Predicted tertiary structure of the plant G-protein coupled receptor (GCR1) coupled with the Galpha Protein (GPA1), with and without ligands.

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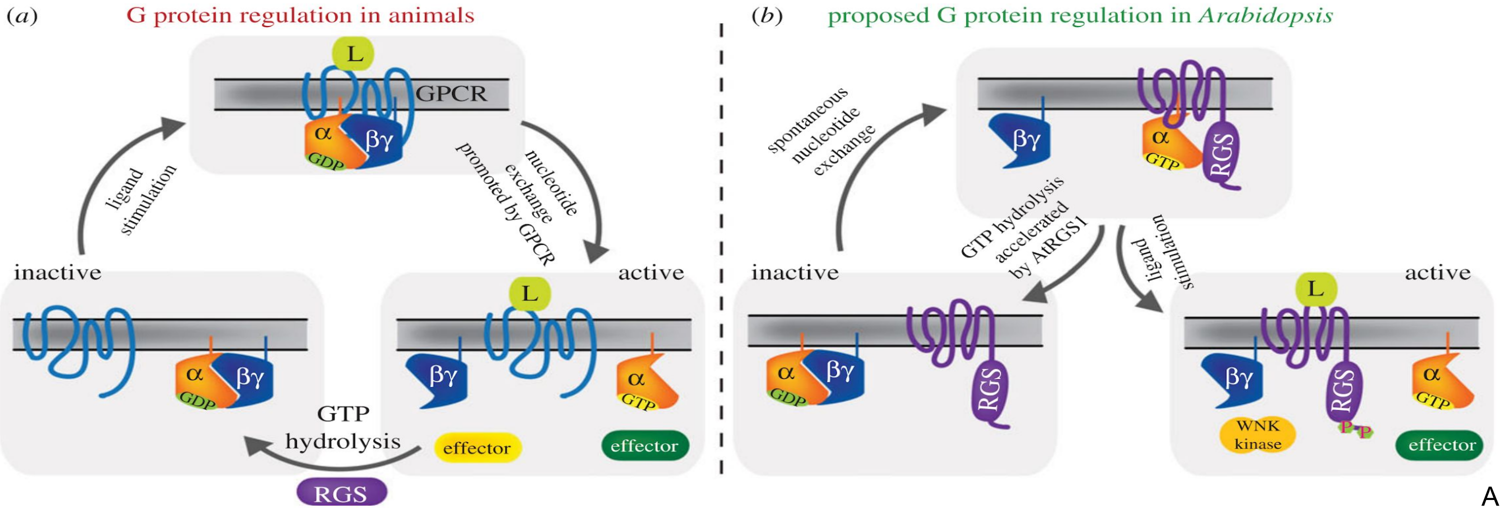
William Goddard, Ph.D.

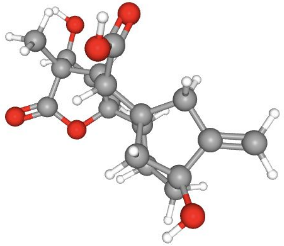
ómicas

Pontificia Universidad
JAVERIANA
Cali

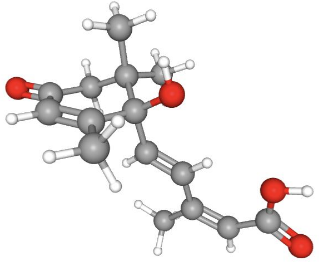
A comparative view of the signaling mechanism through the G-protein in animal and plant cells.



Gibberellin A1



Abscisic Acid



required? Urano et al. Open biology. 2013;3: 120186. (image source)

ation of alpha subunit: Johnston et al. 2007. *Proceedings of the National Academy of Sciences of the United States of America,* **104**(44)

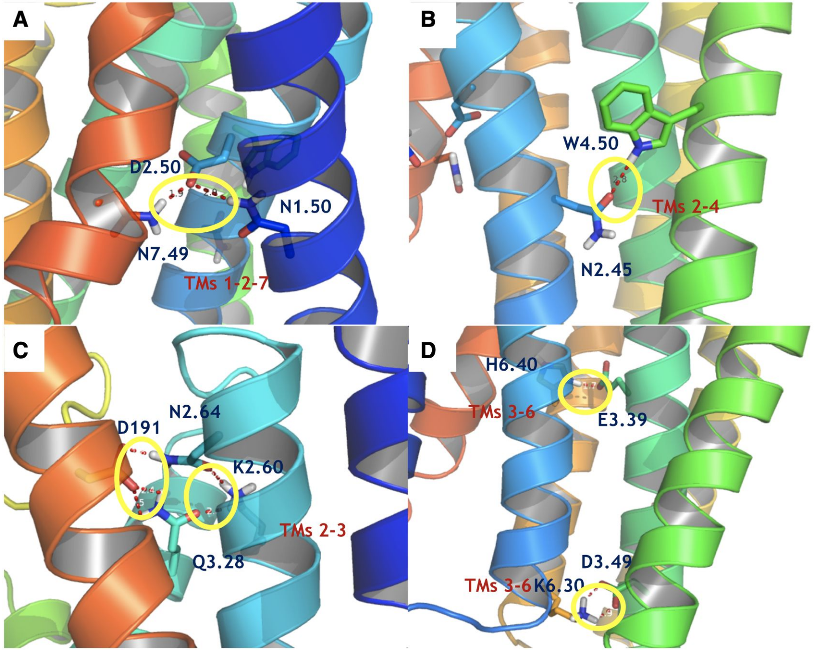
putative Arabidopsis G protein-coupled receptor gene is cell cycle-regulated, and its overexpression abolishes seed dormancy and time to flowering. Colucci et al. 2002. *Proceedings of the National Academy of Sciences.*

GPA1 coupling regulates nitrate , cell wall , immunity and light responses in Arabidopsis. Chakraborty et al. 2019.

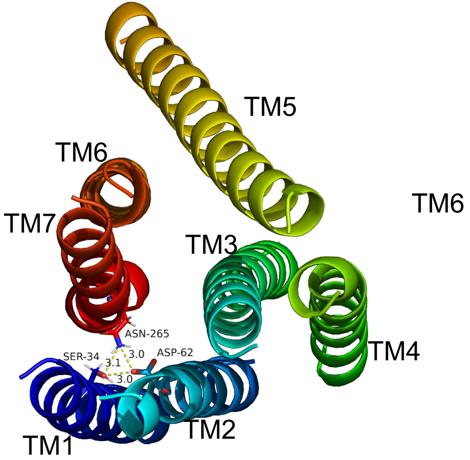
We propose that GCR1 could help regulate the GP

A comparative view between networks found in animal GPCRs and GCR1

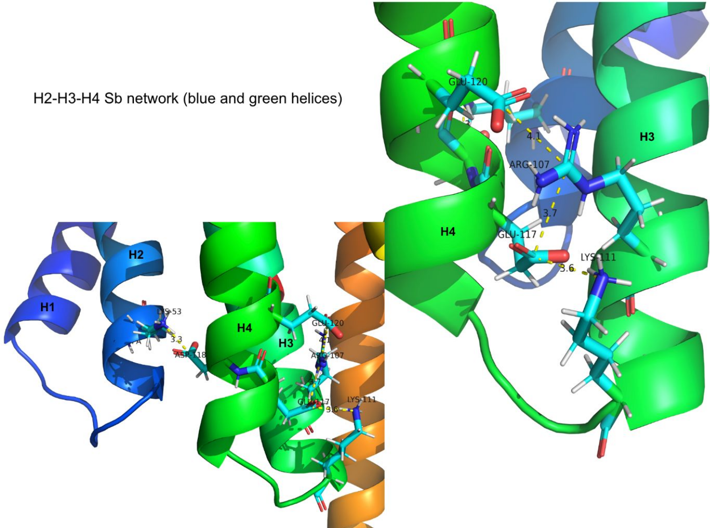
animal GPCR networks

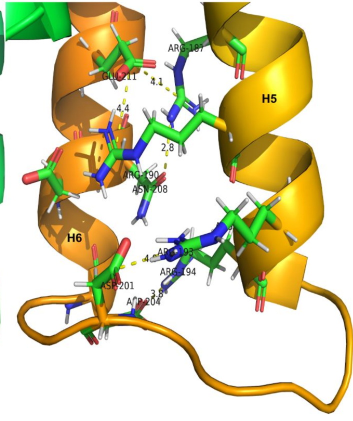


GCR1 network



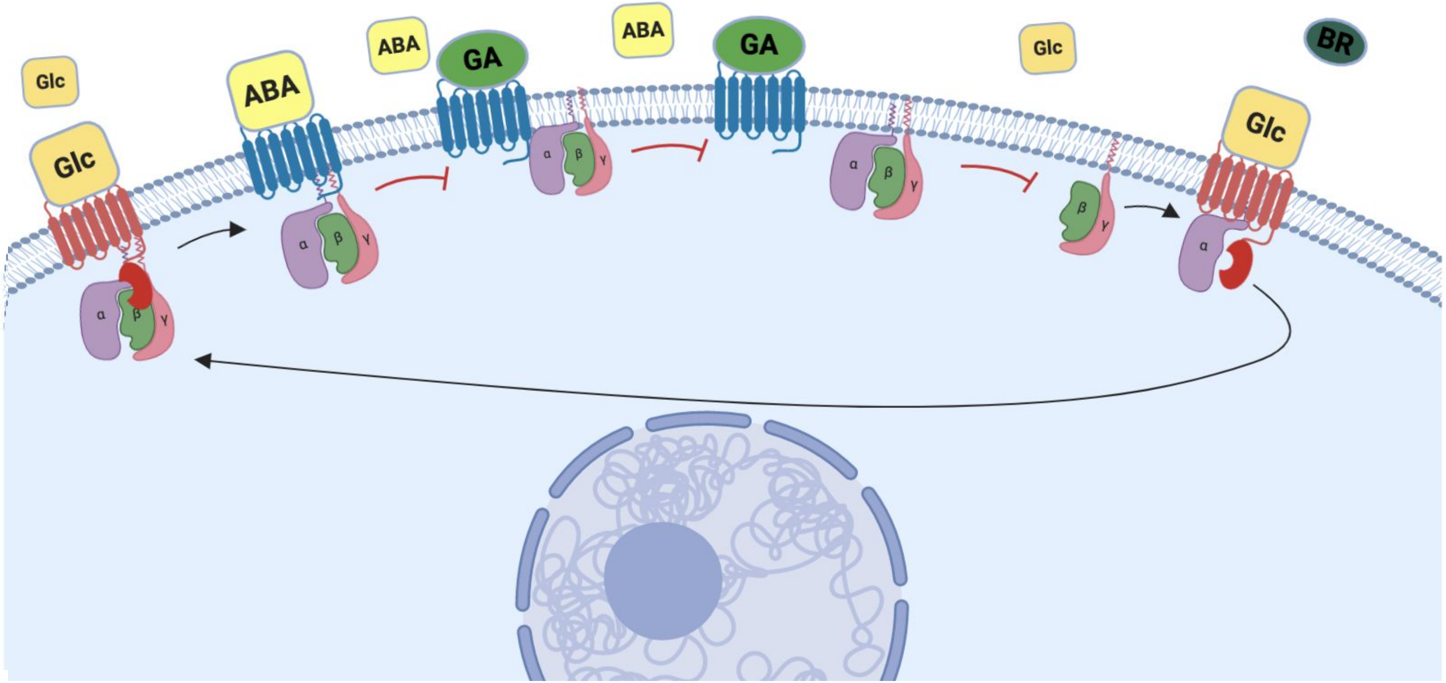
H2-H3-H4 Sb network (blue and green helices)





H5-H6 Sb network (yellow and orange helices)

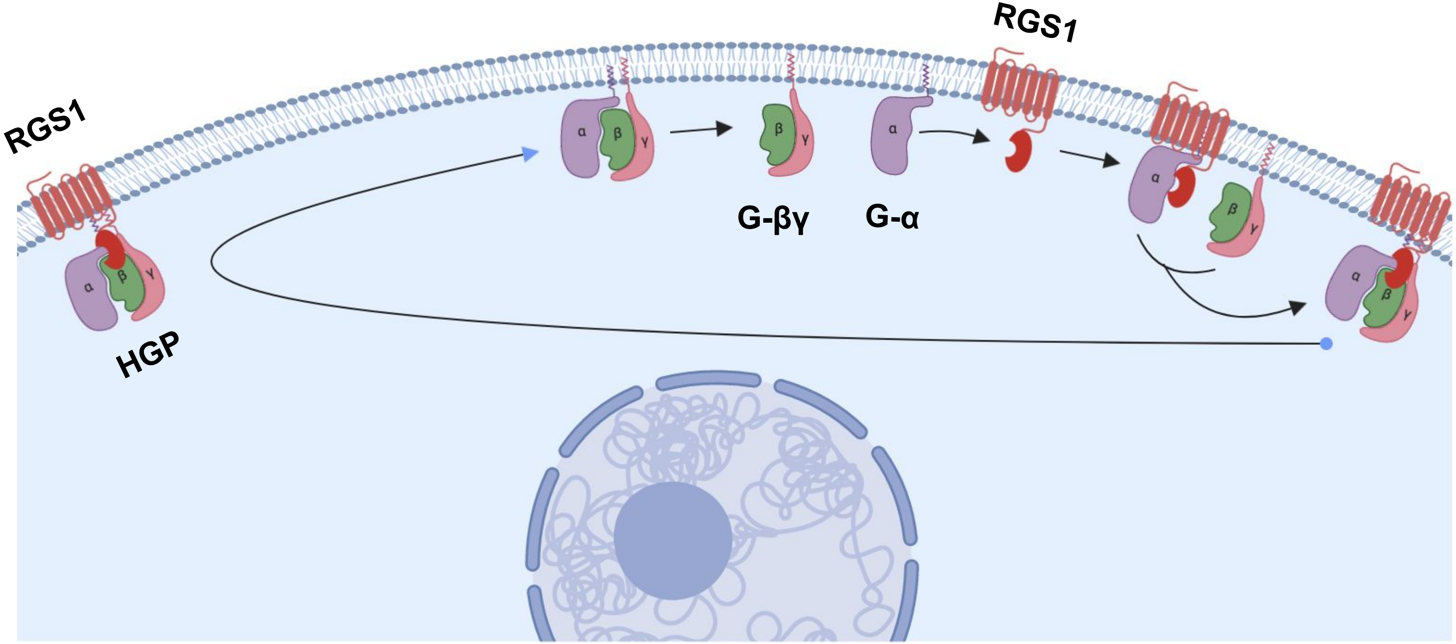
Synergistic activity of RGS1 and GCR1 regulating the G-protein signaling pathway



Although RGS1 is a regulatory protein of GPA1, GCR1 is needed to keep the inactivation on the GP

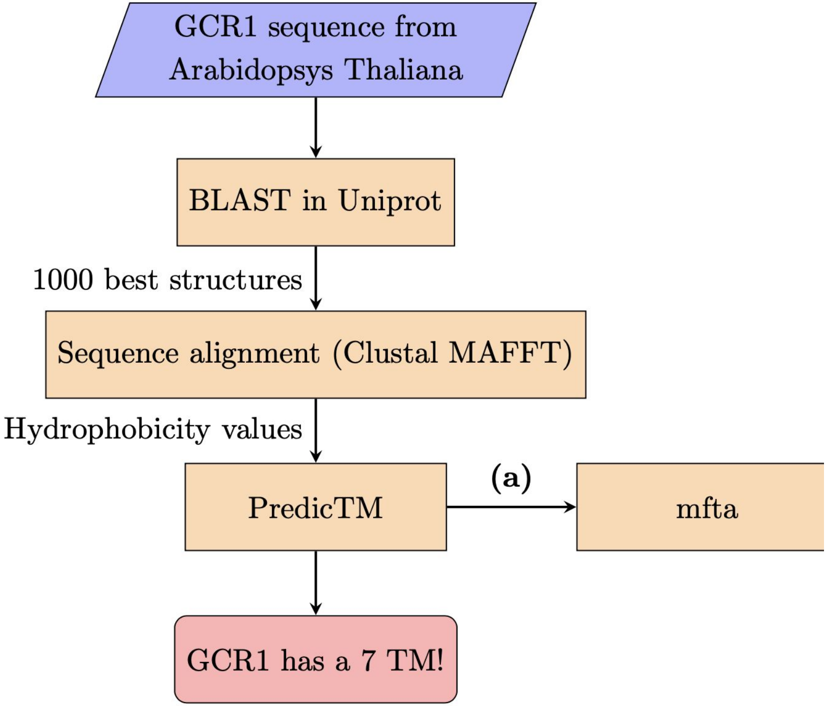
Several active states per GPCR: Gray et al. Proceedings of the National Academy of Sciences of the United States of America 2014 Jan 7,;111(1):12.
Evidence of heterotrimer signalling in plants: *Chen et al. PLANT PHYSIOLOGY 2006,* ***141****(3)*
GCR1 internalization: Pandey and Assmann The Plant Cell, , 2004. **16**(6)
CR1 and GPA1 regulate DNA synthesis through PI- PLC activation and IP_3 and DAG production. Apone et al. PLANT PHYSIOLOGY, 2003. **133**(2)

Futile cycle



Is RGS1 hyper-activated?

Prediction of the starting tertiary structure of GCR1 from its primary sequence



GCR1 sequence from Arabidopsys Thaliana

BLAST in Uniprot

1000 best structures

Sequence alignment (Clustal MAFFT)

Hydrophobicity values

PredicTM

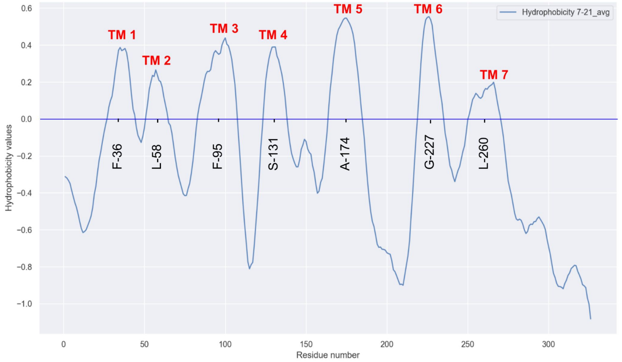
(a)

mfta

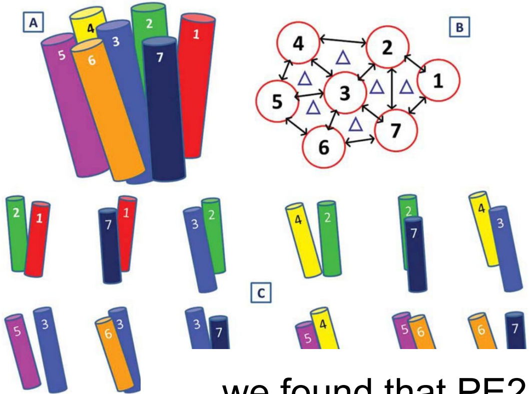
GCR1 has a 7 TM!

(a): Plant based: eta residues, hydrophobic centers, helix size

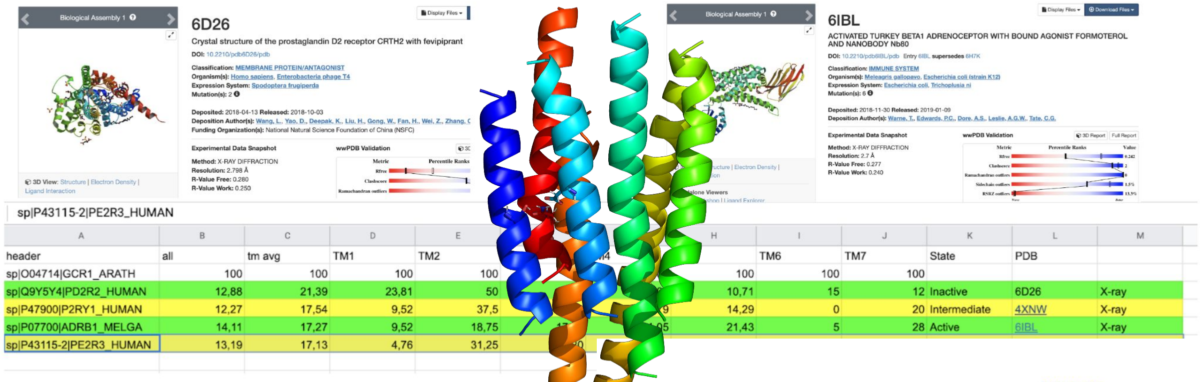
GCR1 has no crystal structure, we predicted its tertiary structure using GEnSeMBLE complete sampling



Prediction of TM regions



Selected templates



6D26

Crystal structure of the prostaglandin D2 receptor CRTH2 with fevipiprant

DOI: 10.2210/pdb6D26/pdb

Classification: MEMBRANE PROTEIN/ANTAGONIST
Organism(s): Homo sapiens, Enterobacteria phage T4
Expression System: Spodoptera frugiperda
Mutation(s): 2

Deposited: 2018-04-13 Released: 2018-10-03
Deposition Author(s): Wang, L., Yao, D., Deepak, K., Liu, H., Gong, W., Fan, H., Wei, Z., Zhang, C
Funding Organization(s): National Natural Science Foundation of China (NSFC)

Experimental Data Snapshot
Method: X-RAY DIFFRACTION
Resolution: 2.798 Å
R-Value Free: 0.280
R-Value Work: 0.250

wwPDB Validation

6IBL

ACTIVATED TURKEY BETA1 ADRENOCEPTOR WITH BOUND AGONIST FORMOTEROL AND NANOBODY Nb80

DOI: 10.2210/pdb6IBL/pdb Entry 6IBL supersedes 6H7K

Classification: IMMUNE SYSTEM
Organism(s): Meleagris gallopavo, Escherichia coli (strain K12)
Expression System: Escherichia coli, Trichoplusia ni
Mutation(s): 6

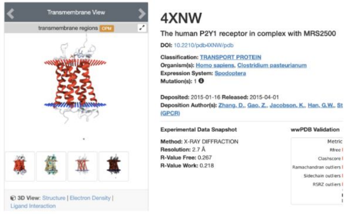
Deposited: 2018-11-30 Released: 2019-01-09
Deposition Author(s): Warne, T., Edwards, P.C., Dore, A.S., Leslie, A.G.W., Tate, C.G.

Experimental Data Snapshot
Method: X-RAY DIFFRACTION
Resolution: 2.7 Å
R-Value Free: 0.277
R-Value Work: 0.240

wwPDB Validation

sp|P43115-2|PE2R3_HUMAN

A	B	C	D	E	F	G	H	I	J	K	L	M
header	all	tm avg	TM1	TM2	[illegible]	M4	[illegible]	TM6	TM7	State	PDB	
sp|O04714|GCR1_ARATH	100	100	100	100	[illegible]	[illegible]	100	100	100			
sp|Q9Y5Y4|PD2R2_HUMAN	12,88	21,39	23,81	50	[illegible]	[illegible]	10,71	15	12	Inactive	6D26	X-ray
sp|P47900|P2RY1_HUMAN	12,27	17,54	9,52	37,5	[illegible]	[illegible]	14,29	0	20	Intermediate	4XNW	X-ray
sp|P07700|ADRB1_MELGA	14,11	17,27	9,52	18,75	[illegible]	[illegible]	21,43	5	28	Active	6IBL	X-ray
sp|P43115-2|PE2R3_HUMAN	13,19	17,13	4,76	31,25	[illegible]							



4XNW

The human P2Y1 receptor in complex with MRS2500

DOI: 10.2210/pdb4XNW/pdb

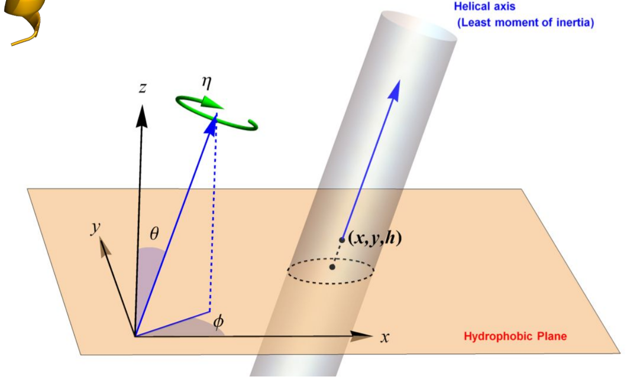
Classification: TRANSPORT PROTEIN
Organism(s): Homo sapiens, Clostridium pasteurianum
Expression System: Spodoptera
Mutation(s): 1

Deposited: 2015-01-16 Released: 2015-04-01
Deposition Author(s): Zhang, D., Gao, Z., Jacobson, K., Han, G.W., St
(GPCR)

Experimental Data Snapshot
Method: X-RAY DIFFRACTION
Resolution: 2.7 Å
R-Value Free: 0.267
R-Value Work: 0.218

wwPDB Validation

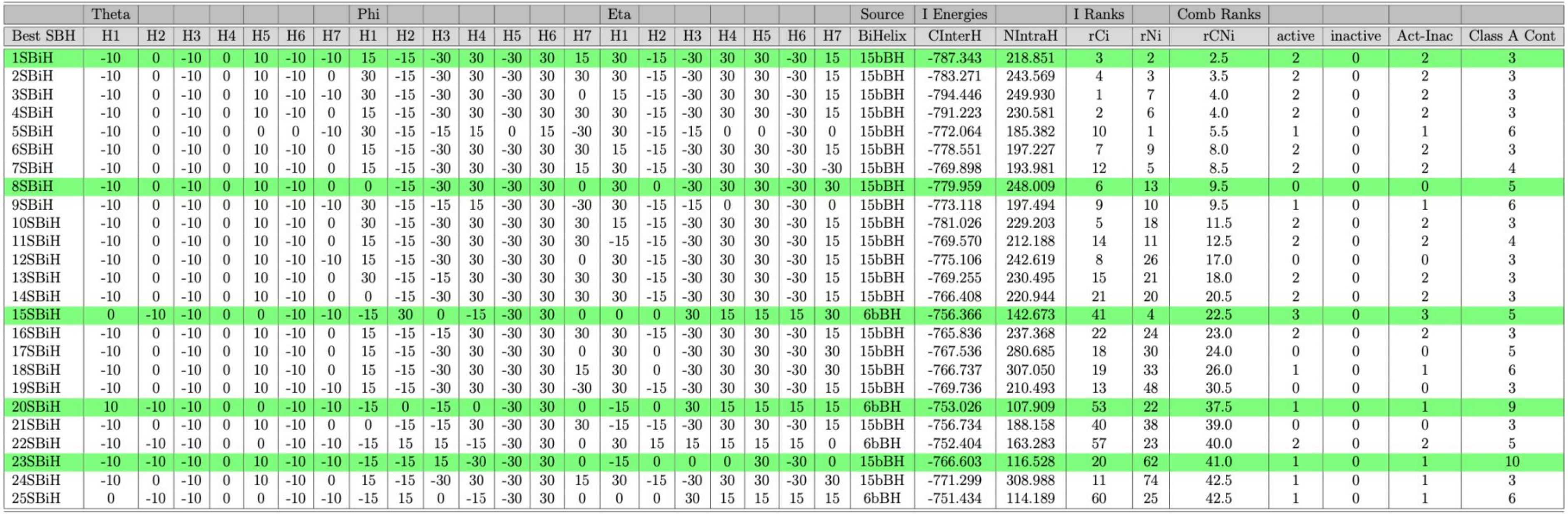
BiHelix and SuperBiHelix sampling



we found that PE2R3 is the best template to generate the initial GCR1 structure.

Bray, J. K.; Abrol, R., Goddard, W. A., et al. Proceedings of the National Academy of Sciences. 2014

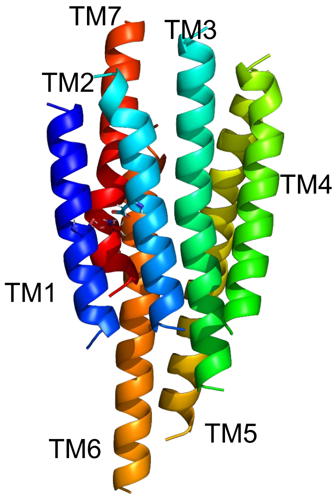
Prediction of the tertiary structure of GCR1 from its primary sequence

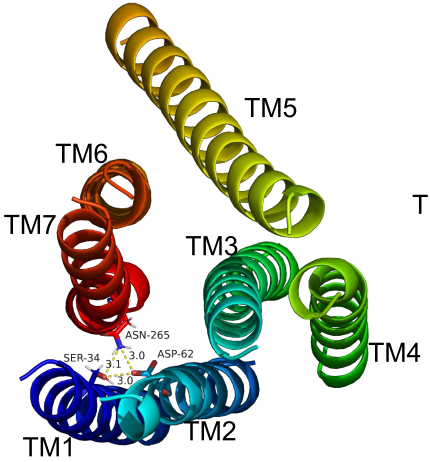


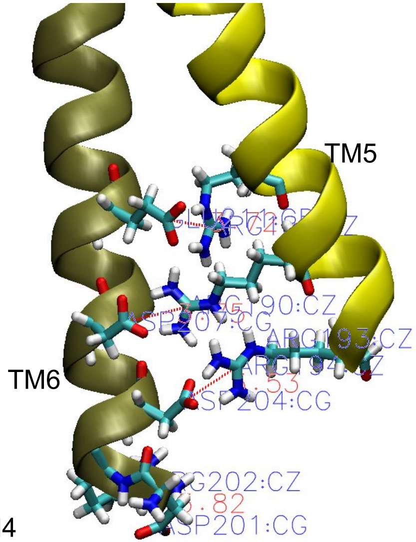
	Theta							Phi							Eta							Source	I Energies		I Ranks		Comb Ranks				
Best SBH	H1	H2	H3	H4	H5	H6	H7	H1	H2	H3	H4	H5	H6	H7	H1	H2	H3	H4	H5	H6	H7	BiHelix	CInterH	NIntraH	rCi	rNi	rCNi	active	inactive	Act-Inac	Class A Cont
1SBiH	-10	0	-10	0	10	-10	-10	15	-15	-30	30	-30	30	15	30	-15	-30	30	30	-30	15	15bBH	-787.343	218.851	3	2	2.5	2	0	2	3
2SBiH	-10	0	-10	0	10	-10	0	30	-15	-30	30	-30	30	30	30	-15	-30	30	30	-30	15	15bBH	-783.271	243.569	4	3	3.5	2	0	2	3
3SBiH	-10	0	-10	0	10	-10	-10	30	-15	-30	30	-30	30	0	15	-15	-30	30	30	-30	15	15bBH	-794.446	249.930	1	7	4.0	2	0	2	3
4SBiH	-10	0	-10	0	10	-10	0	15	-15	-30	30	-30	30	30	30	-15	-30	30	30	-30	15	15bBH	-791.223	230.581	2	6	4.0	2	0	2	3
5SBiH	-10	0	-10	0	0	0	-10	30	-15	-15	15	0	15	-30	30	-15	-15	0	0	-30	0	15bBH	-772.064	185.382	10	1	5.5	1	0	1	6
6SBiH	-10	0	-10	0	10	-10	0	15	-15	-30	30	-30	30	30	15	-15	-30	30	30	-30	15	15bBH	-778.551	197.227	7	9	8.0	2	0	2	3
7SBiH	-10	0	-10	0	10	-10	0	15	-15	-30	30	-30	30	15	30	-15	-30	30	30	-30	-30	15bBH	-769.898	193.981	12	5	8.5	2	0	2	4
8SBiH	-10	0	-10	0	10	-10	0	0	-15	-30	30	-30	30	0	30	0	-30	30	30	-30	30	15bBH	-779.959	248.009	6	13	9.5	0	0	0	5
9SBiH	-10	0	-10	0	10	-10	-10	30	-15	-15	15	-30	30	-30	30	-15	-15	0	30	-30	0	15bBH	-773.118	197.494	9	10	9.5	1	0	1	6
10SBiH	-10	0	-10	0	10	-10	0	30	-15	-30	30	-30	30	30	15	-15	-30	30	30	-30	15	15bBH	-781.026	229.203	5	18	11.5	2	0	2	3
11SBiH	-10	0	-10	0	10	-10	0	15	-15	-30	30	-30	30	30	-15	-15	-30	30	30	-30	15	15bBH	-769.570	212.188	14	11	12.5	2	0	2	4
12SBiH	-10	0	-10	0	10	-10	-10	15	-15	-30	30	-30	30	0	30	-15	-30	30	30	-30	15	15bBH	-775.106	242.619	8	26	17.0	0	0	0	3
13SBiH	-10	0	-10	0	10	-10	0	30	-15	-15	30	-30	30	30	30	-15	-30	30	30	-30	15	15bBH	-769.255	230.495	15	21	18.0	2	0	2	3
14SBiH	-10	0	-10	0	10	-10	0	0	-15	-30	30	-30	30	30	30	-15	-30	30	30	-30	15	15bBH	-766.408	220.944	21	20	20.5	2	0	2	3
15SBiH	0	-10	-10	0	0	-10	-10	-15	30	0	-15	-30	30	0	0	0	30	15	15	15	30	6bBH	-756.366	142.673	41	4	22.5	3	0	3	5
16SBiH	-10	0	-10	0	10	-10	0	15	-15	-15	30	-30	30	30	30	-15	-30	30	30	-30	15	15bBH	-765.836	237.368	22	24	23.0	2	0	2	3
17SBiH	-10	0	-10	0	10	-10	0	15	-15	-30	30	-30	30	0	30	0	-30	30	30	-30	30	15bBH	-767.536	280.685	18	30	24.0	0	0	0	5
18SBiH	-10	0	-10	0	10	-10	0	15	-15	-30	30	-30	30	15	30	0	-30	30	30	-30	30	15bBH	-766.737	307.050	19	33	26.0	1	0	1	6
19SBiH	-10	0	-10	0	10	-10	-10	15	-15	-30	30	-30	30	-30	30	-15	-30	30	30	-30	15	15bBH	-769.736	210.493	13	48	30.5	0	0	0	3
20SBiH	10	-10	-10	0	0	-10	-10	-15	0	-15	0	-30	30	0	-15	0	30	15	15	15	15	6bBH	-753.026	107.909	53	22	37.5	1	0	1	9
21SBiH	-10	0	-10	0	10	-10	0	0	-15	-15	30	-30	30	30	-15	-15	-30	30	30	-30	15	15bBH	-756.734	188.158	40	38	39.0	0	0	0	3
22SBiH	-10	-10	-10	0	0	-10	-10	-15	15	15	-15	-30	30	0	30	15	15	15	15	15	0	6bBH	-752.404	163.283	57	23	40.0	2	0	2	5
23SBiH	-10	-10	-10	0	10	-10	-10	-15	-15	15	-30	-30	30	0	-15	0	0	0	30	-30	0	15bBH	-766.603	116.528	20	62	41.0	1	0	1	10
24SBiH	-10	0	-10	0	10	-10	0	15	-15	-30	30	-30	30	15	30	-15	-30	30	30	-30	30	15bBH	-771.299	308.988	11	74	42.5	1	0	1	3
25SBiH	0	-10	-10	0	0	-10	-10	-15	15	0	-15	-30	30	0	0	0	30	15	15	15	15	6bBH	-751.434	114.189	60	25	42.5	1	0	1	6

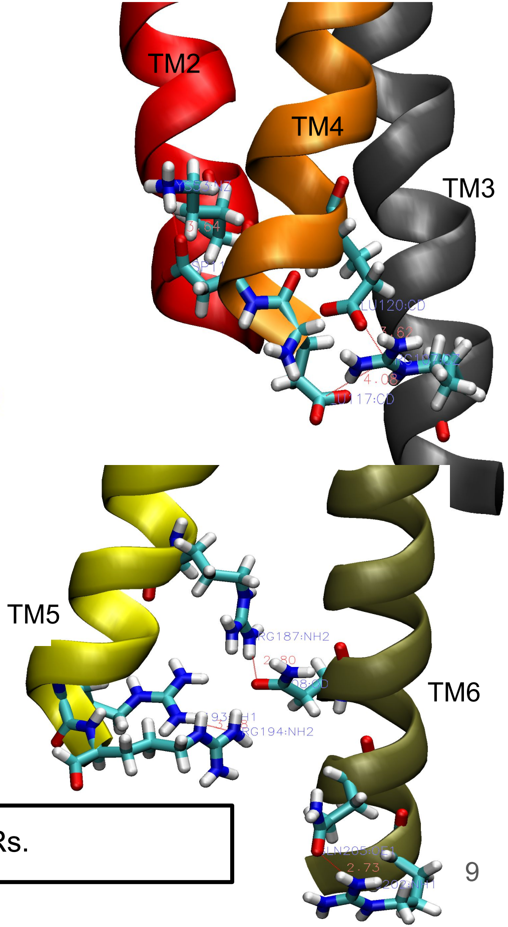
Selected 7TM bundles based on energy and diversity of the angles evaluated.

Prediction of the tertiary structure of GCR1 using the GEnSemBLE complete sampling.



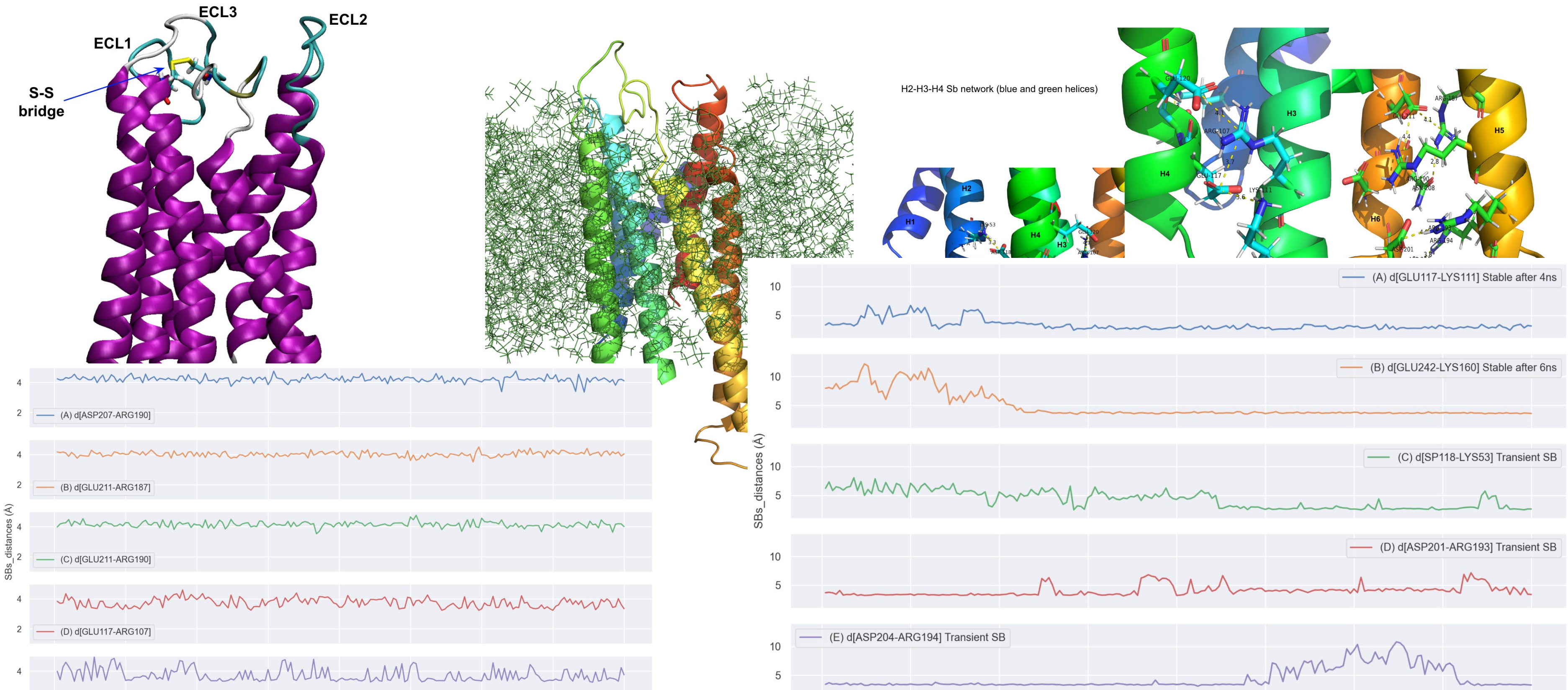






GCR1 has the 7TMD similar to those found in animal GPCRs.

Annealing and MD simulation of SuperBiHelix's newly constructed GCR1 structures

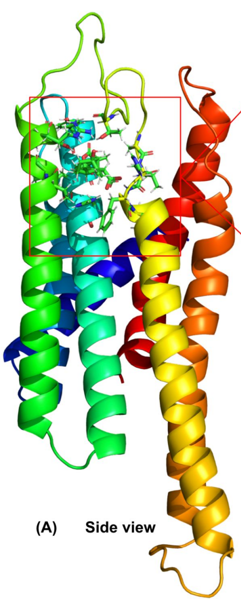


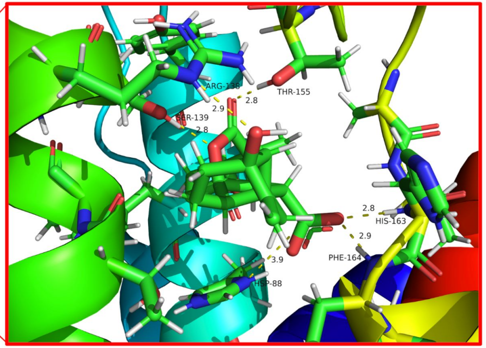
GCR1 has an stable packaging similar to that found in animal GPCRs, then, GCR1 would be a first confirmed plant homolog GPCR

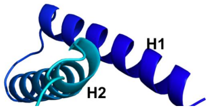
Stable SBs

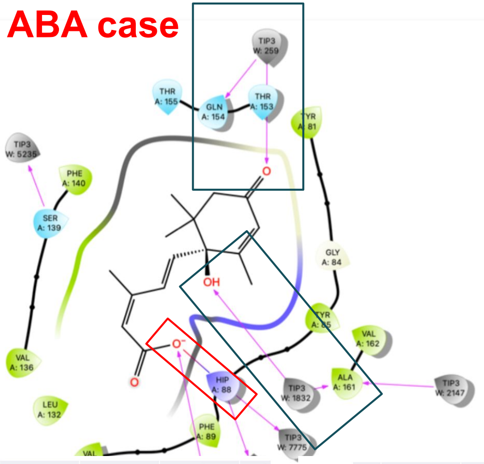
Transient SBs

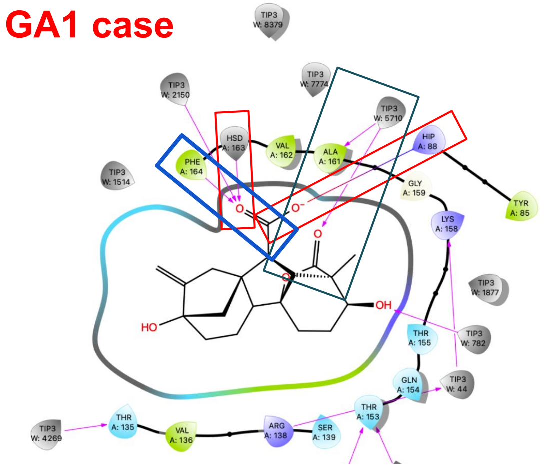
Docking and MD simulation of GCR1 with abscisic acid (ABA) and gibberellin A1 (GA1)

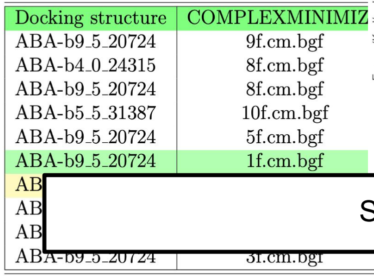




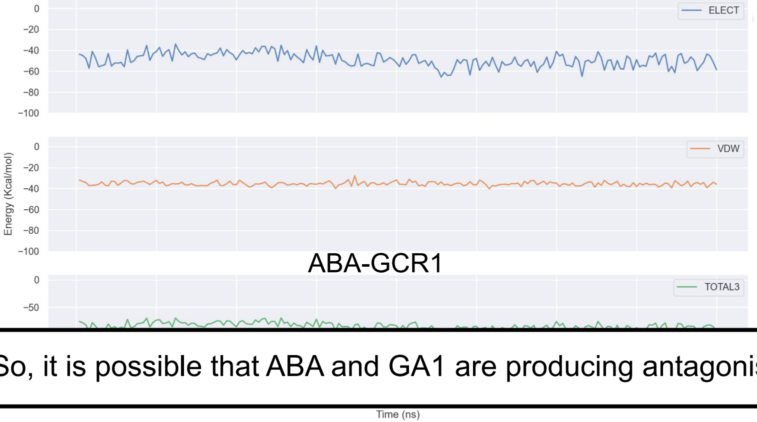


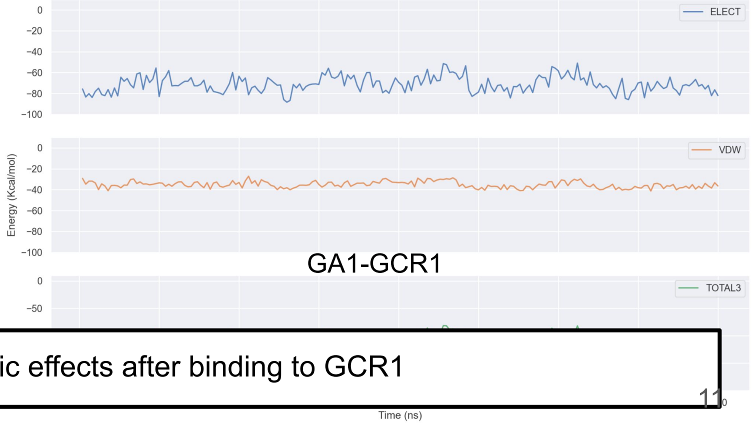

ABA case


GA1 case



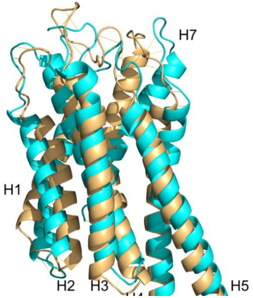
Docking structure	COMPLEXMINIMIZ
ABA-b9_5_20724	9f.cm.bgf
ABA-b4_0_24315	8f.cm.bgf
ABA-b9_5_20724	8f.cm.bgf
ABA-b5_5_31387	10f.cm.bgf
ABA-b9_5_20724	5f.cm.bgf
ABA-b9_5_20724	1f.cm.bgf
AB	
AB	
AB	
ABA-b9_5_20724	3f.cm.bgf


ABA-GCR1

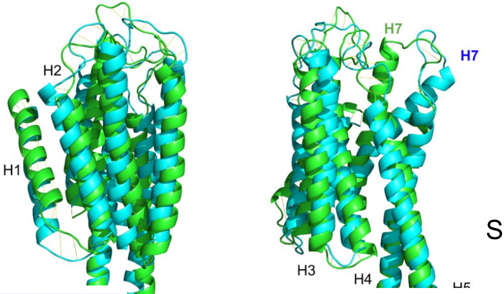

GA1-GCR1

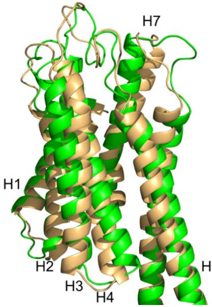
So, it is possible that ABA and GA1 are producing antagonistic effects after binding to GCR1

Docking and MD simulation of GCR1 with abscisic acid (ABA) and gibberellin A1 (GA1)



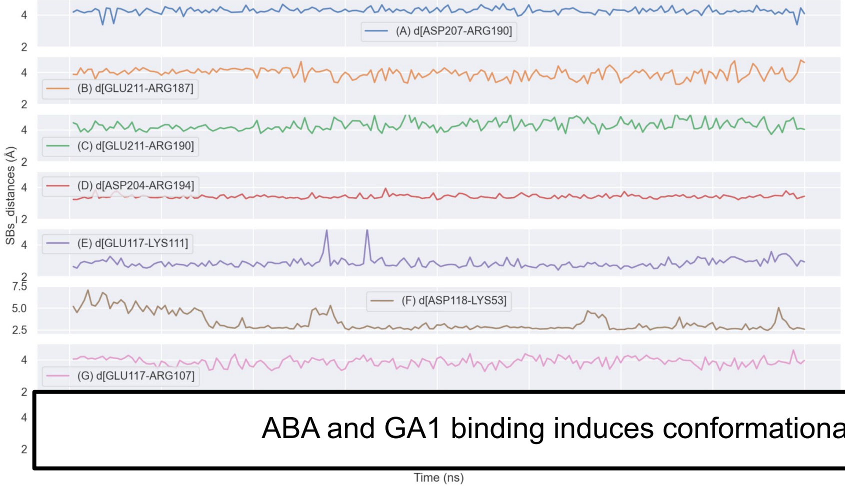
Stable SBs in GA1-GCR1 docked structure



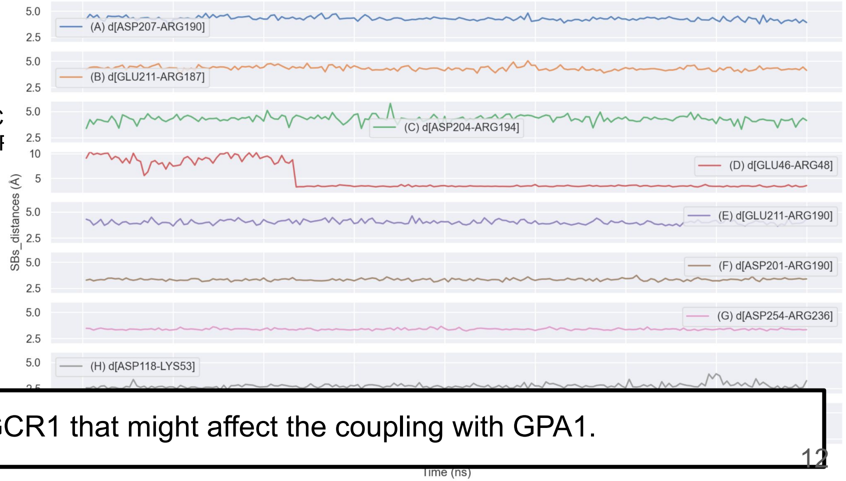


Stable SBs in ABA-GCR1 docked structure

(B)



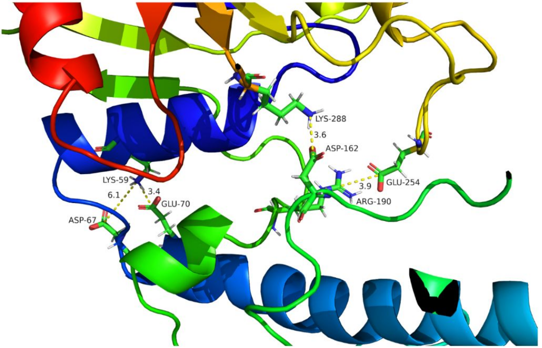
green = GA1-GC
cyan = ABA-GCF



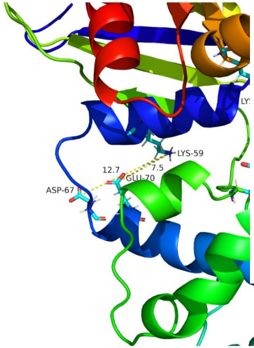
ABA and GA1 binding induces conformational changes in GCR1 that might affect the coupling with GPA1.

Docking and MD simulation of GCR1 with abscisic acid (ABA) and gibberellin A1 (GA1)

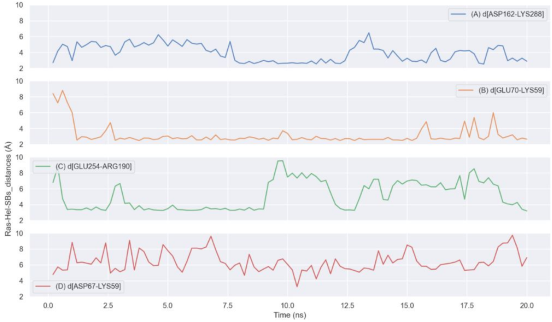
(A) ras-hel Sbs distances in ABA-GCR1-GPA1 Complex



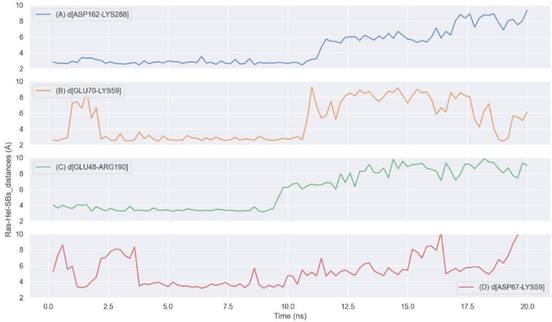
(B) ras-hel Sbs distan
Co



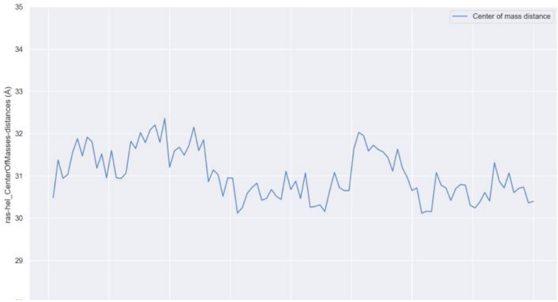
(A) ras-hel Sbs distances in ABA-GCR1-GPA1 Complex



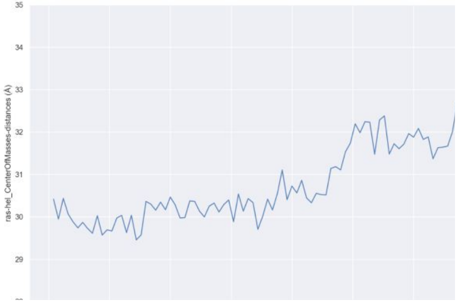
(B) ras-hel Sbs distances in GA1-GCR1-GPA1 Complex



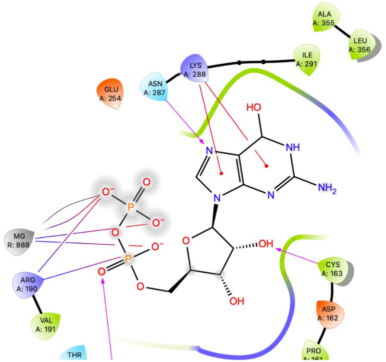
(A) Center of mass distance for ras-hel domains in ABA-GCR1-GPA1 Complex



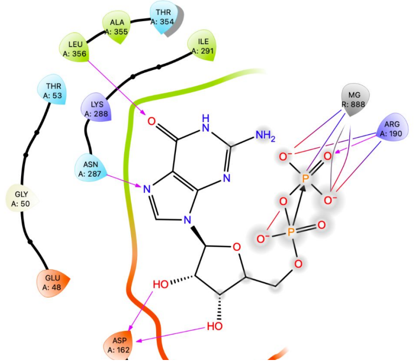
(B) Center of mass distance for ras-hel don
GA1-GCR1-GPA1 Complex



(A) GDP-Ligand interaction diagram in ABA-GCR1-GPA1 Complex



(B) GDP-Ligand interaction diagram in GA1-GCR1-GPA1 Complex



GCR1 can be mediating agonist and negative agonist mechanisms to activate or inactivate GPA1

The crystal structure of a self-activating g protein α-subunit reveals its distinct mechanism of signal initiation. Jones, J. C.; Duffy, et al. Science Signaling 2011

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