras GTPase-activating protein

