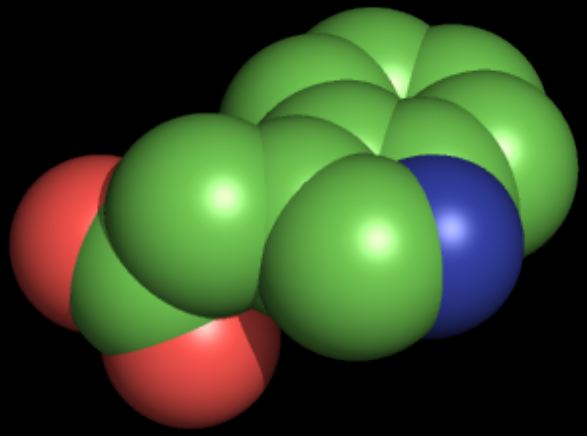
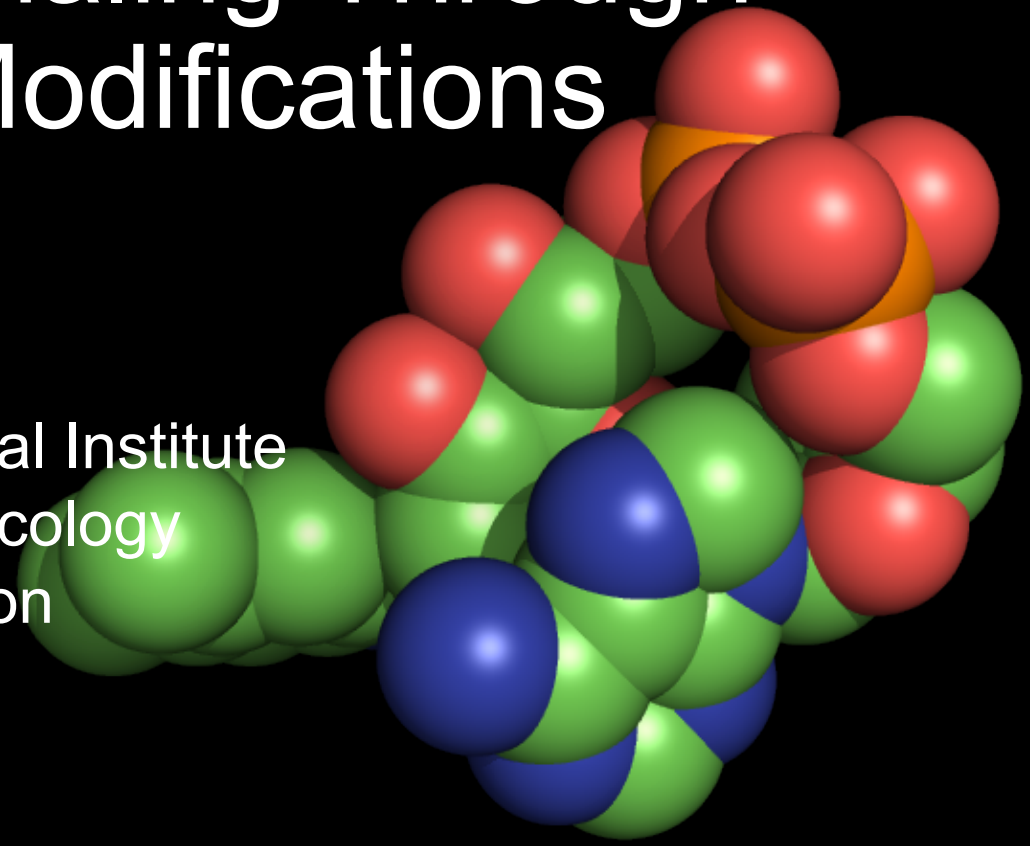


Cellular Signaling Through Protein Modifications

Ning Zheng
Howard Hughes Medical Institute
Department of Pharmacology
University of Washington
Seattle



We Could Discuss About

Structural and Regulatory Mechanisms of Major PTM enzymes

Kinases
Phosphatases
Ubiquitin Ligases
Deubiquitinases
Acetyl-transferases
Deacetylases
Methyl-transferases
Demethylases
Proteases
Isomerases

Instead ……

Using Structural Approaches to Address Biological Problems

Signaling via Ubiquitination

Challenges In Structural Biology

Data Collection
Data Interpretation

Are You Serious?

SEATTLE STRUCTURAL GENOMICS CENTER FOR INFECTIOUS DISEASE

Hello,

My name is [redacted] and I'm one of the crystallographers for the Seattle Structural Genomics Center For Infectious Diseases<http://www.ssgcid.org/home/index.asp> (SSGCID). I'm writing to you because of a your groups' interest in membrane proteins. Here at SSGCID have deposited over 500 protein structures to the protein data bank in the last 4.5 years, including over 50 structures for one of our community requestors, [redacted].

We would like to work on some more difficult targets, specifically membrane proteins. There are a number of either essential, drug resistance related, or antigen targets for vaccine development. These proteins are AmtBs (ammonium transporters), Omps (outer membrane proteins), and K+ channel proteins that can be found in our parasitic, yeast, or bacterial pathogenic organisms. Since you are in the local area and interested in membrane protein structure I thought I would reach out to you. Additionally, if there are any E3 proteins in our eukaryotic organisms you could request that we attempt to crystallize them.

The results of these studies would be published, and any materials such as expression plasmids, purified proteins, and expression/purification protocols would be freely available to you since SSGCID is a publicly funded structural biology center. These materials could be used by your laboratory for any future studies as you see fit.

Perhaps most importantly, this would all be done at no cost to you or your group. We would also hope that you or someone in your lab would be willing to help us co-author papers or do biochemical assays on protein and structures produced through this effort, although it is not a requirement. This is an excellent opportunity for graduate students or postdocs to become first or co-first authors on structures and publications.

I have compiled a list of AmtB, Omp, and K-channels from our target pathogenic organisms that I have attached to this email.

If you are interested in this project, we would really like for you to request these targets through our formal target request process. This requires around 15 minutes of effort to compose an email to our target request email address located here: http://www.ssgcid.org/home/Email_target_submission.asp.

If you are pressed for time, I would be happy to fill out the short form required for the email.

Thanks for your time and consideration.
Please feel free to contact me by phone or email,

RCSB PDB PROTEIN DATA BANK

An Information Portal to 105025 Biological Macromolecular Structures

Search by PDB ID, author, macromolecule, sequence, or ligands

Advanced Search | Browse by Annotations | Search History (4) | Previous Results (13080)

PDB-101 | PDB | EMDataBank

13080 Structure Hits | 84 Unreleased Structures | 1896 Citations | 1319 Ligand Hits

Query Parameters: Query Details | Save Query to MyP

Structural Genomics Search :

Query Refinements: *Select an item or pie chart* Sh

Organism | Taxonomy | Exp. Method | X-ray Resolution | Release Date | Polymer Type

Enzyme Classification | SCOP Classification | Protein Symmetry | Protein Stoichiometry | Membrane Proteins

Refine Query with Advanced Search

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9 Related Molecule of the Month articles Sh

Serotonin Receptor | Vitamin D Receptor | Crystallins | Epidermal Growth Factor | Auxin and TIR1 Ubiquitin Ligase | Selenocysteine Synthase | Adrenergic Receptors | Luciferase | Aminoacyl-tRNA Synthetases

Showing 1 - 25 of 13080 Results

Results : 25 Page: 1 of 524

Filter: Check All View: Detailed Download Results Reports: Select one... Sort: Release Date

4PED Mitochondrial ADCK3 employs an atypical protein kinase-like fold to enable coenzyme Q biosynthes

Authors: Bingman, C.A. , Smith, R. , Joshi, S. , Stefely, J.A. , Reidenbach, A.G. , Ulbrich, A. , Oruganty, O. , Floyd, B.J. , Jochem, A. , Saunders, J.M. , Johnson, I.E. , Wrobel, R.L. , Barber, G.E. , Lee, D. , Li, S. , Kannan, N. , Coon, J.J. , Pagliarini, D.J. , Mitochondrial Protein Partnership

Release: 2014-11-19

Experiment: X-RAY DIFFRACTION with resolution of 1.64 Å **Residue Count** 393

Compound: 1 Polymer [*Display Full Polymer Details* | *Display for All Results*]
1 Ligand [*Display Full Ligand Details* | *Display for All Results*]

Citation: PubMed ID is not available.

4PFZ X-ray Crystal Structure of 5-carboxymethyl-2-hydroxymuconate delta-isomerase from Mycobacterium smegmatis

Authors: Horanyi, P.S. , Abendroth, J. , Lorimer, D. , Edwards, T. , Seattle Structural Genomics Center for Infectious Disease

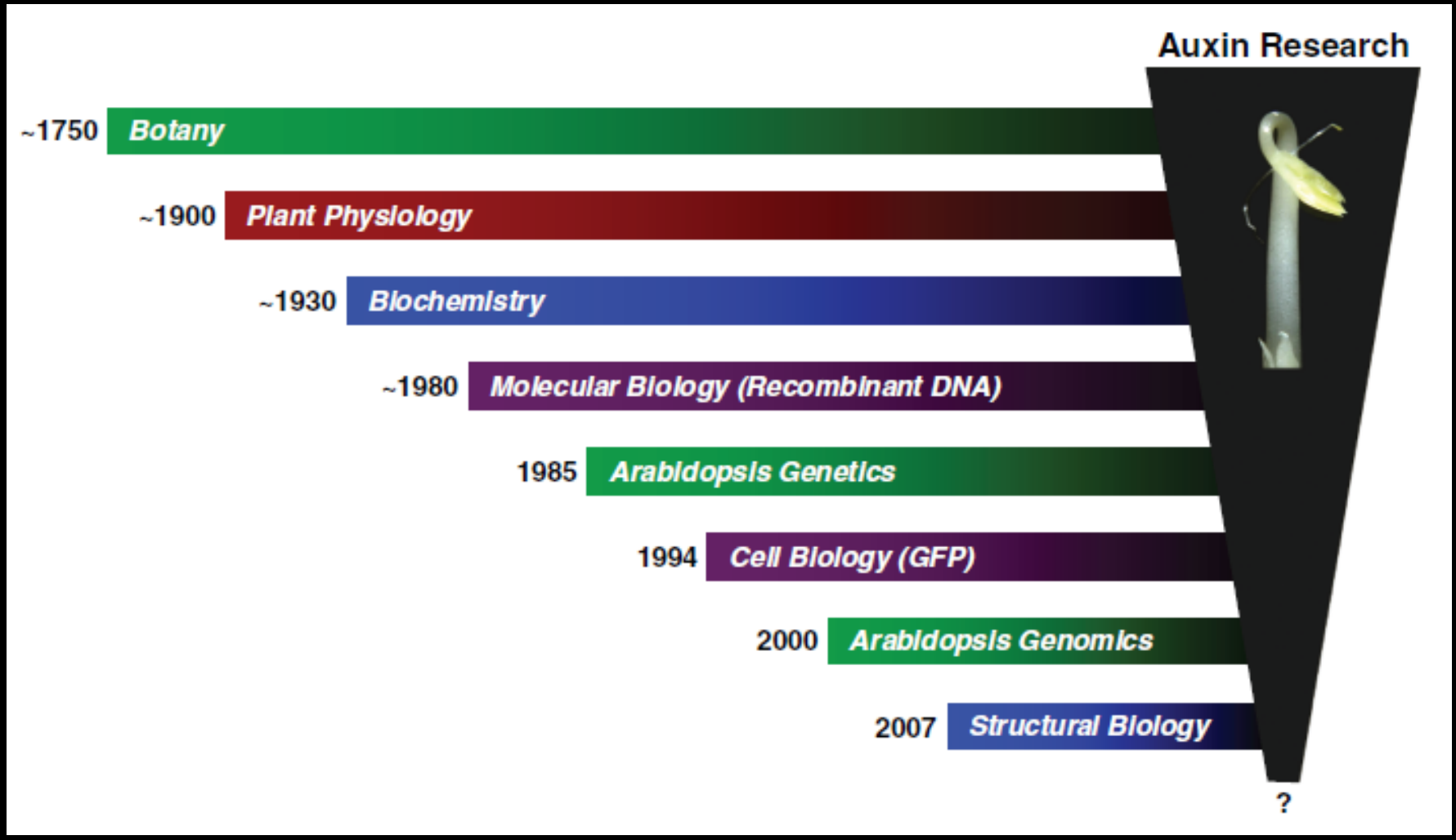
Release: 2014-11-19

Experiment: X-RAY DIFFRACTION with resolution of 1.80 Å **Residue Count** 518

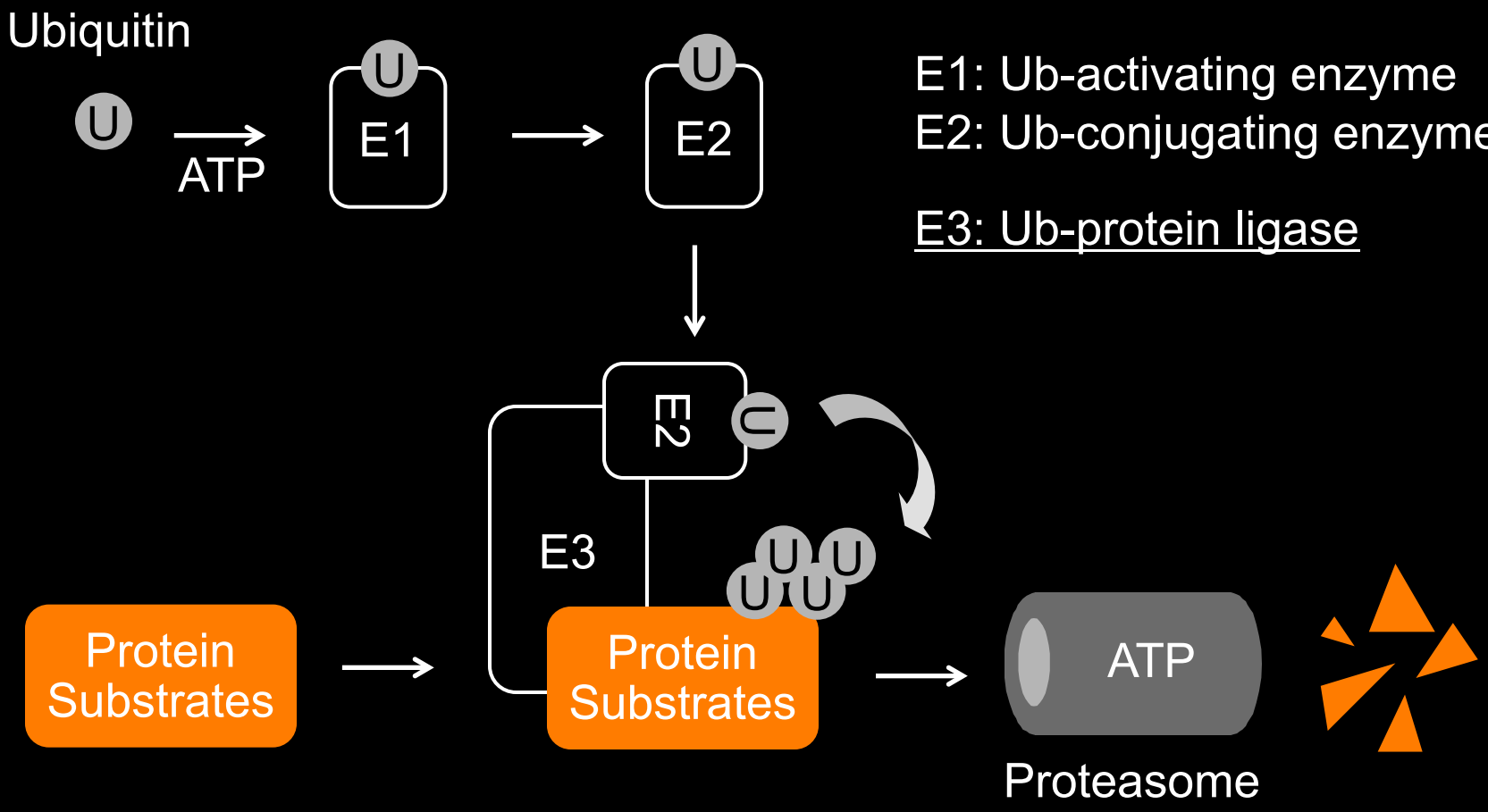
Compound: 1 Polymer [*Display Full Polymer Details* | *Display for All Results*]
1 Ligand [*Display Full Ligand Details* | *Display for All Results*]

Citation: PubMed ID is not available.

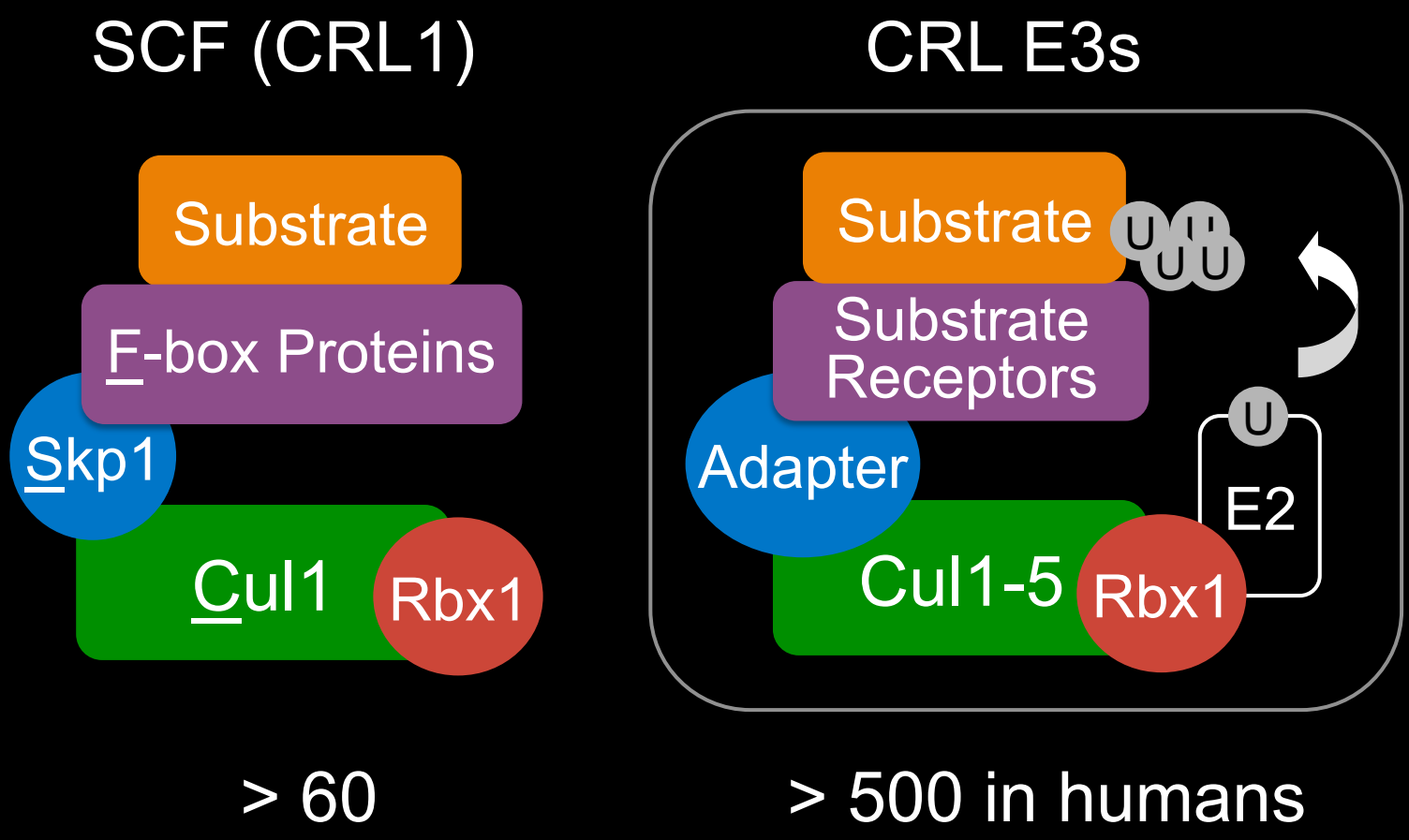
Structural Biology Makes A Difference



Ubiquitin Proteasome System



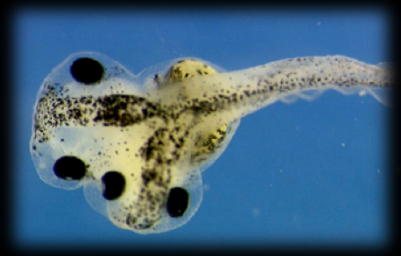
Cullin-RING Ubiquitin Ligases (CRLs)



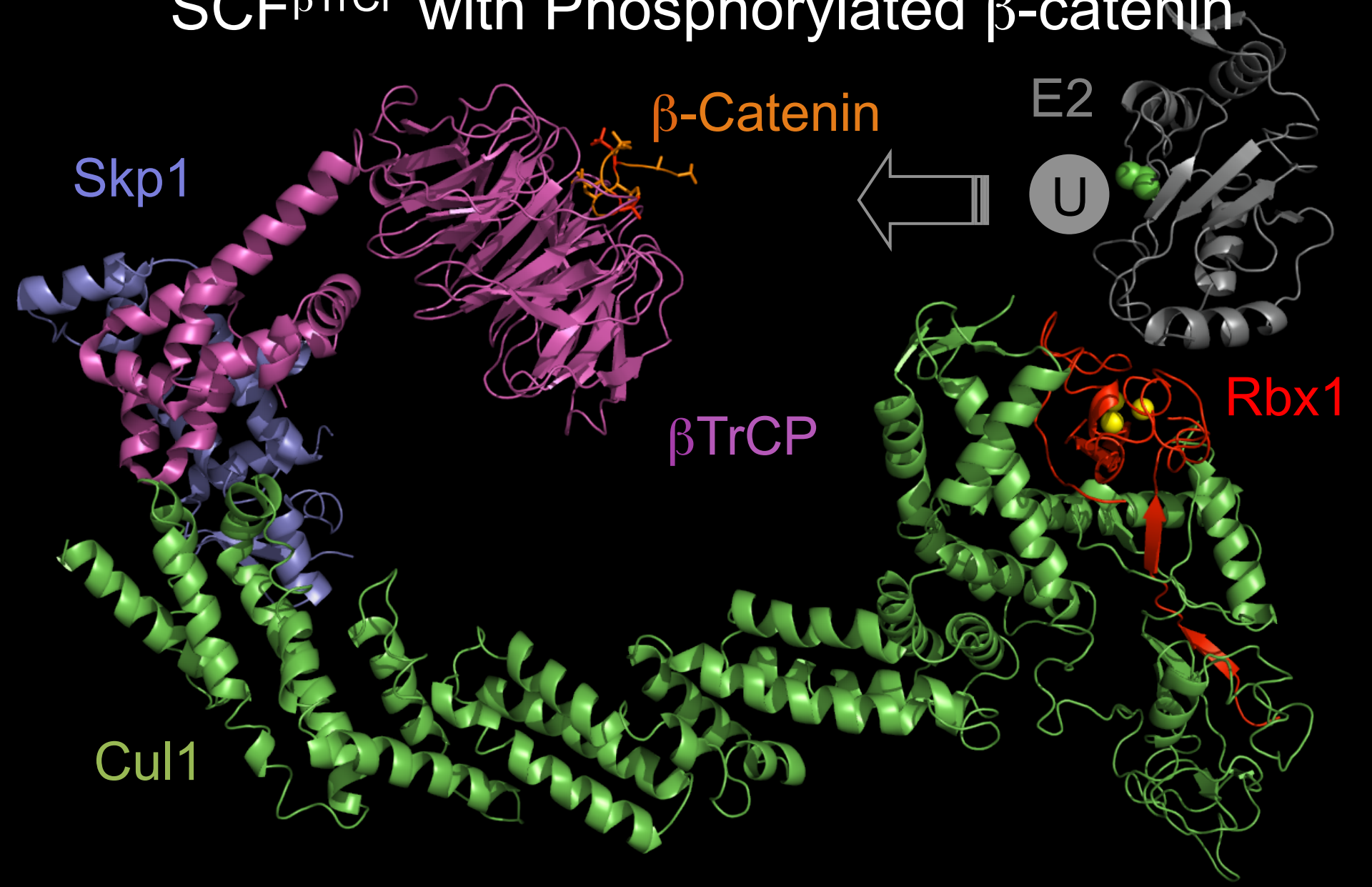
> 60

> 500 in humans

$SCF^{\beta\text{-}TrCP}$ E3 Ligase Regulates Wnt Signaling

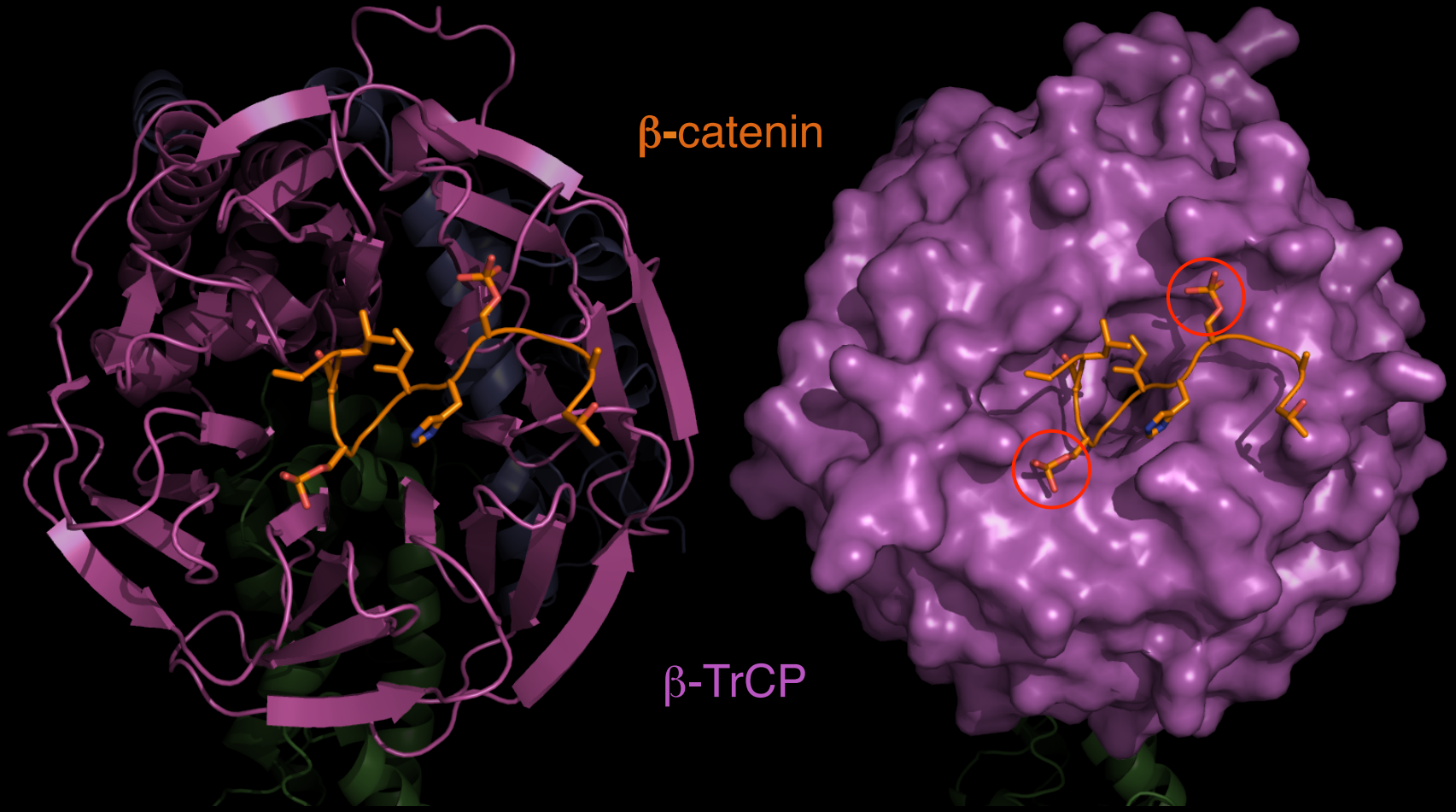


$SCF^{\beta TrCP}$ with Phosphorylated β-catenin



(Zheng et al. 2002 NATURE 416:703; Wu et al. 2003 Mol. Cell 11:1445.)

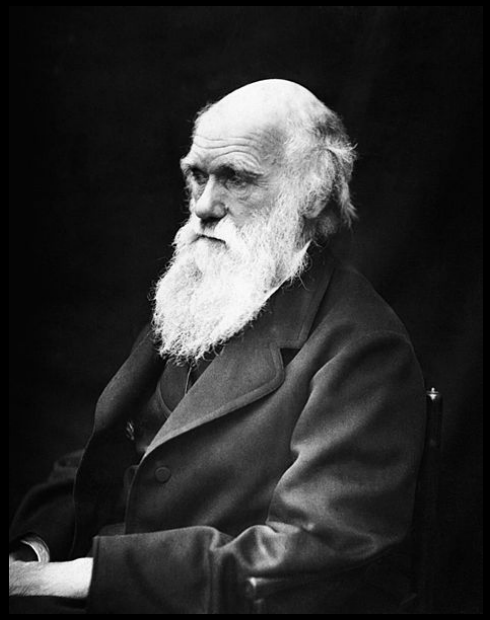
Phosphorylation-Enabled Binding of β-catenin Peptide to β-TrCP

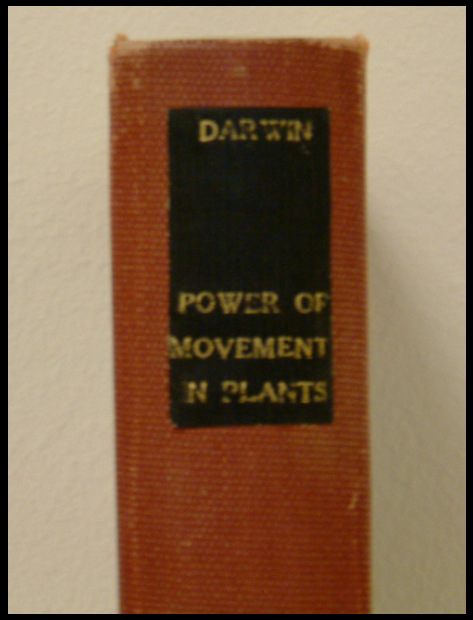


(Wu et al. 2003 Mol. Cell 11:1445.)


A New Paradigm of SCF – Substrate Recognition

Darwin's "Influence"





1880

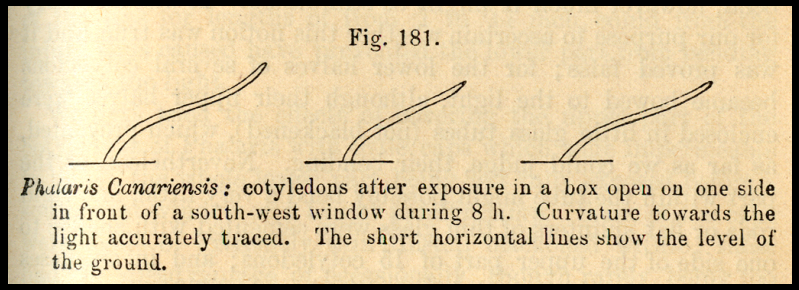


Fig. 181.

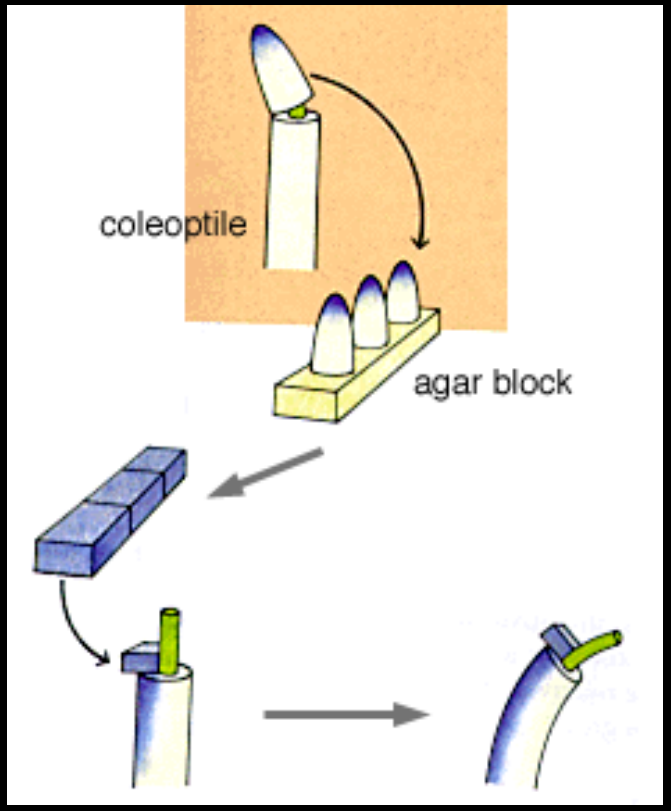
Phalaris Canariensis: cotyledons after exposure in a box open on one side in front of a south-west window during 8 h. Curvature towards the light accurately traced. The short horizontal lines show the level of the ground.

Plants in Motion, Roger Hangarter, Indiana University

"We must therefore conclude that when seedlings are freely exposed to a lateral light some influence is transmitted from the upper to the lower part, causing the latter to bend."

Darwin's "Influence" = A Diffusible Chemical

Fritz Went

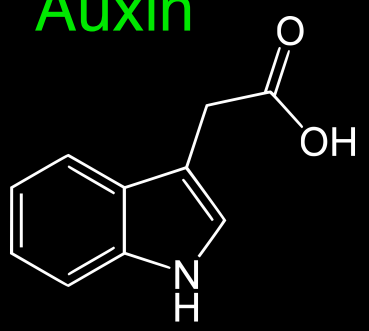


1928

Went, F.W. (1935) Auxin, the plant growth-hormone.
Bot. Rev. 1: 162-182.

Darwin's "Influence" = A Diffusible Chemical = Auxin, a Pivotal Plant Hormone

Auxin



Indole-3-acetic acid

Kenneth V. Thimann

Embryo development

Fruit development

Apical dominance

Phototropism

Vascular differentiation

Gravitropism

Root initiation

Lateral root development

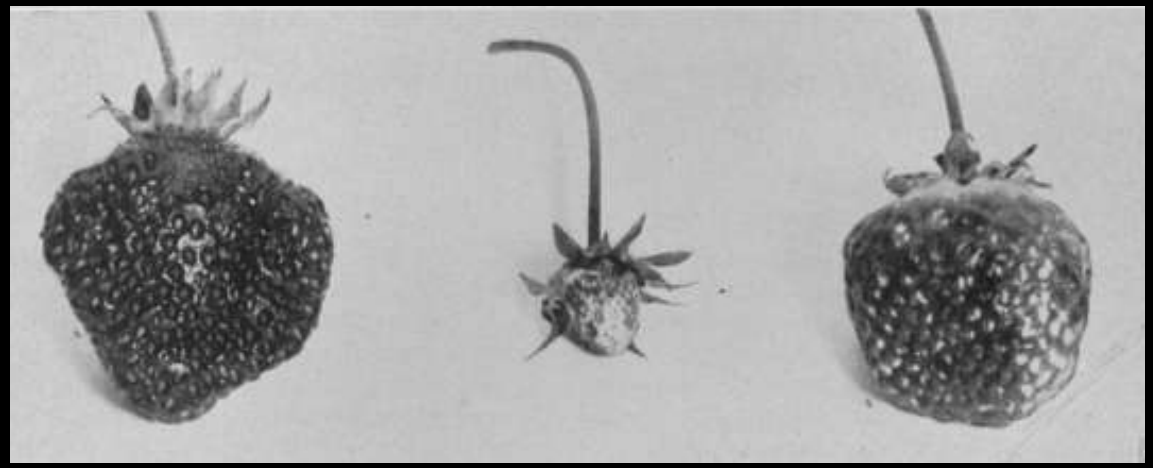
Classic Examples of Auxin Action

Fruit Development

Normal

Seed Removed

Seed removed Auxin applied



(J. P. Nitsch 1949 American J. Botany 37:211)

Structure-Activity Relationships of Auxin

Auxin induced curvature of slit pea stem

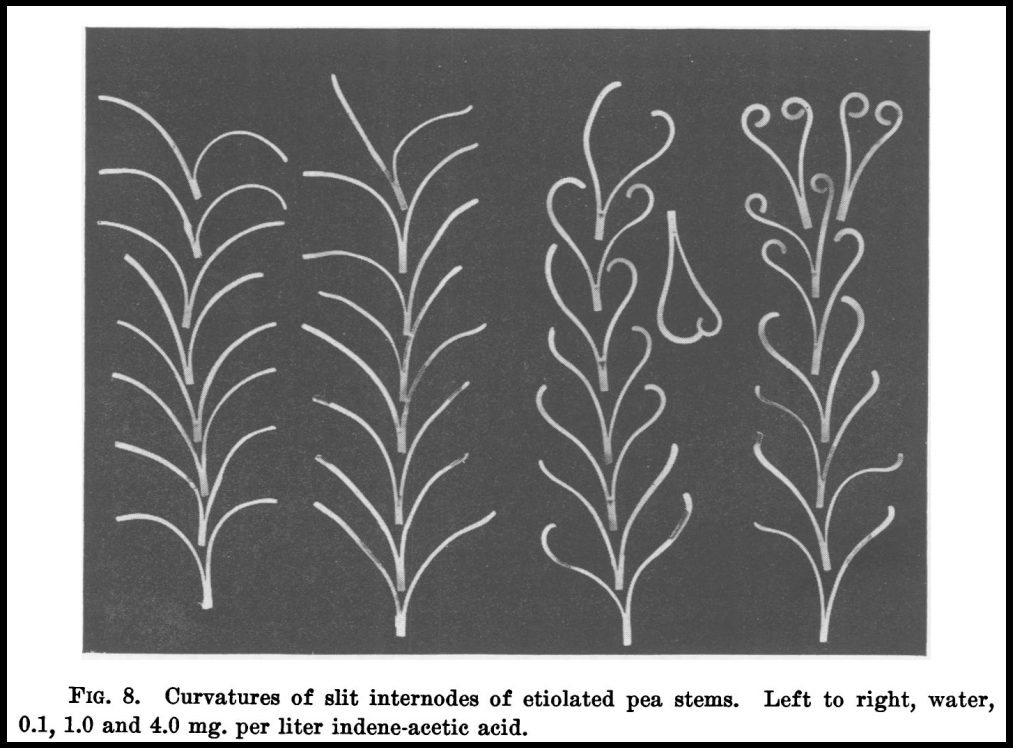
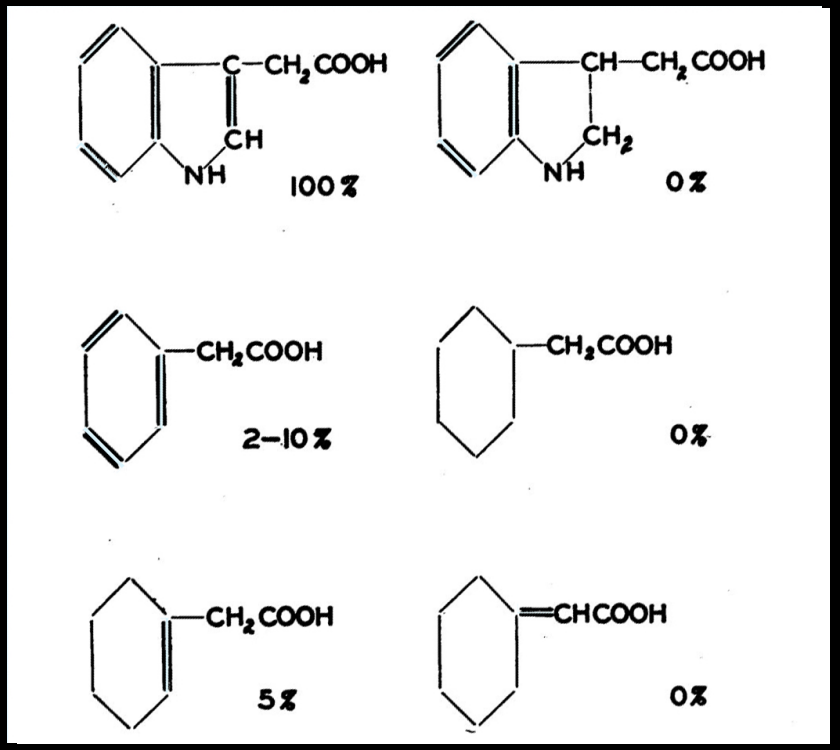


FIG. 8. Curvatures of slit internodes of etiolated pea stems. Left to right, water, 0.1, 1.0 and 4.0 mg. per liter indene-acetic acid.

A mysterious *"Key & Lock"* problem

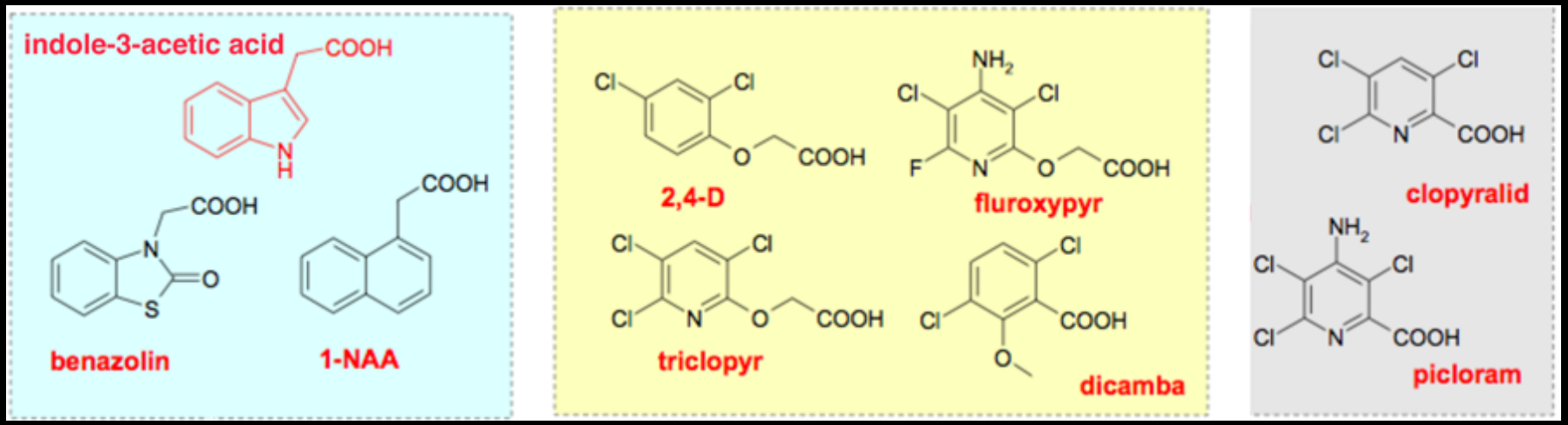


Thimann, K.V. (1938). Hormones and the analysis of growth. Plant Physiol. 13: 437-449

[Auxin] (nM – μM)

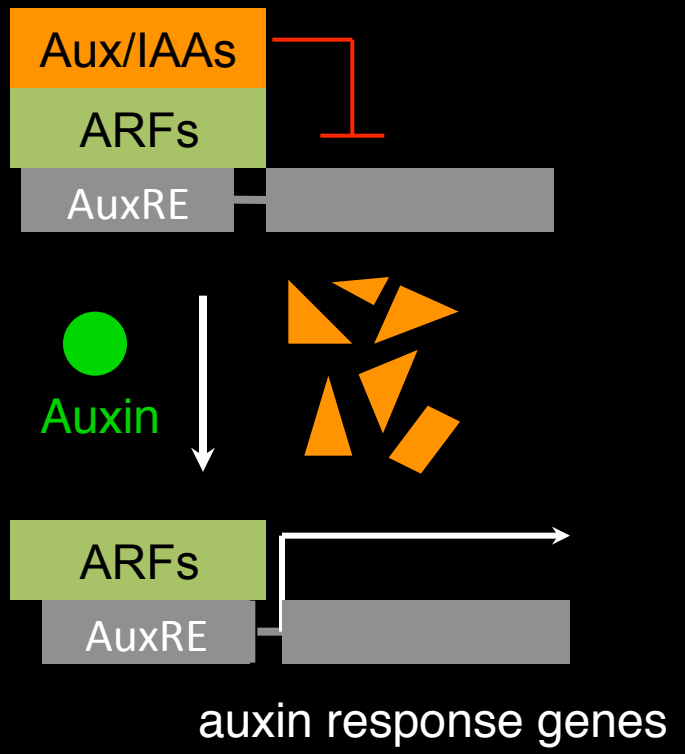
Diverse Auxin and Auxin Analogues

Natural and synthetic auxins (1940 -1970)



2,4-dichlorophenoxyacetic acid

Auxin Regulates Transcription

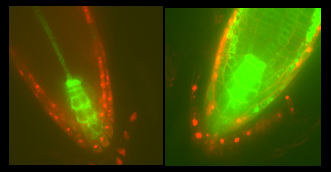


Auxin alters transcription programs by inducing rapid degradation of Aux/IAA transcription repressors.

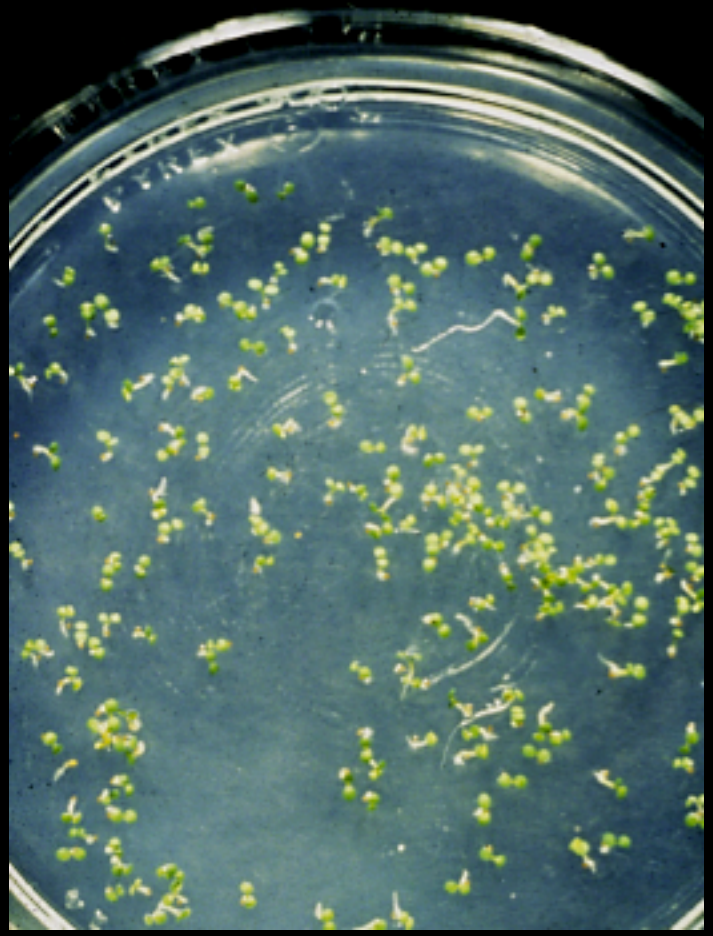
A. thaliana genome encodes 29 Aux/IAAs and 25 ARFs.

- auxin + auxin

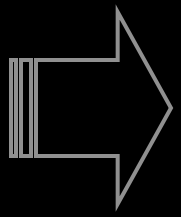
Col-0 DR5-GFP

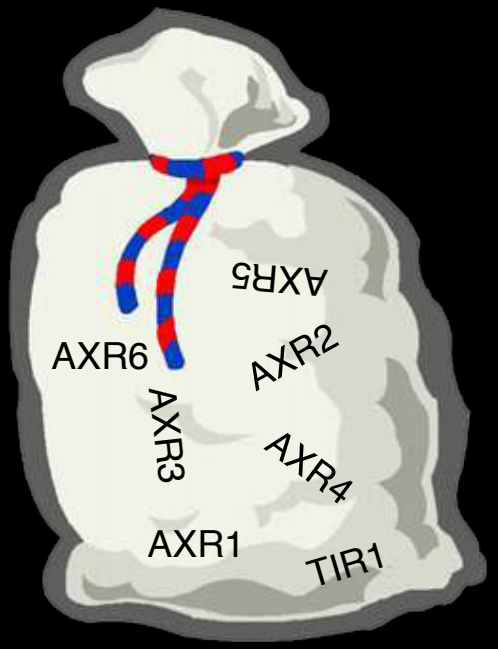


Identification of Auxin Response Mutants



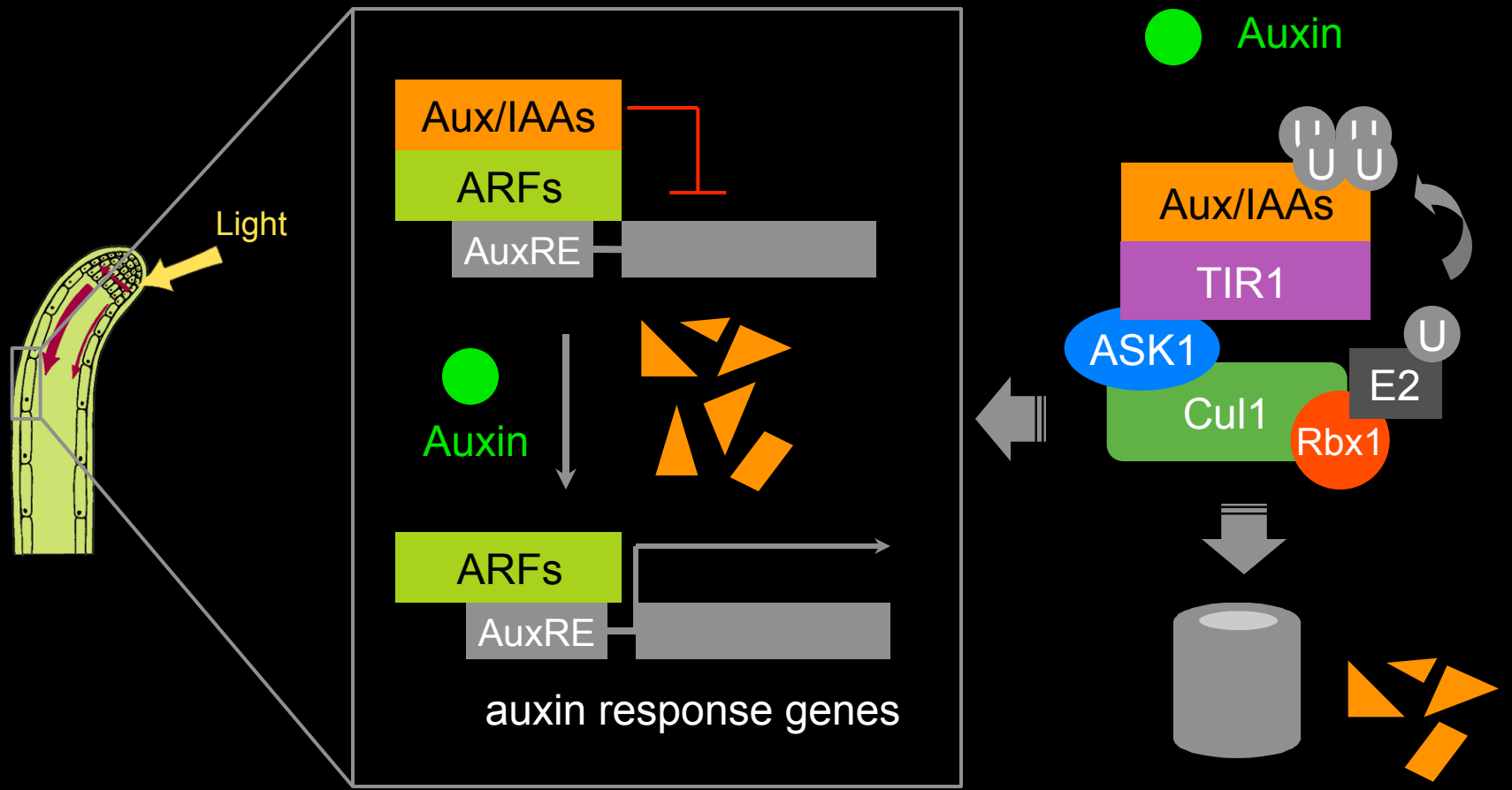
+ 2,4-D ⊣ Root elongation





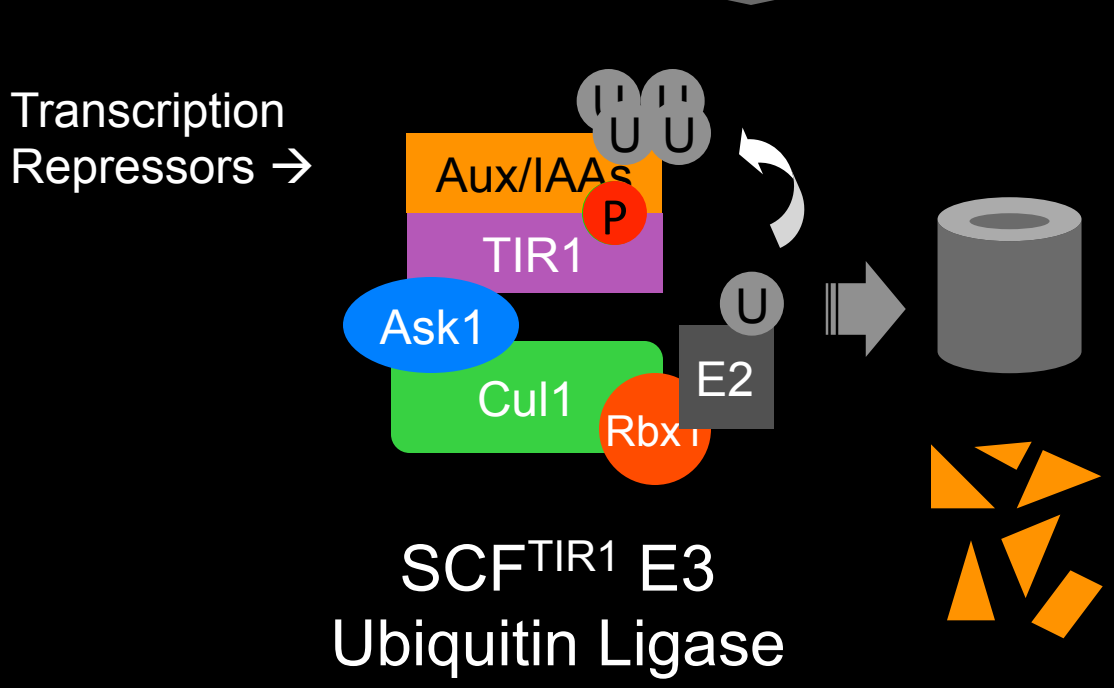
Mark Estelle
(UCSD)

Plant SCF^{TIR1} Mediates Auxin Signaling

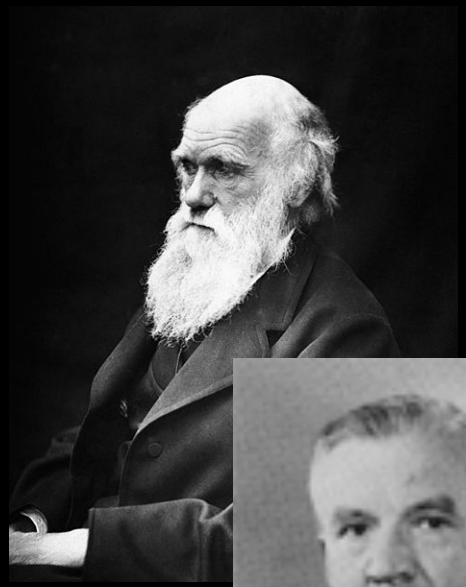


How Do Plants Sense Auxin?

A New Paradigm of Hormone Signaling



Ready to Solve the *Key* & *Lock* Problem

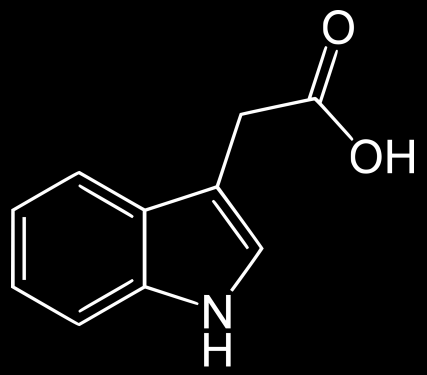


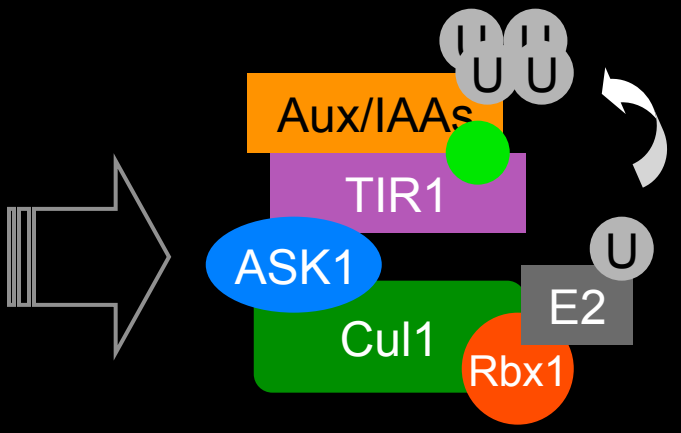
1880

1924

1938

Auxin

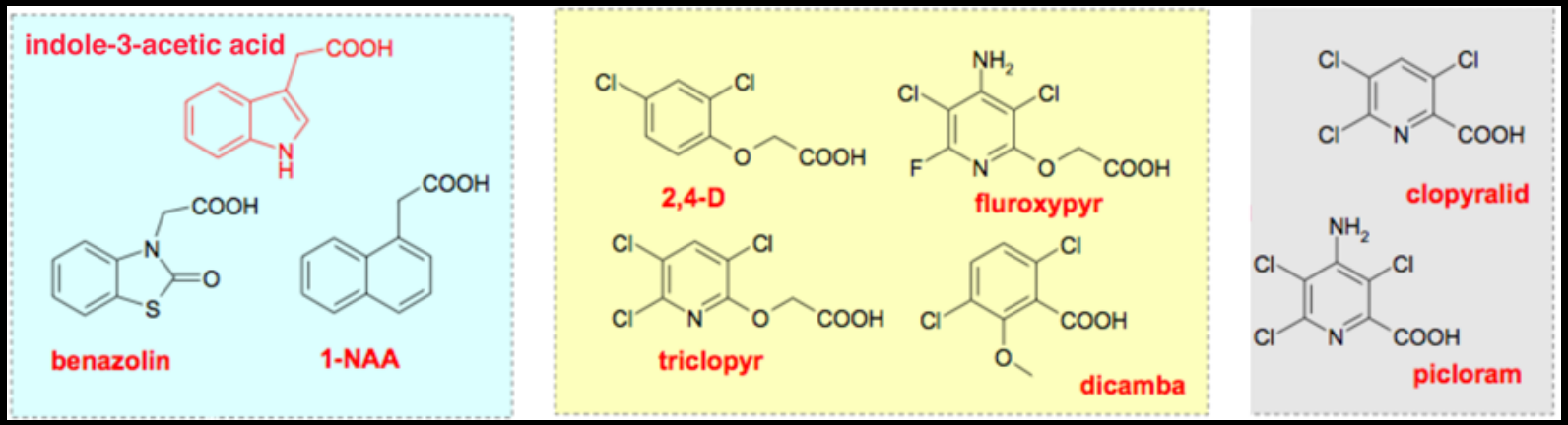




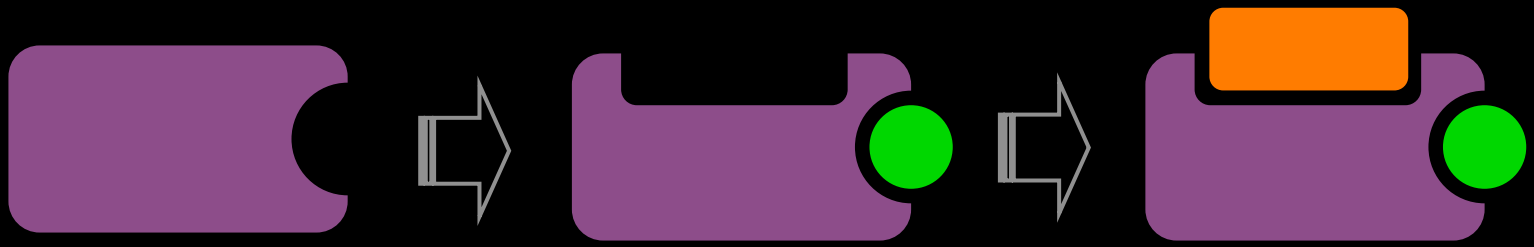
2007

Two Questions on Auxin Action

1. How does TIR1 recognize auxin and more than 200 different analogues?

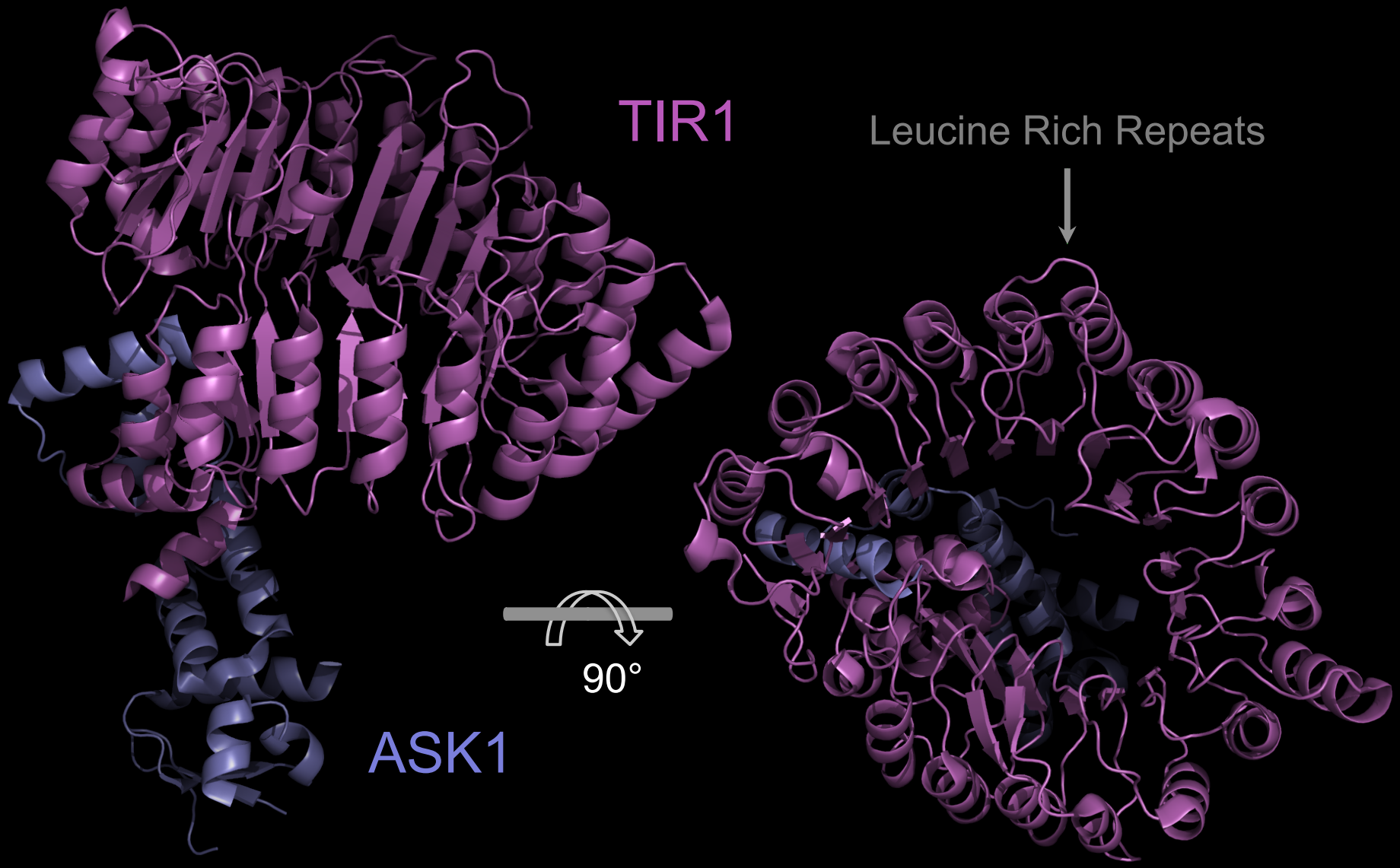


2. How does the small molecule auxin “activate” SCF-TIR1 ubiquitin ligase?



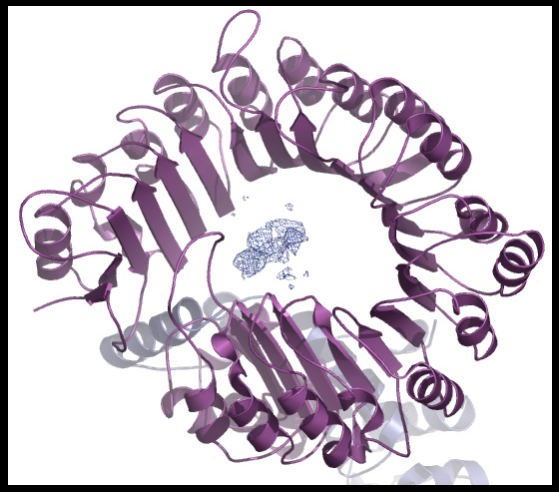
An Allosteric Switch Model

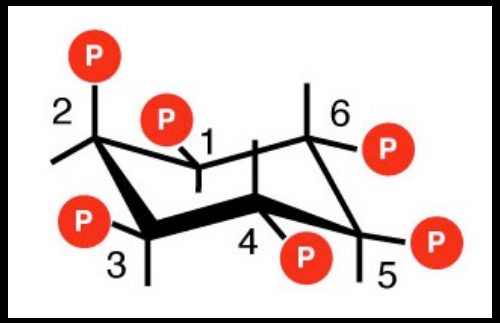
Overall Structure of the TIR1-ASK1 Complex

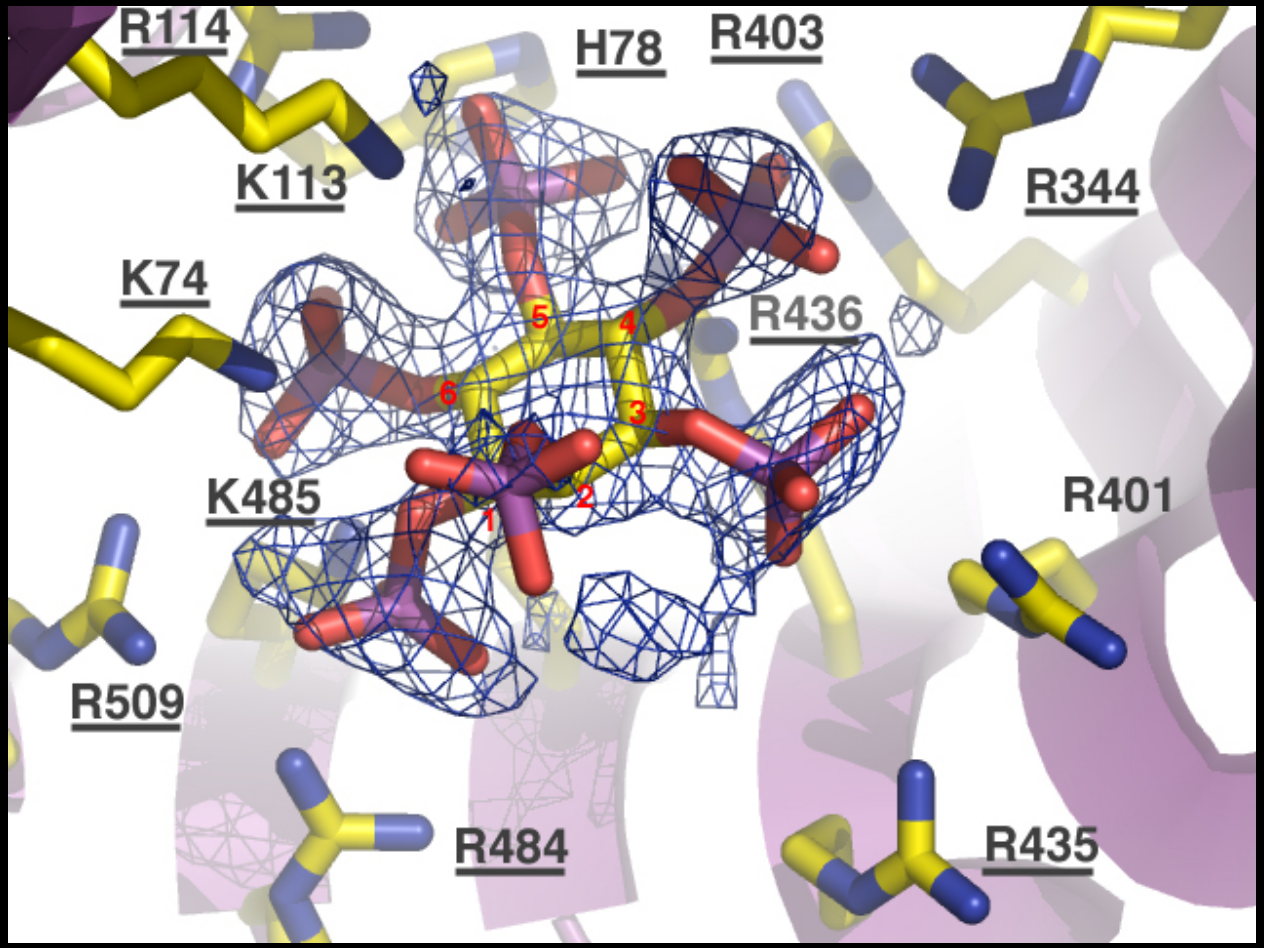


Mysterious Density in TIR1

Inositol hexakisphosphate





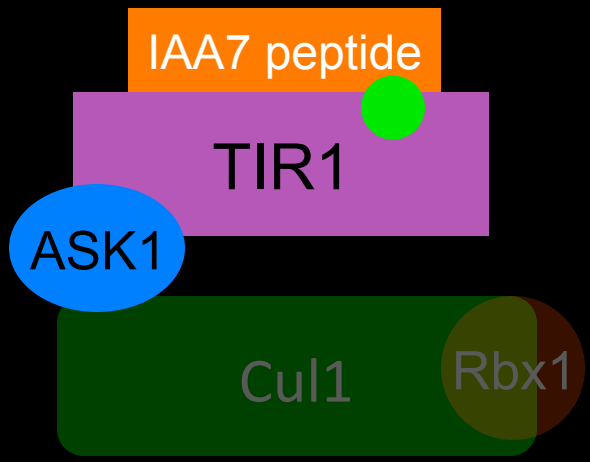


A Conserved Degron in SCF^{TIR1} Substrates

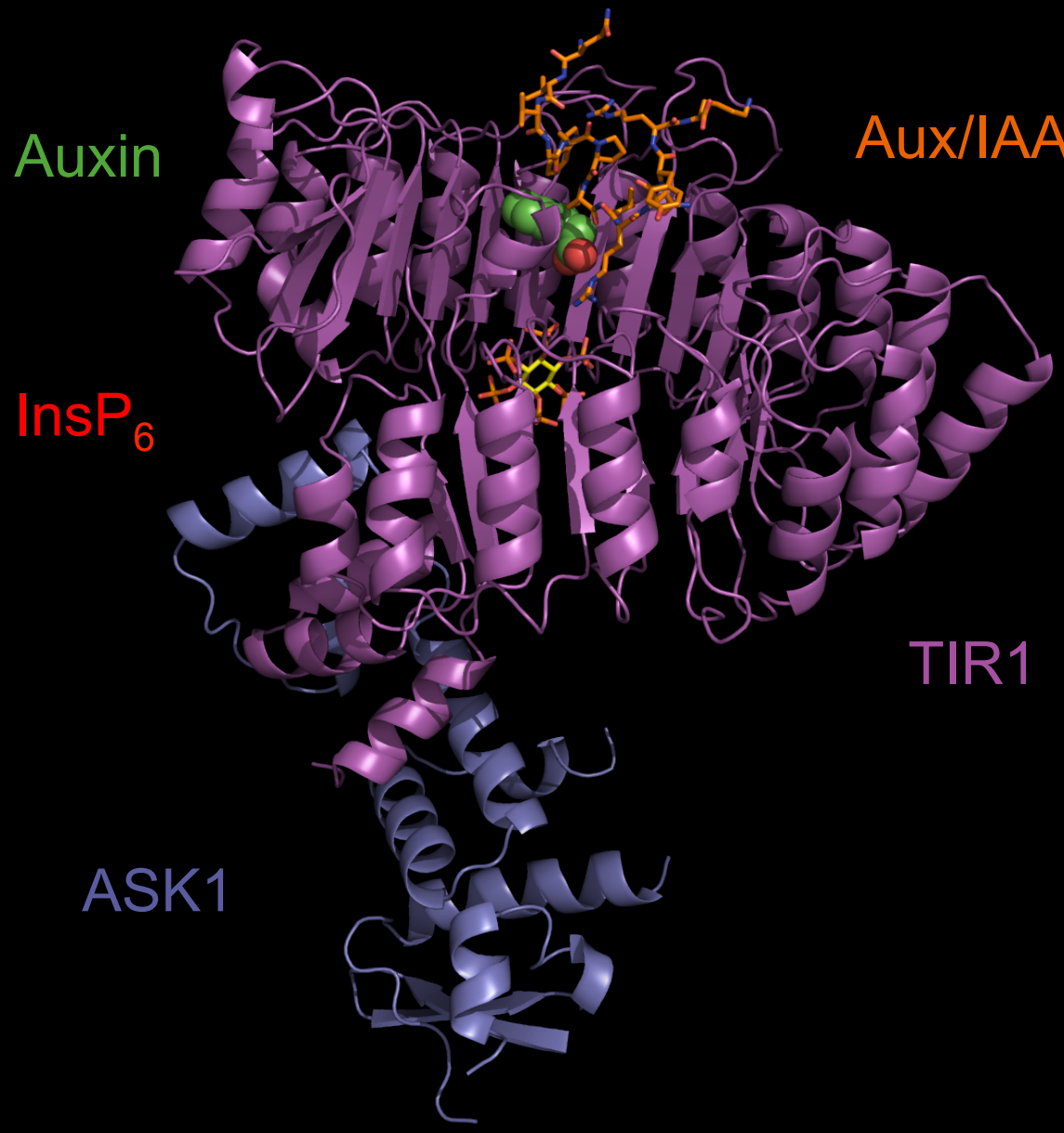
Aux/IAA 1 – 29

…PVVGWPPVRSFRR… IAA26/PAP1
…QIVGWPPVRSYRK… IAA3 /SHY2
…QVVGWPPVRSYRK… IAA17/AXR3
…QIVGWPPIRNYRK… IAA7 /AXR2
…QVVGWPPVCSYRK… IAA14/SLR1
…KIVGWPPIRSYRK… IAA19/MSG2
…QVVGWPPIGLHRM… IAA12/BDL
…QLVGWPPVATARK… IAA15

gvVGWPPvrsyrk



TIR1-ASK1-IAA7-Auxin-(InP_6) Complex



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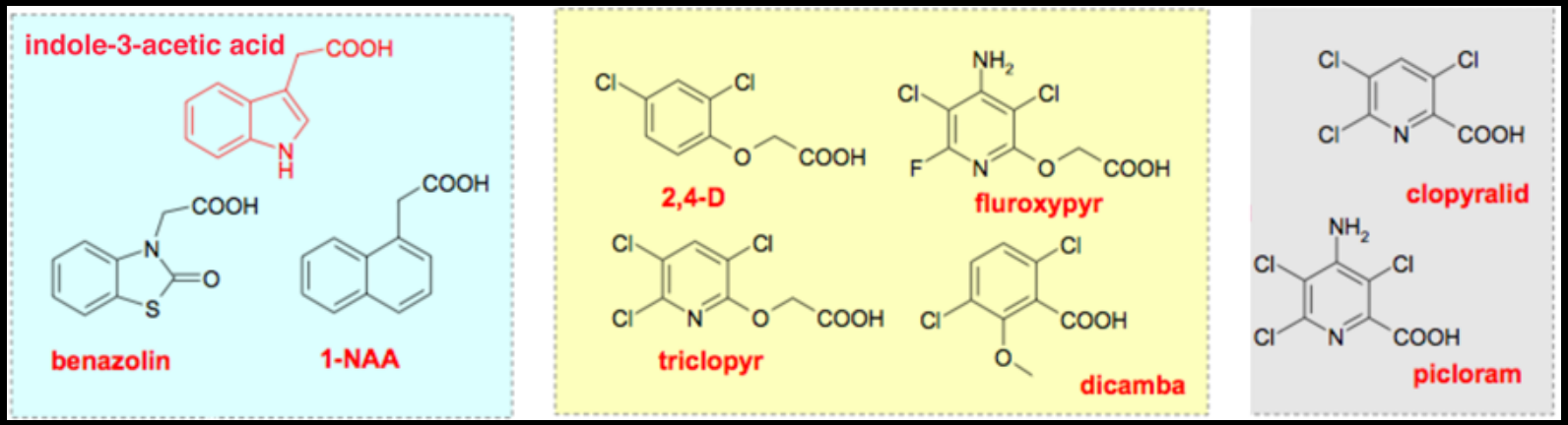
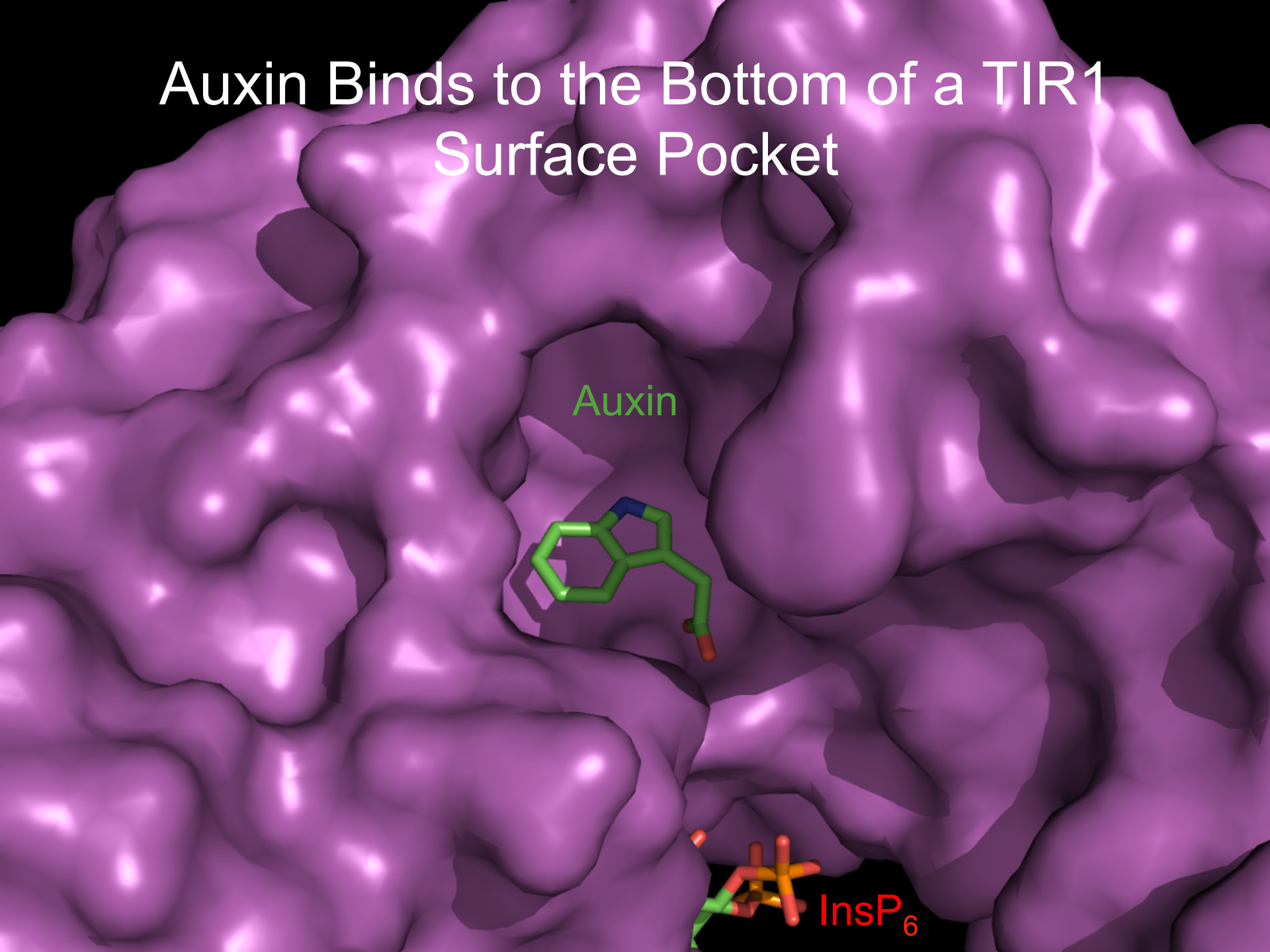


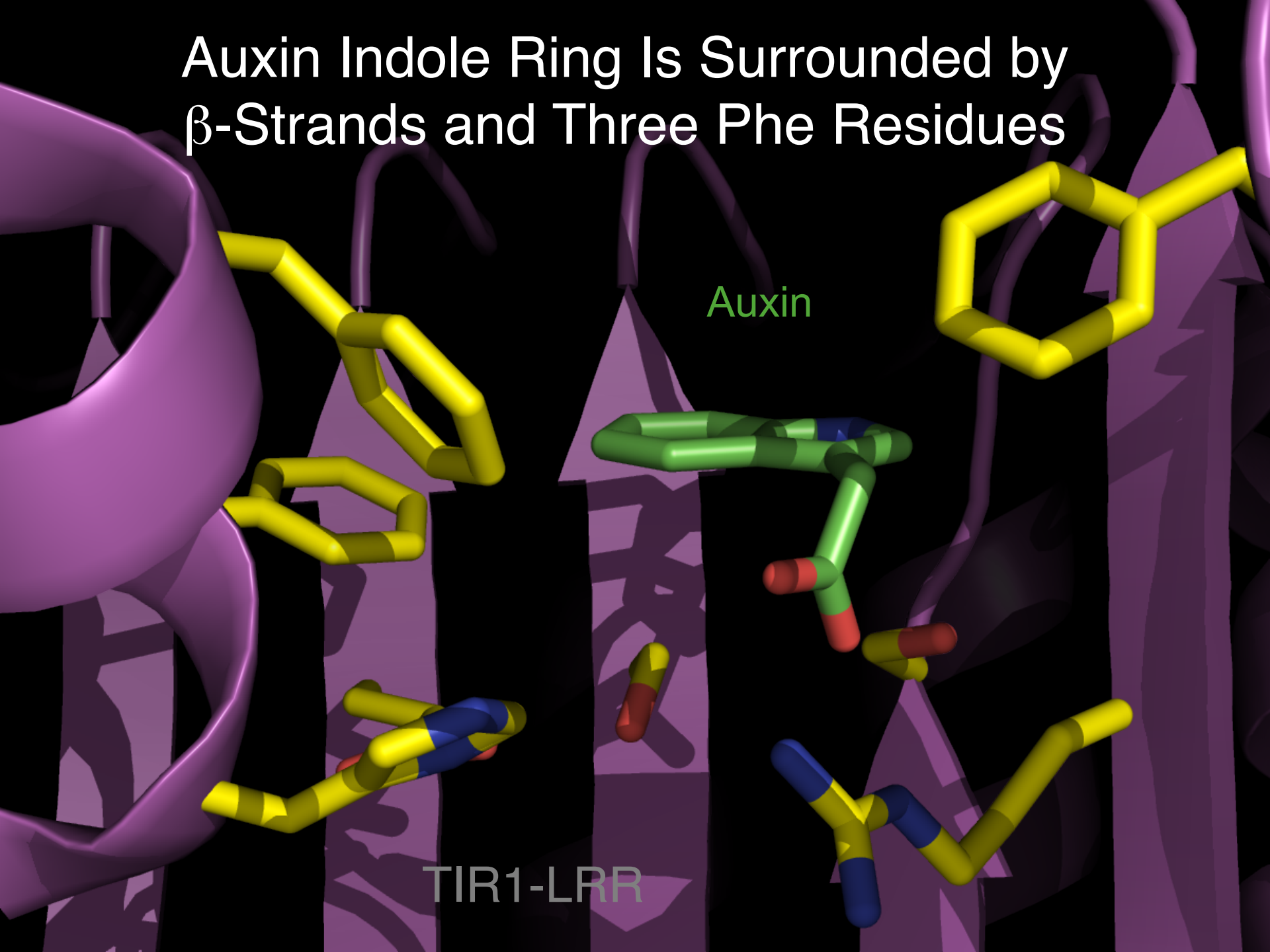
2. How does the small molecule auxin “activate” SCF-TIR1 ubiquitin ligase?

An Allosteric Switch Model

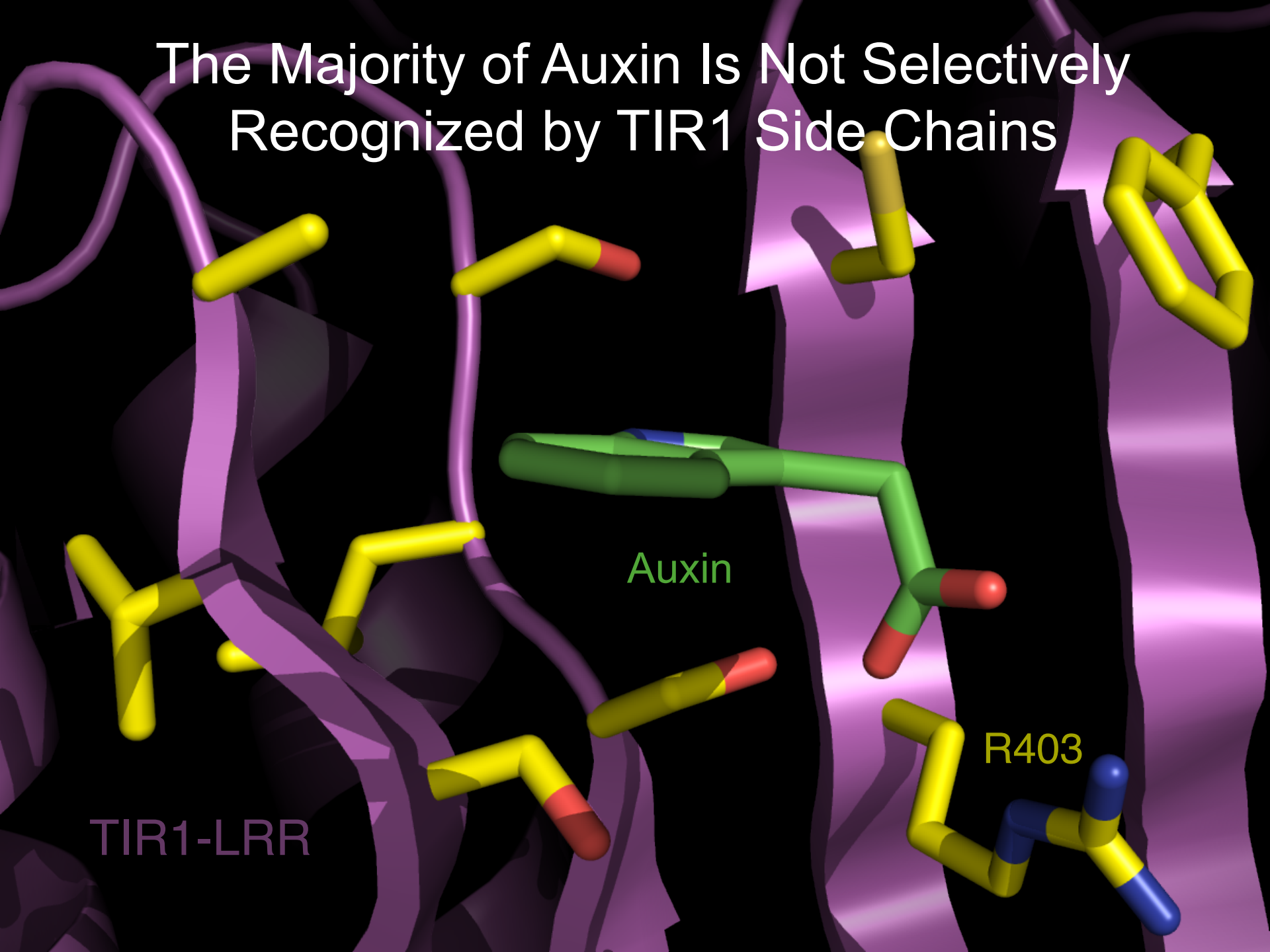
Auxin Binds to the Bottom of a TIR1 Surface Pocket



Auxin Indole Ring Is Surrounded by β-Strands and Three Phe Residues

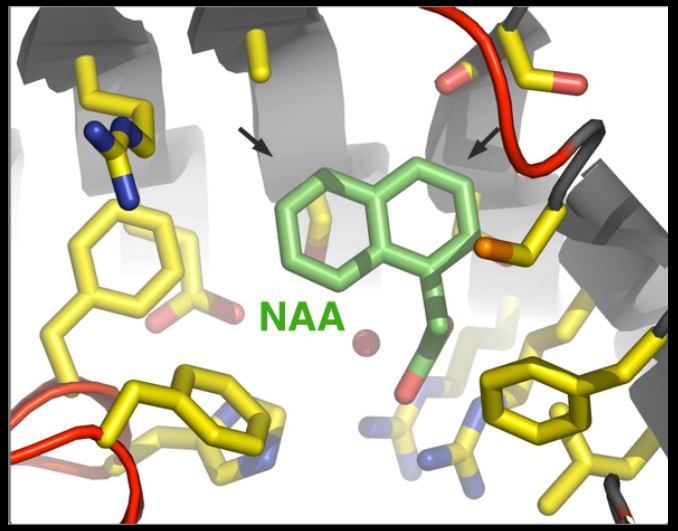


The Majority of Auxin Is Not Selectively Recognized by TIR1 Side Chains

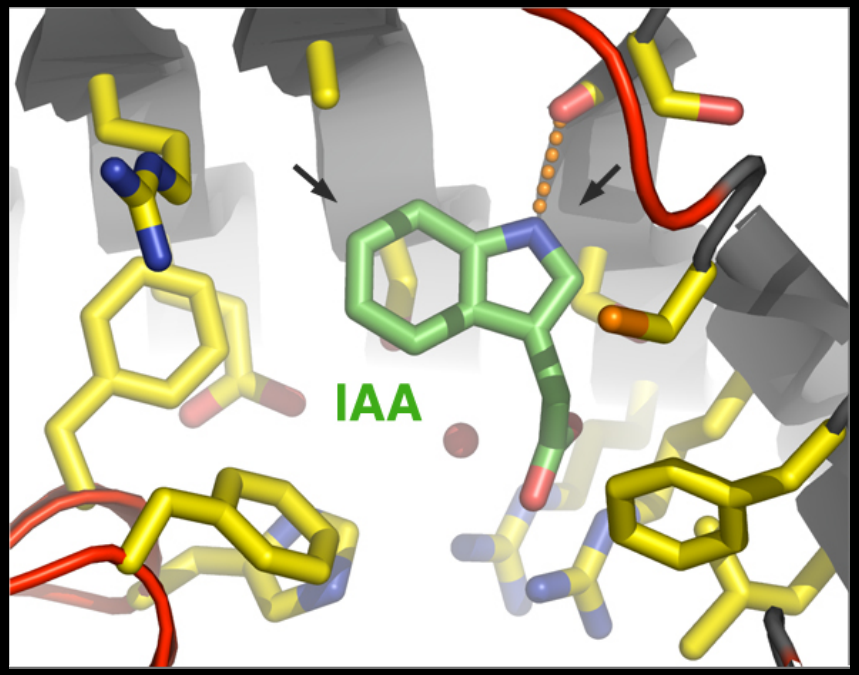


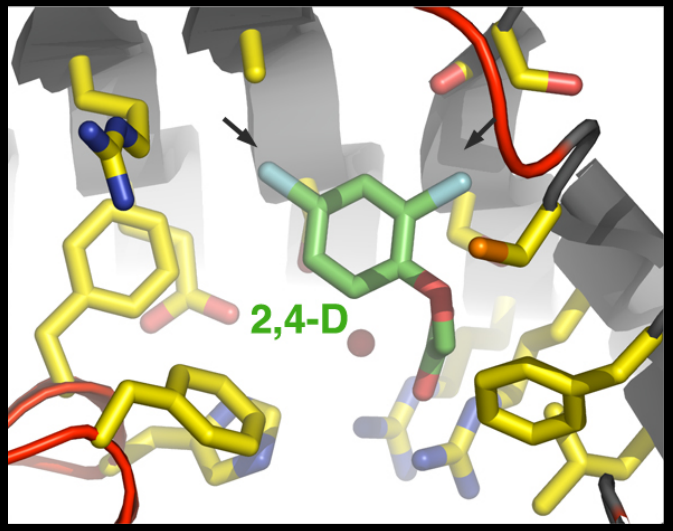
Common Binding Mode of Different Auxin Compounds

1-NAA
1-Naphthaleneacetic acid



IAA
Indole-3-Acetic Acid

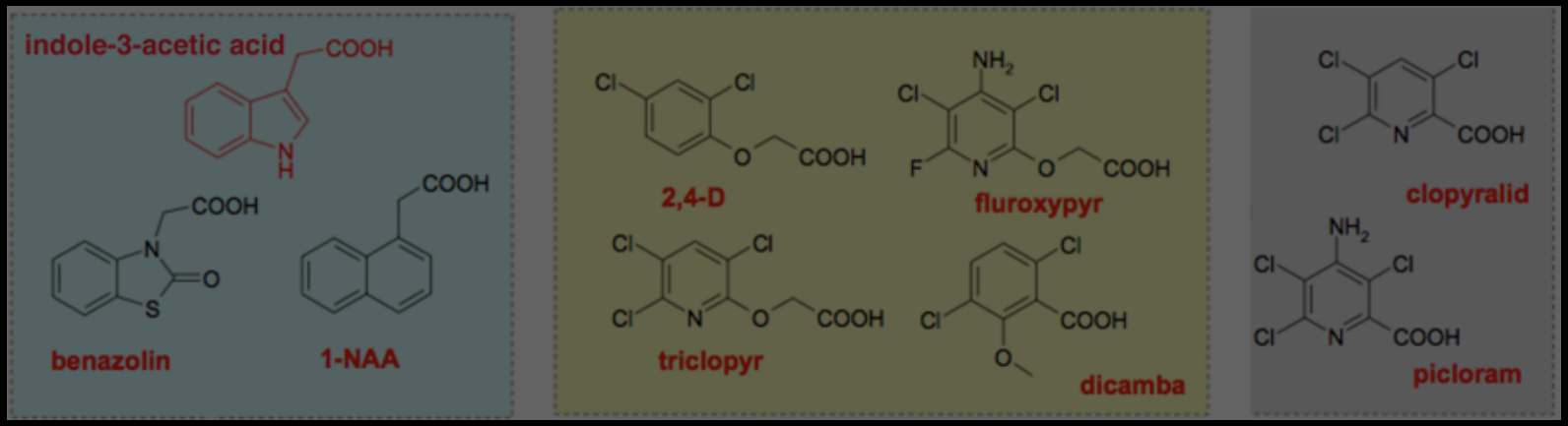




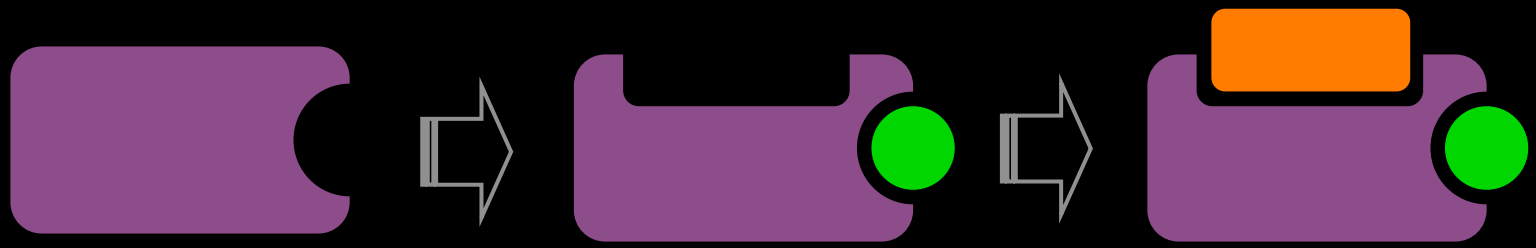
2,4-D
2,4-dichlorophenoxyacetic acid

Two Questions on Auxin Action

1. How does TIR1 recognize auxin and more than 200 different analogues?

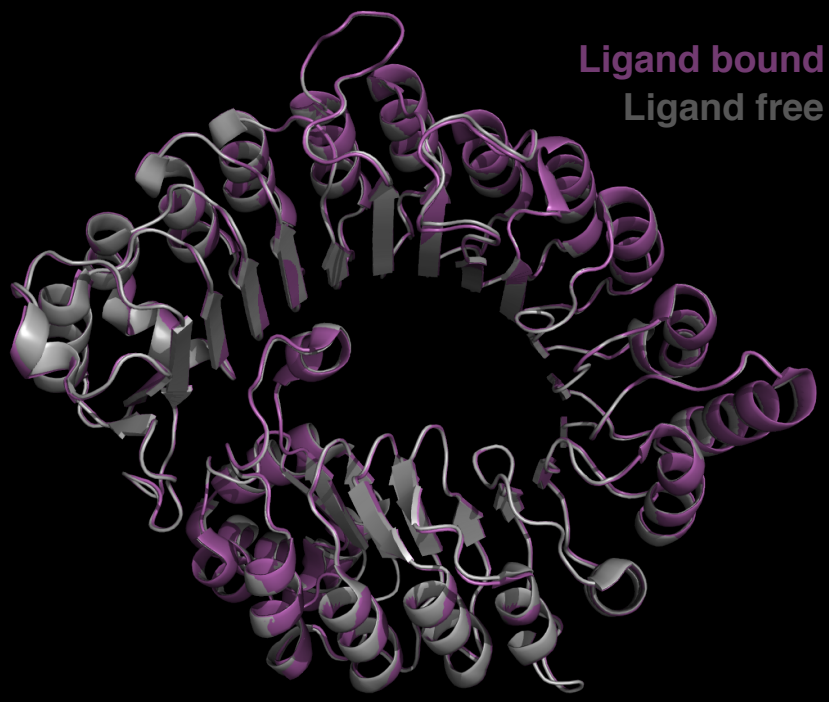


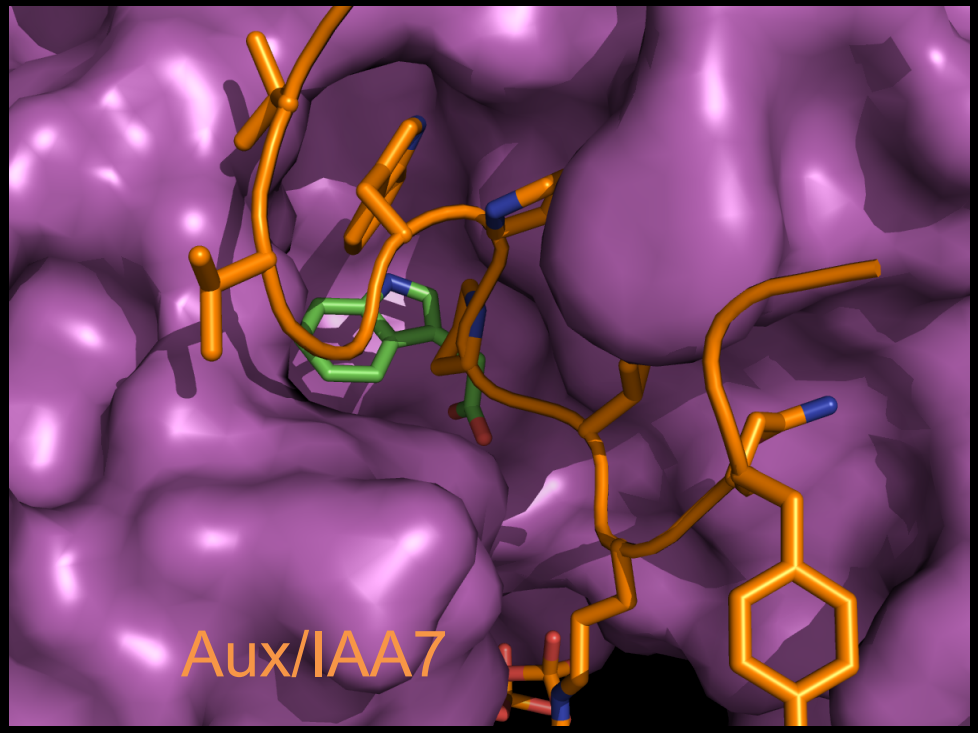
2. How does the small molecule auxin “activate” SCF-TIR1 ubiquitin ligase?



An Allosteric Switch Model

Auxin is NOT an Allosteric Switch

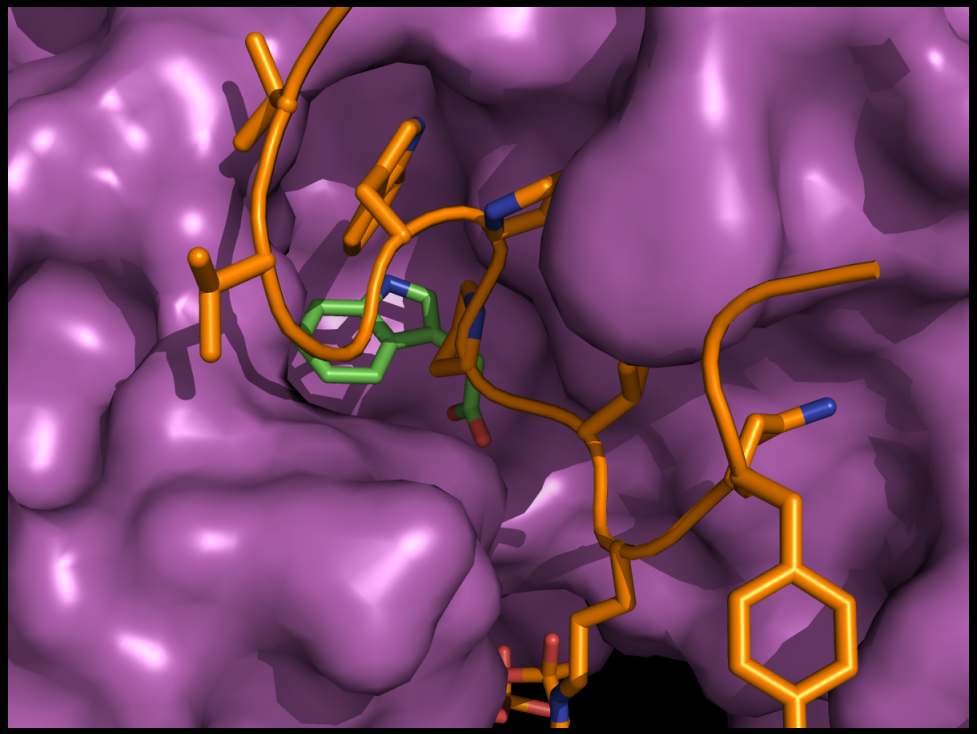


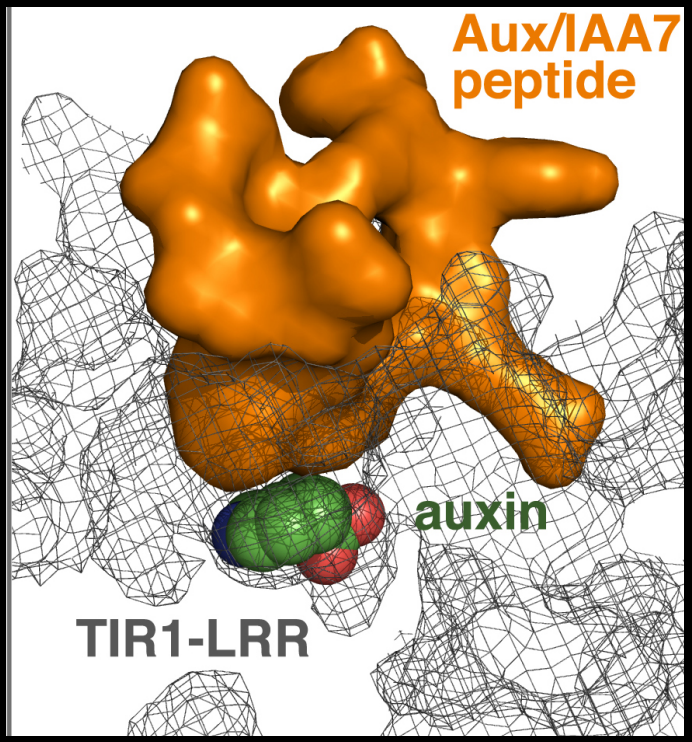


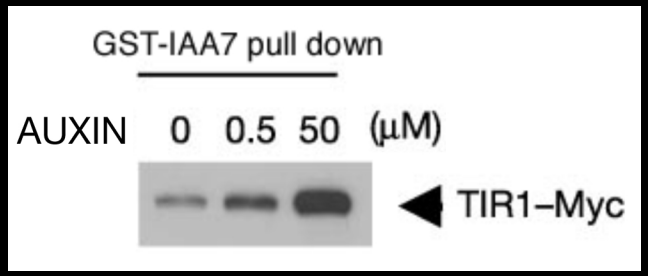
(1) Auxin does not induce any major conformational change of TIR1

(2) Auxin binds to the same pocket where the substrate peptide binds

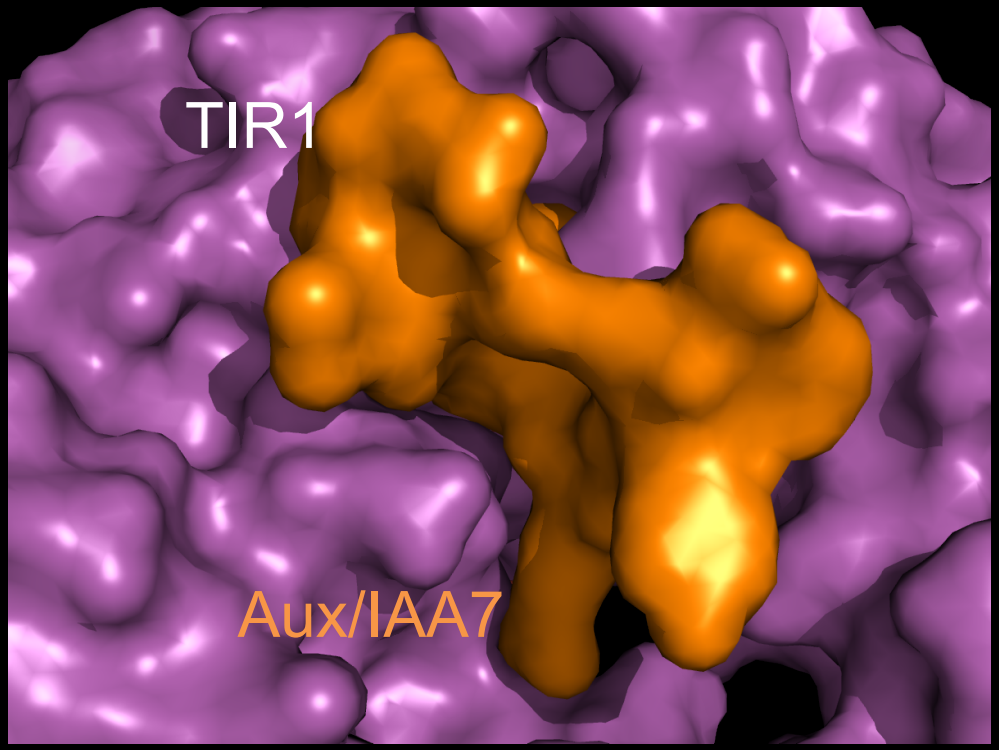
Auxin Fills A Gap at Protein Interface

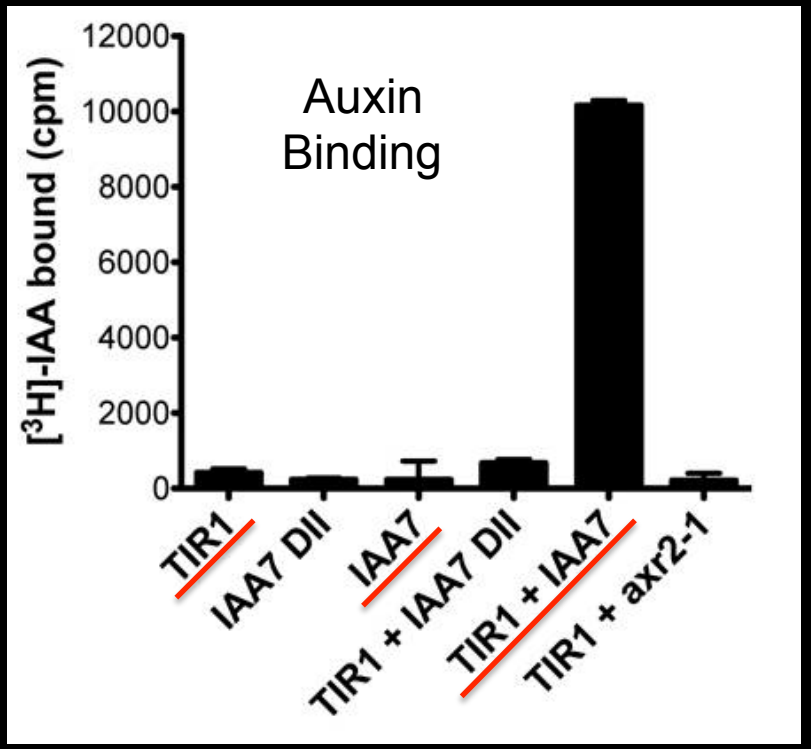






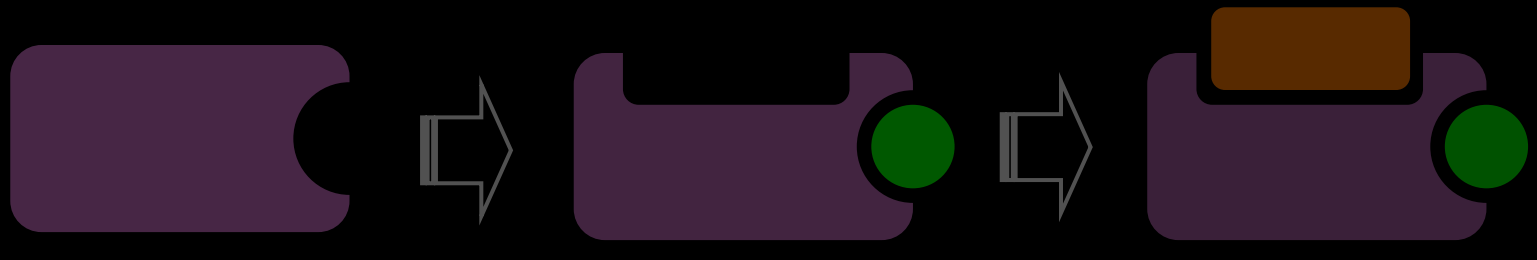
TIR1 – Aux/IAA7 (Ubiquitin Ligase – Substrate) as A High Affinity Hormone Co-receptor



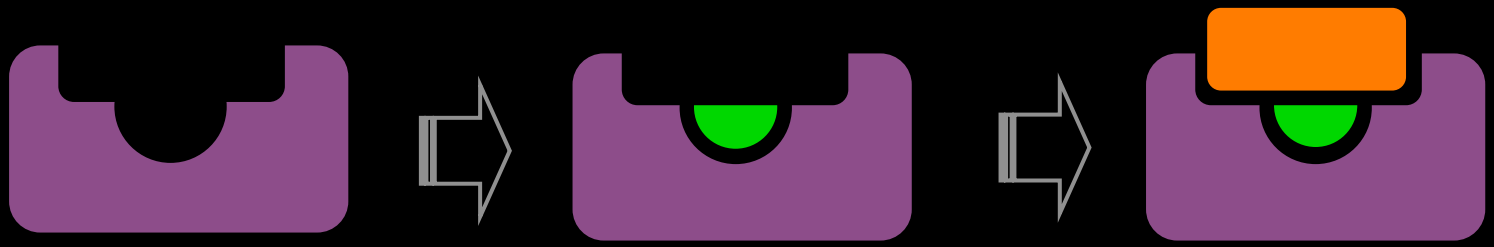


A possible reason behind the failure of conventional biochemical approaches for purifying auxin receptors.

Two Distinct Models of Auxin Action



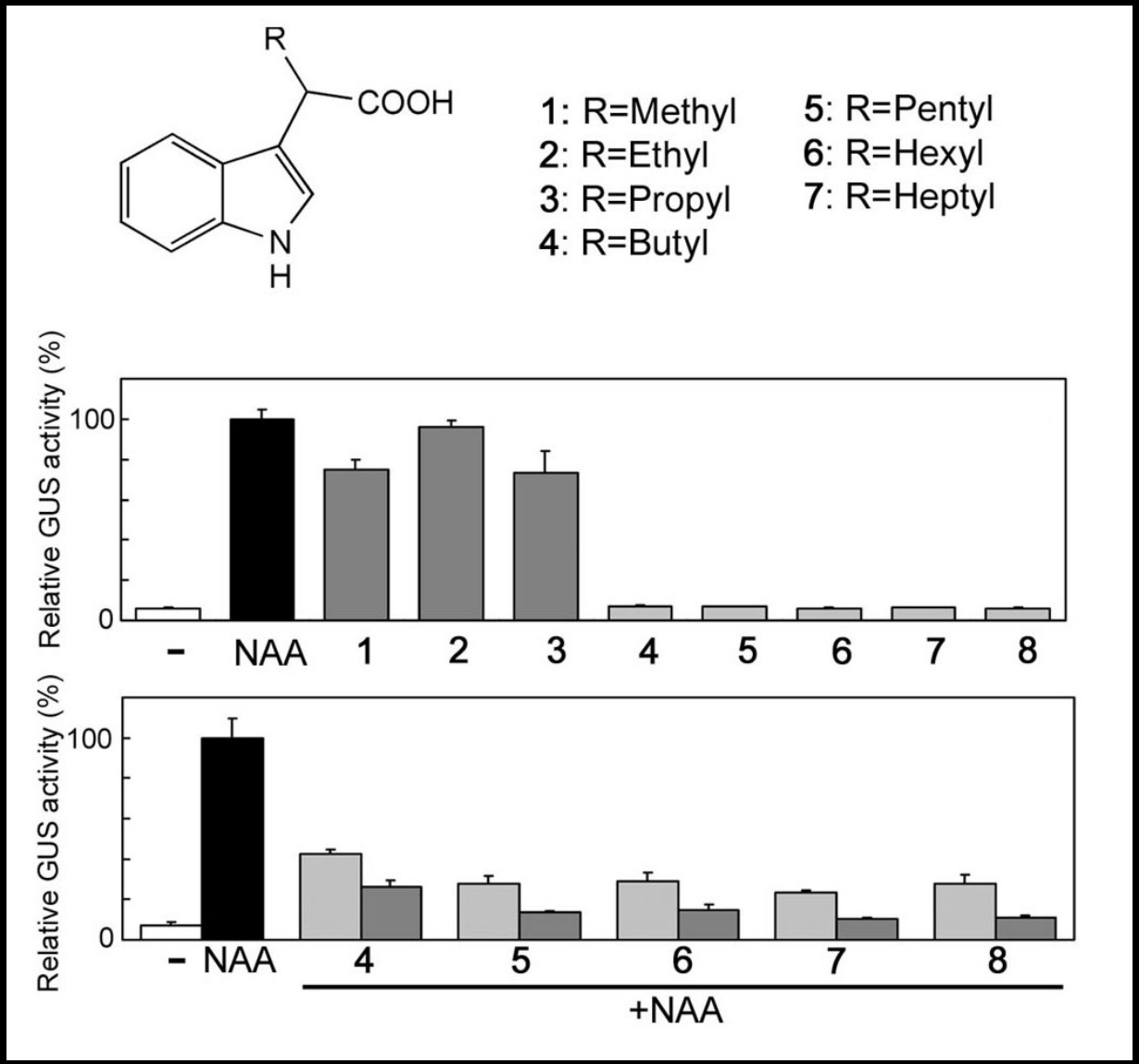
A Molecular Glue Model

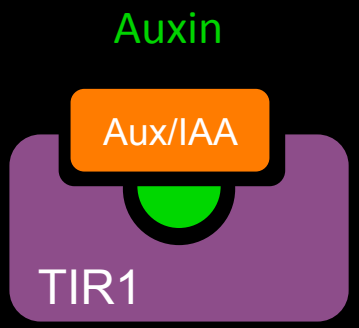


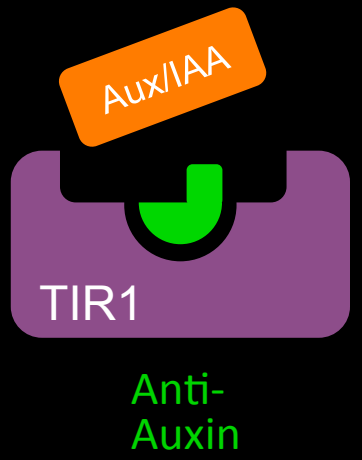
Molecular Glue ⟺ Promiscuity

Phosphorylation of SCF substrates is essentially a type of molecular glue too (covalent).

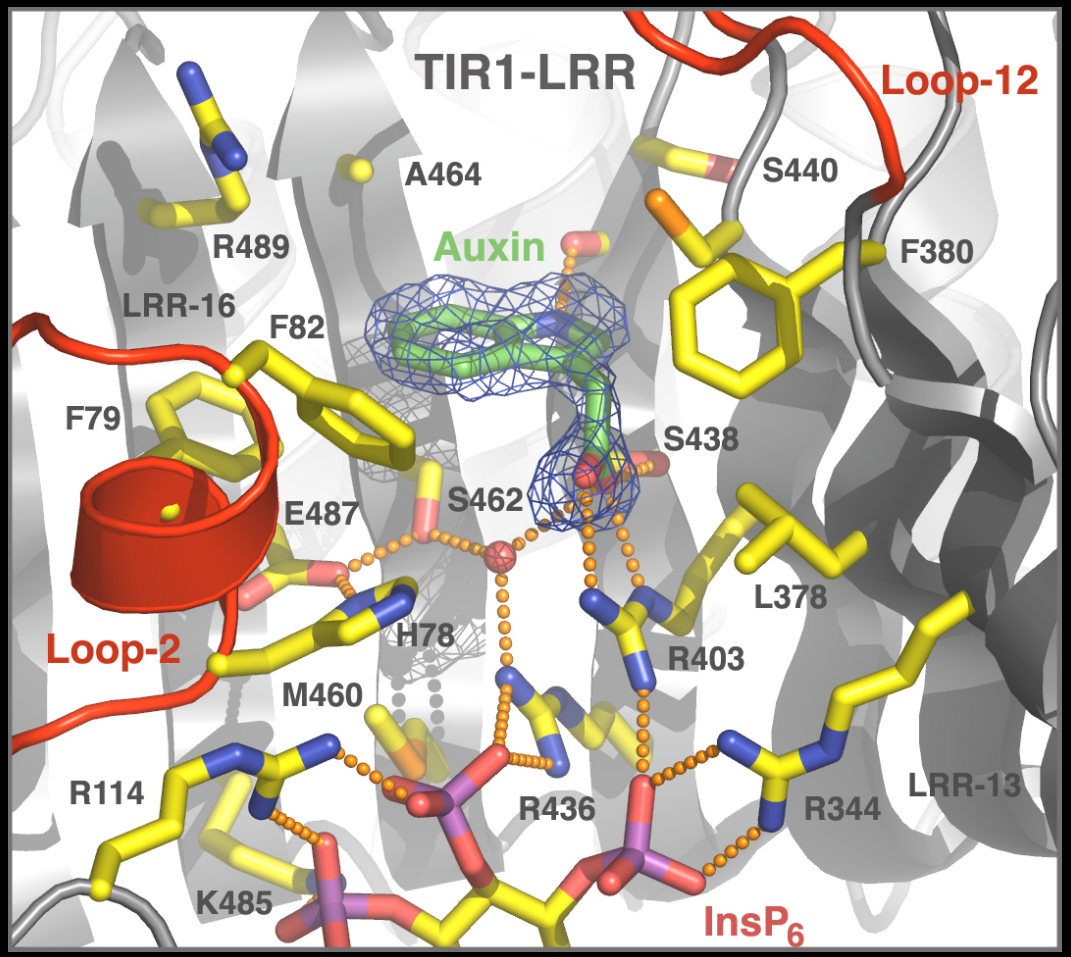
Converting Auxin to Anti-Auxins







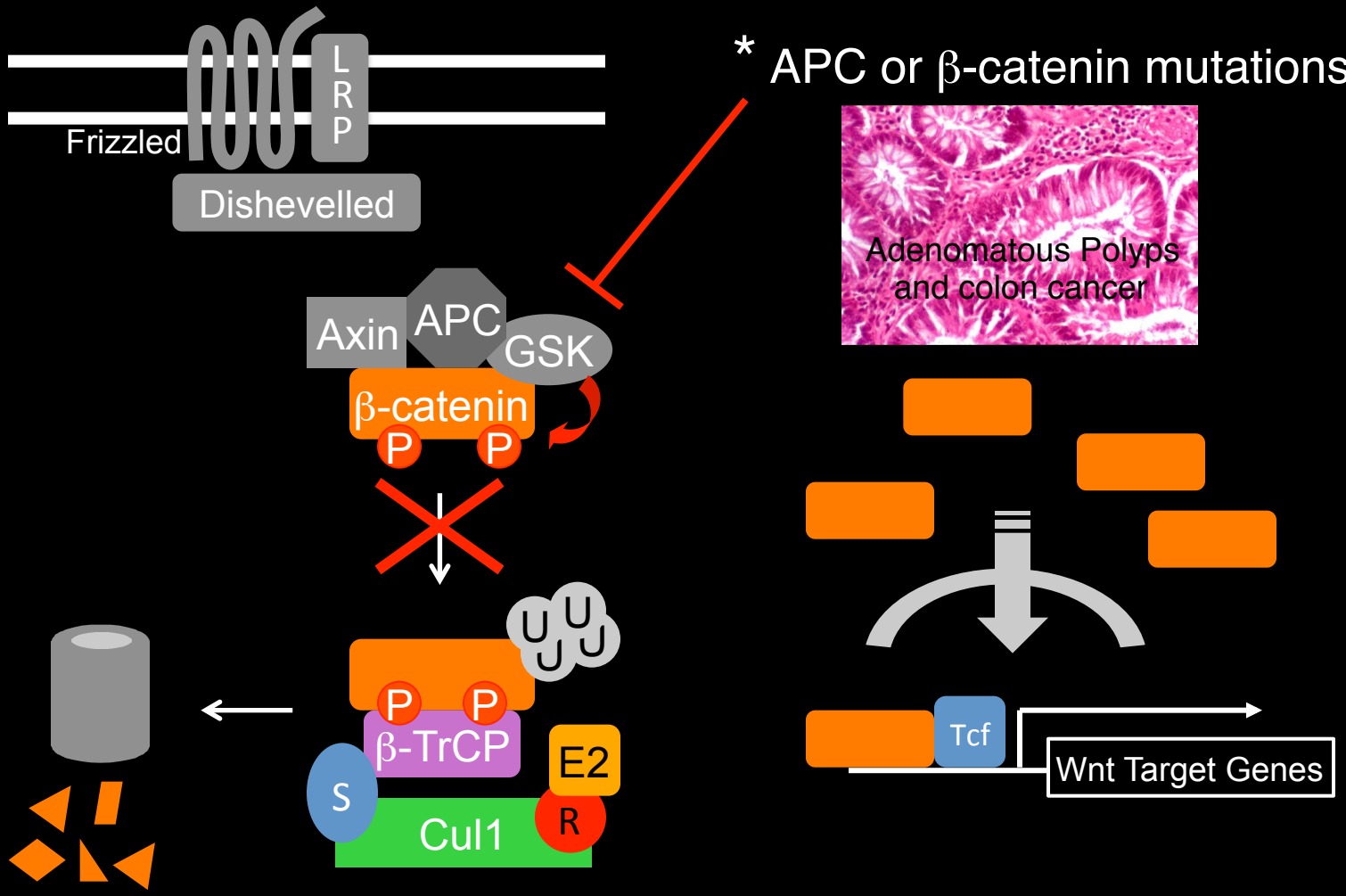
The Role of $InsP_6$ Remains Unknown



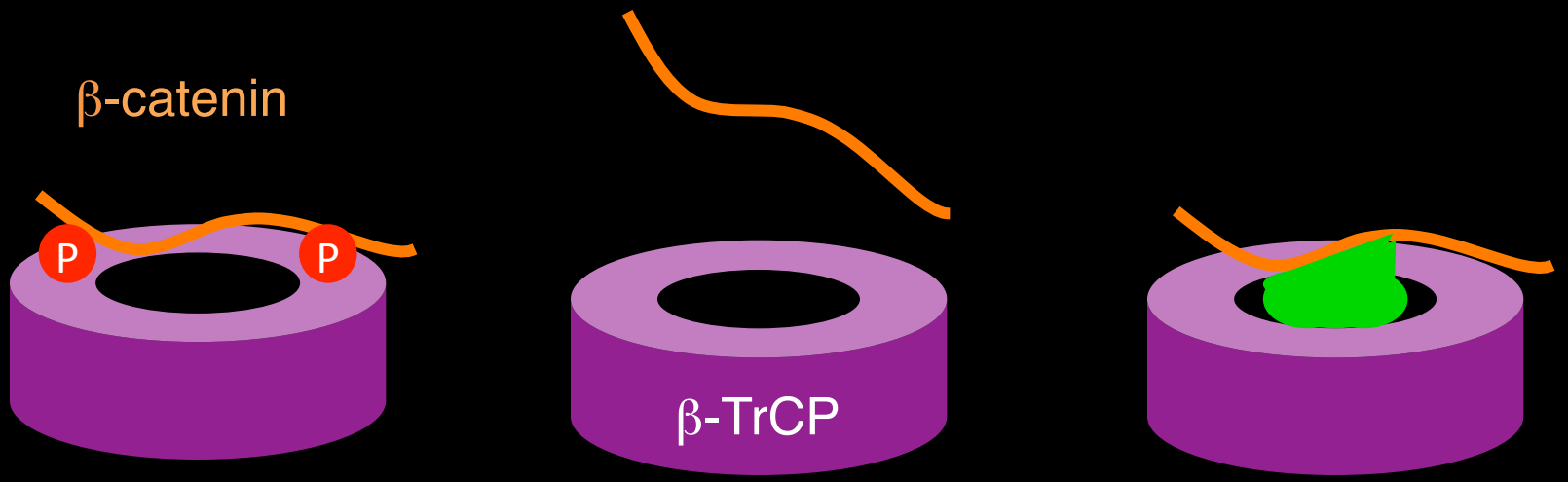
Tons of “phytic acid” a.k.a Inositol hexakisphosphate

A folding cofactor, a signaling molecule, or an artifact?

Impaired β-TrCP – β-catenin Interaction in Colon Cancer



Auxin-Inspired Drug Discovery Effort



Normal State

Disease State

Mutations in upstream kinase complex prevents β-catenin degradation.

Rescued by Drugs

Acknowledgement

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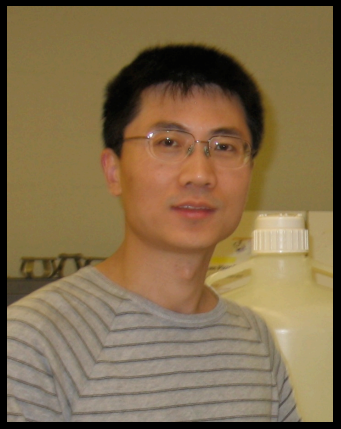

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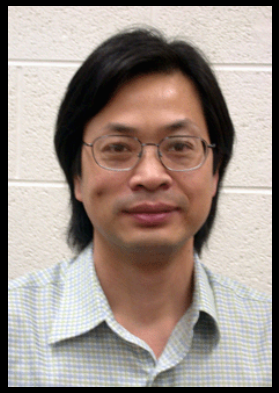

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