Investigation of Activation Mechanism of Heterotrimeric G protein Signaling in Plants

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Heterotrimeric G Proteins in Plants

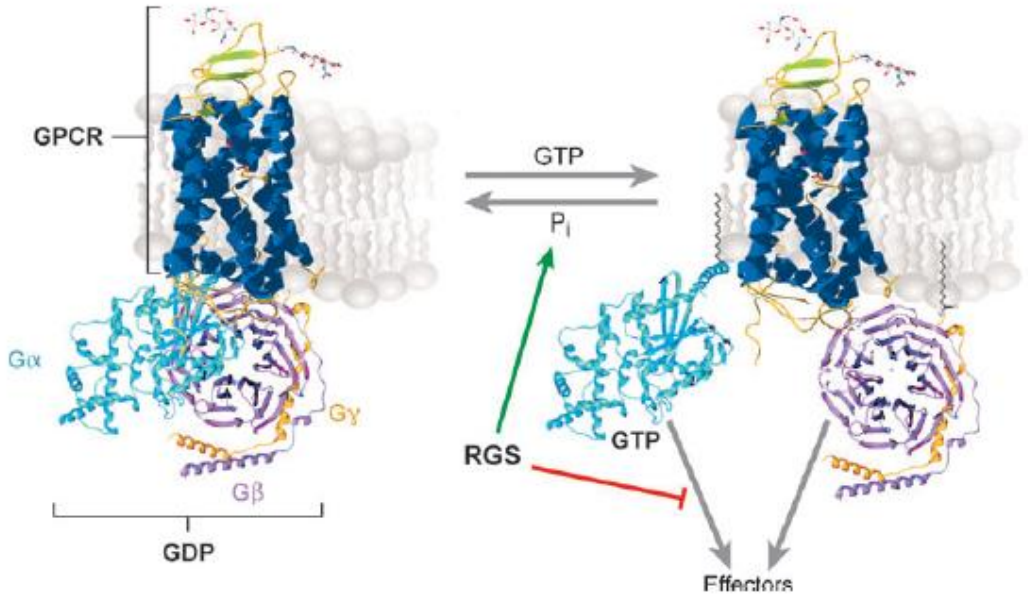
In plants G proteins are involved in

- Biotic / abiotic stress responses
- Regulation of seed growth & germination
- Cell-cycle control
- Light responses

We are intersted in arabidopsis and rice plants.

Heterotrimeric G Proteins

In mammals G proteins coupled GPCRs are involved in initiating sensory signals, cell growth and differentiation

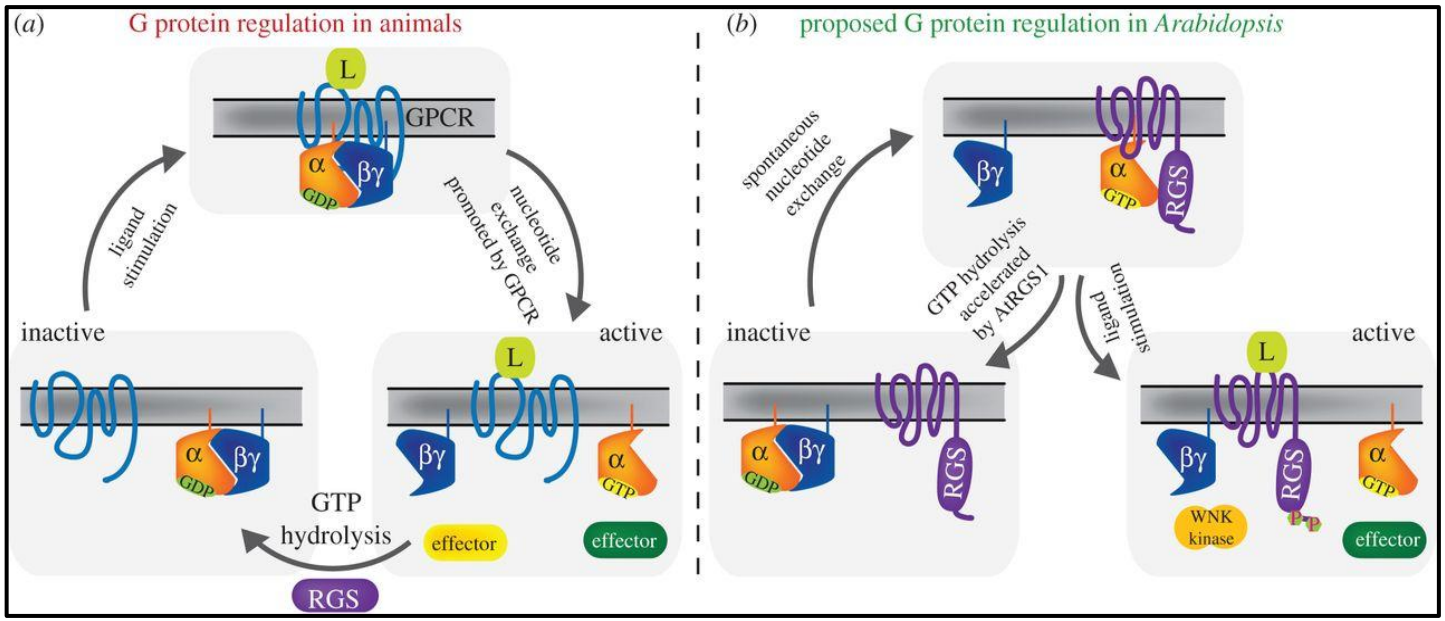


GPCR: 7 TM G-protein coupled receptor (rhodopsin)
Heterotrimeric G-protein complex: α, β and γ subunits
Release of GDP rate limiting step
Ga-GDP →inactive Ga-GTP →active
RGS: Regulator of G-protein signaling

GPCRs are targets for 80% of drugs in the market.

Temple and Jones(2007)

G Protein Dependent Signaling: Mammals vs Plants



Urano et al 2014

Arabidopsis subunits:

GPA1: ~44,482 Da, GTP binding and GTPase activity

AGB1: ~41,000 Da, ~40% identical to mammalian Gβ subunits.

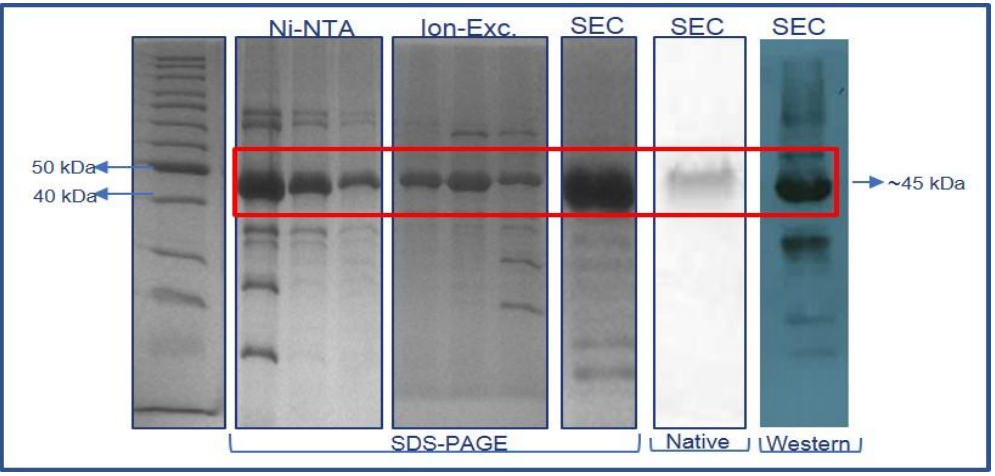
AGG1/2: ~11,000 Da,50% similarity to mammalian subunits

GPCR: G-protein coupled receptor.

L: Ligand

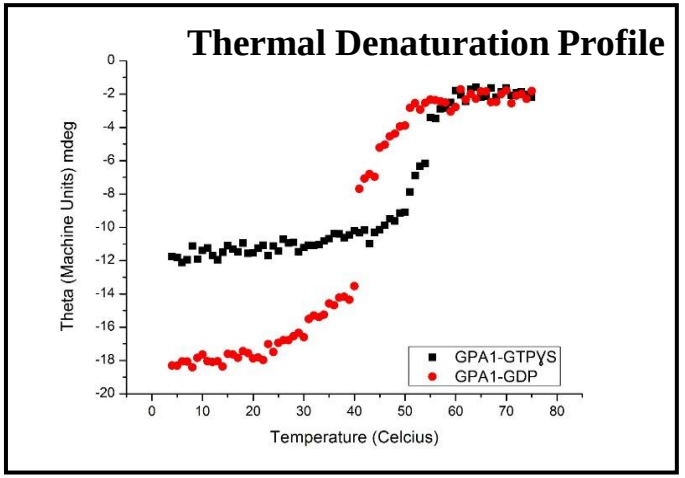
RGS: Regulator of G-protein signaling.

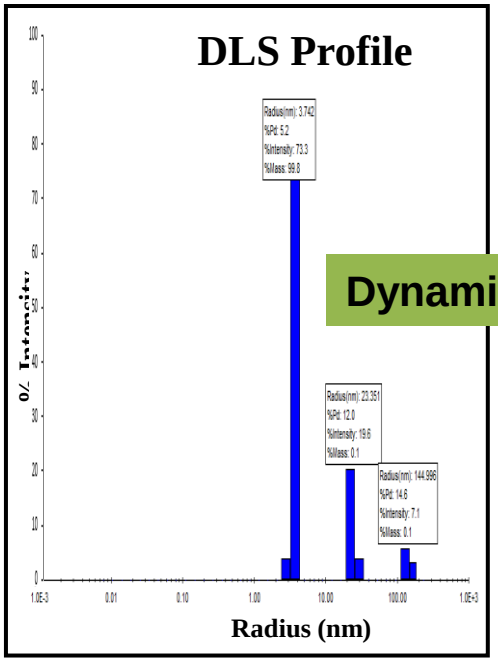
GPA1 Subunit



SDS- Native PAGE

Circular Dichroism Spectroscopy

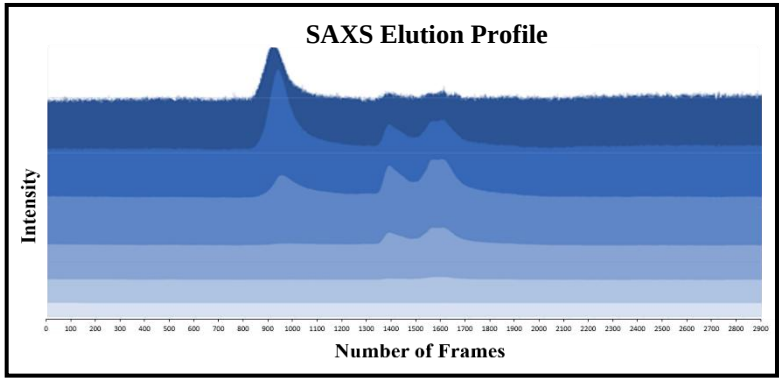


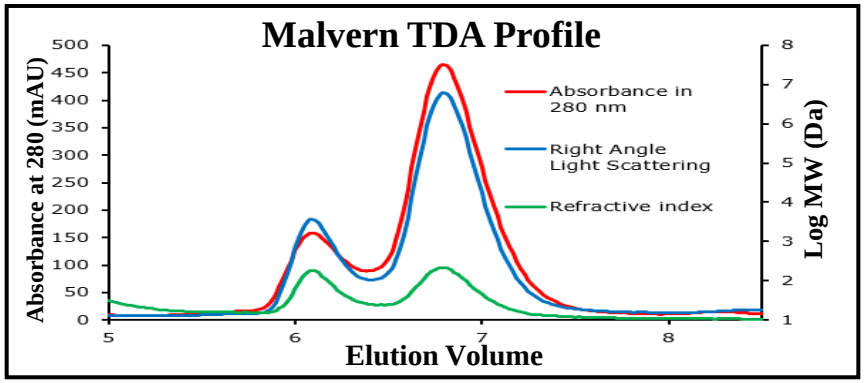


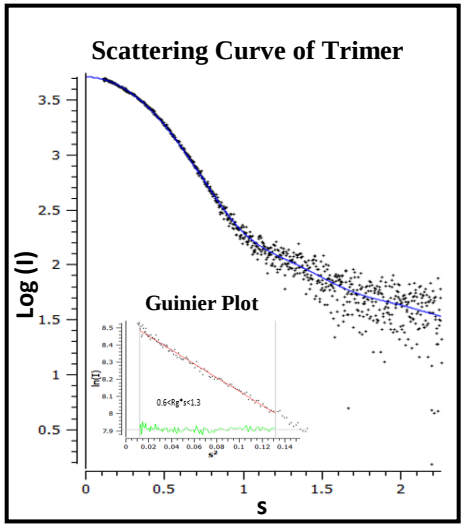
Dynamic Light Scattering

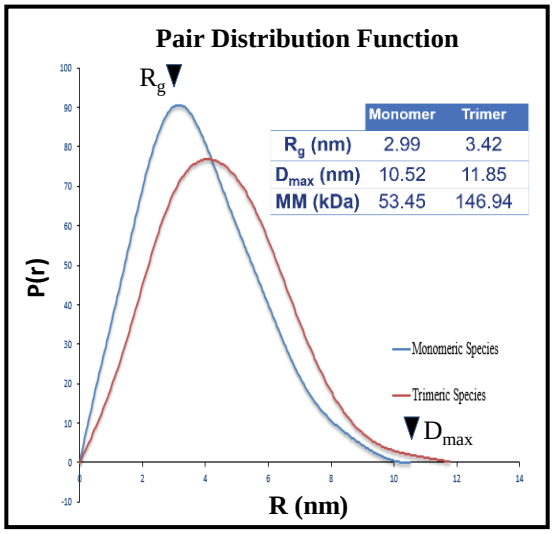
SECONDARY STRUCTURE PREDICTION (CDSSTR method set 4)					
Sample Protein	Helix %	Strand %	Turn %	Unordered%	NRMSD
GPA1 -GDP	49	16	15	19	0.003
Crys. Str of GPA1	49	12	-	-	-

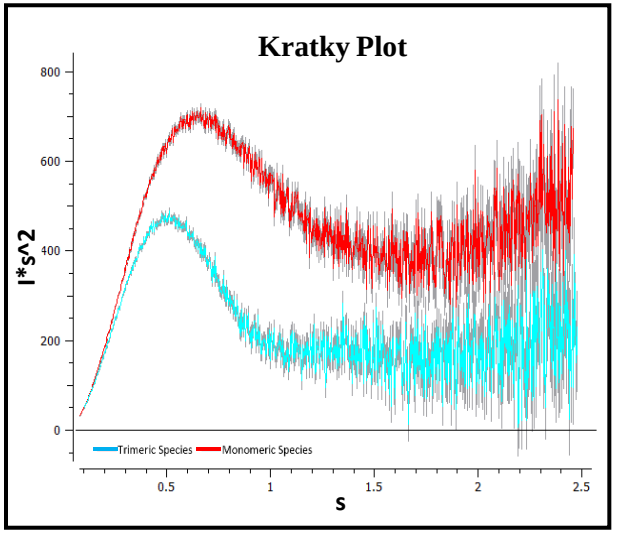
GPA1 Structural Analyses





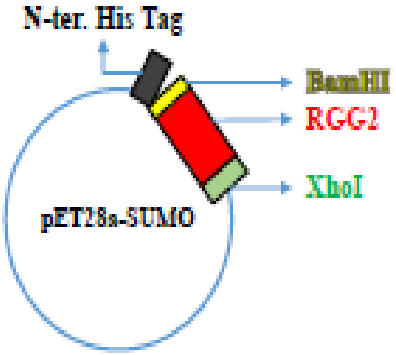


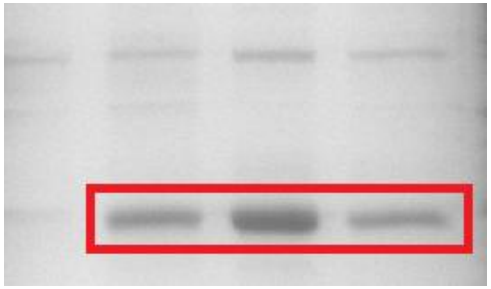




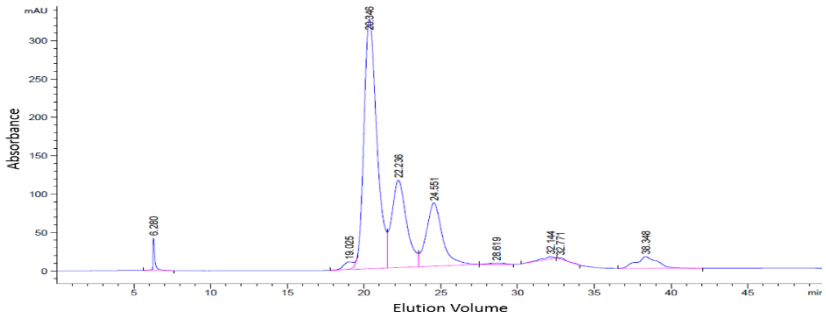
RGG2 Subunit

RGG2 from rice has been cloned, expressed and purified from E. coli



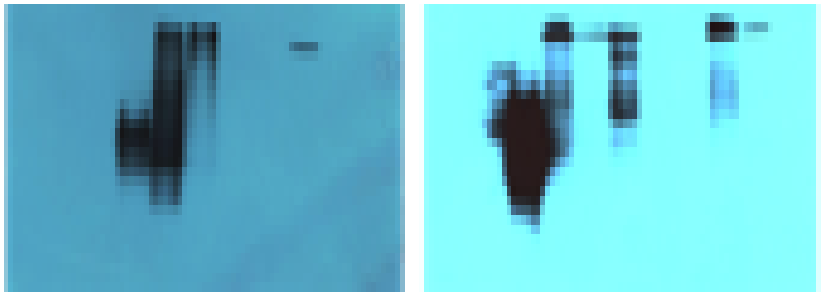


Preliminary measurements showed oligomerization of RGG2.



AGB1 and AGG2 Subunits

His-gamma+beta **His-beta+gamma**

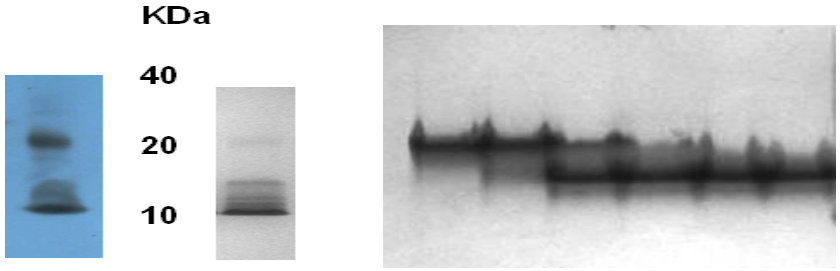


AGB1 and AGG2 can be co-expressed; mostly in inclusion bodies

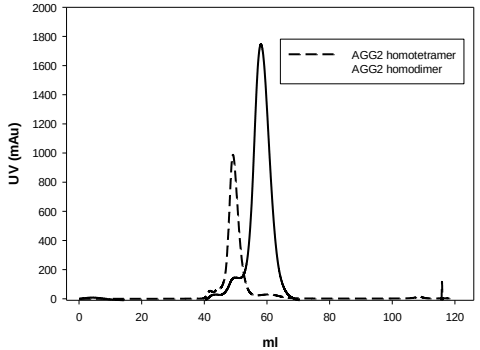
AGG2 Subunit

AGG2

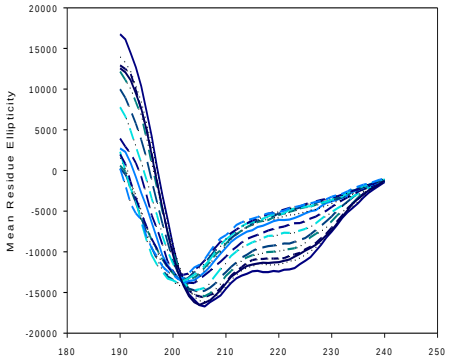
pQE-80L + AGG2 (Qiagen)

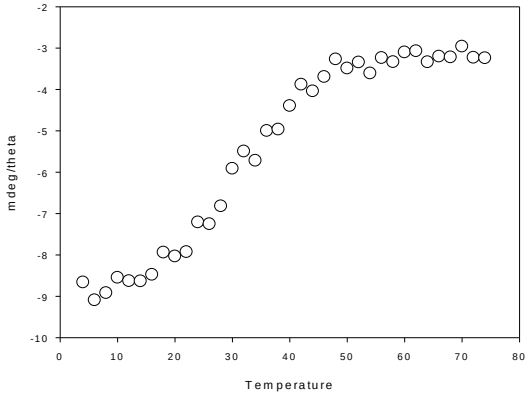


Purity and effect of DTT on the oligomerzation state of AGG2



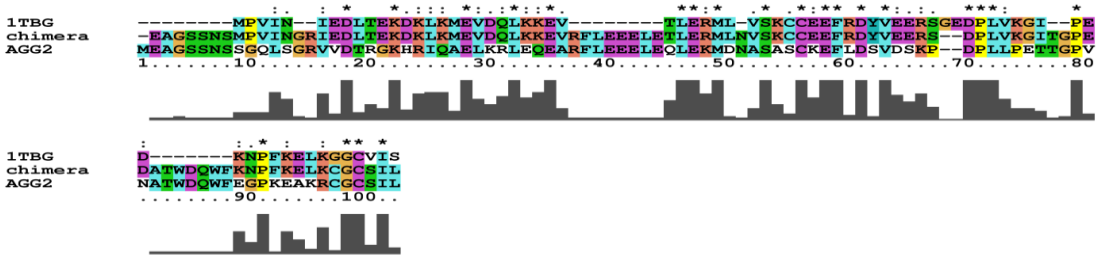
Thermal denaturation of AGG2





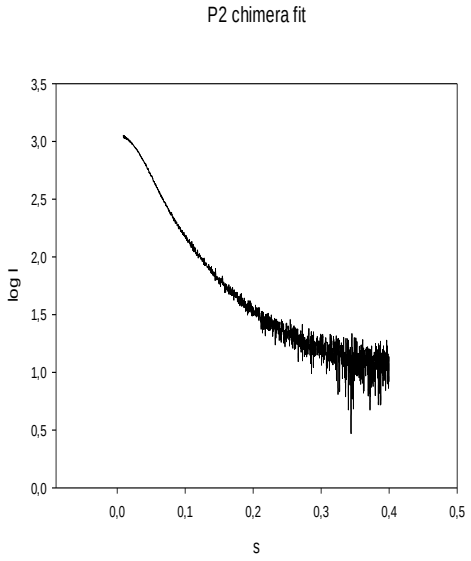
CD measurements: AGG2 conformers with α-helical core and terminal disordered regions

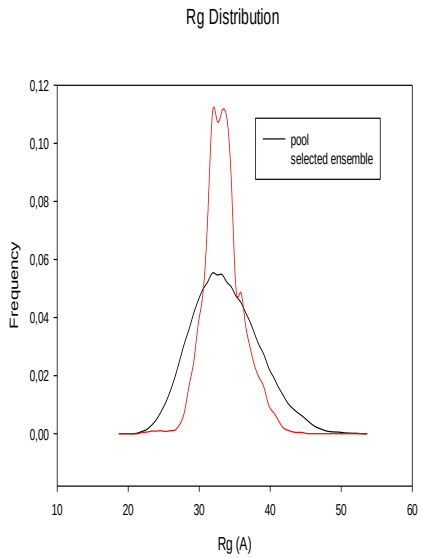
AGG2 Structural Analyses

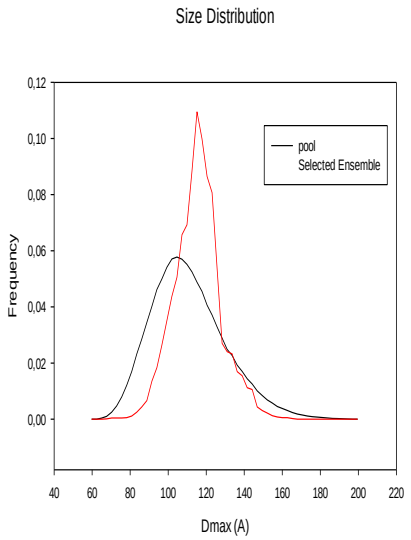


Chimera: mammalian and AGG2

EOM Calculations for likely size distribution in conformer population







In the absence of the natural partner β-subunit the γ-subunit structure is stabilized by dimerizarion.

In vivo Investigations

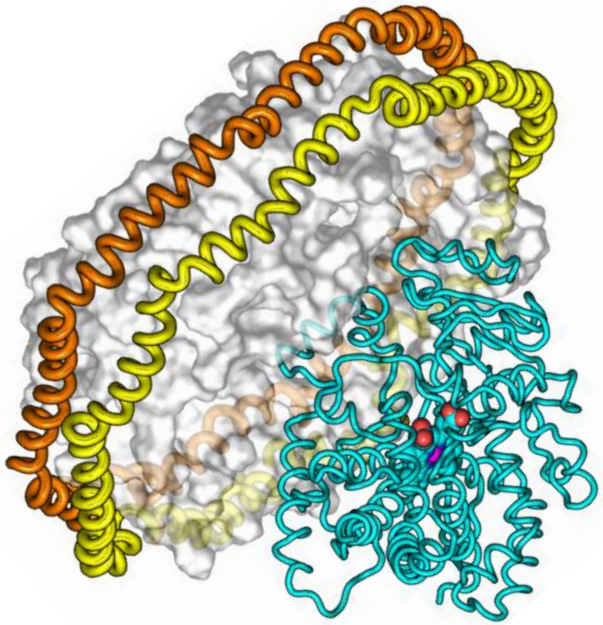
In situ studies to complement AGG2 deficiency by RGG2 to understand functional complementarity between the two proteins.

Investigation of role of the gamma proteins in seed size control in rice.

Nanodisc-Protein Interactions

Nanodiscs: 10 nm diameter lipid structure with protein wrapped around. Can be used to mimic protein-membrane interactions.

How do the individual subunits interact with the membrane?

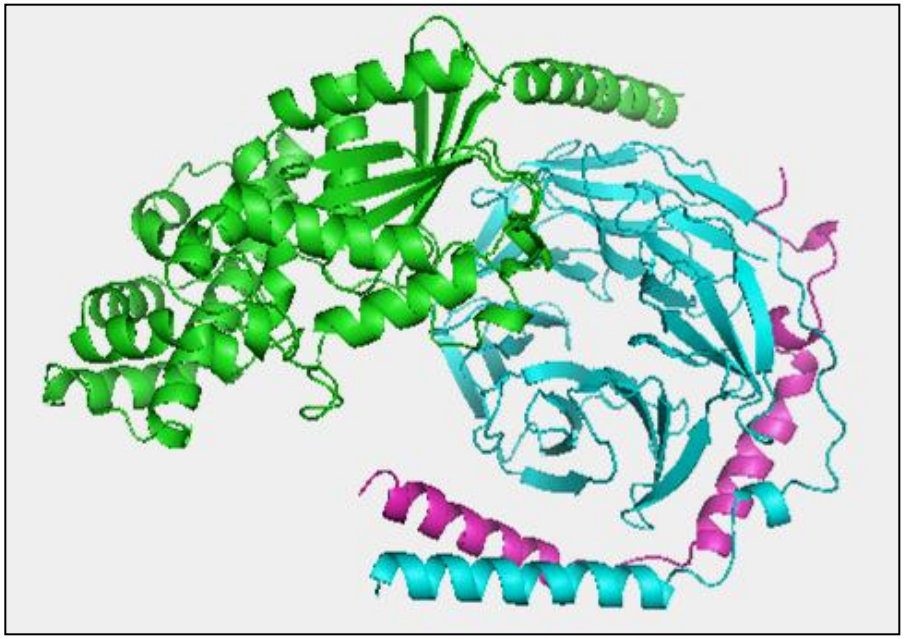


Nanodisc-CYP3A4 interaction

Subunits:
GPA1
AGG2
RGG2

http://depts.washington.edu/wmatkins/nanodiscs.html

Dynamics of Complex Formation



1GOT.pdb: GtAlpha-GiAlpha chimera-Gtbeta-gamma
Green: alpha, blue: beta purple: gamma subunit
Lambright et al 1996

Conformational changes during assembly

Conformational changes associated with GDP and GTP binding.

Conformational changes associated with binding to the membrane