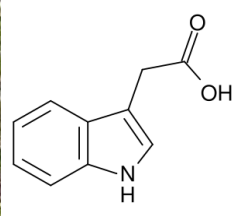
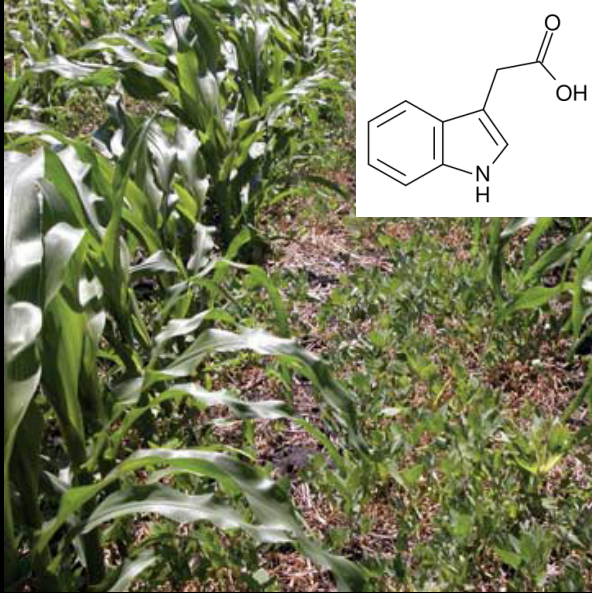


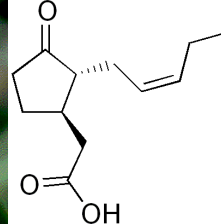
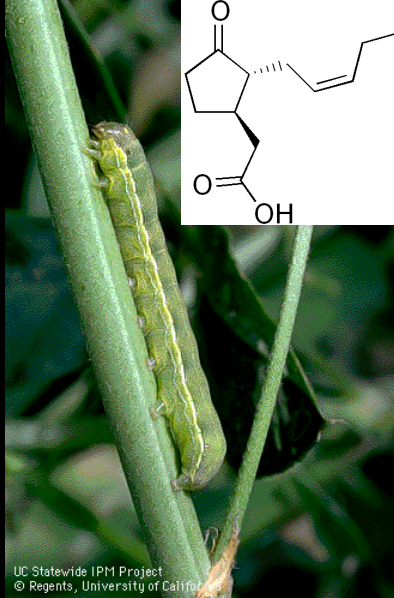
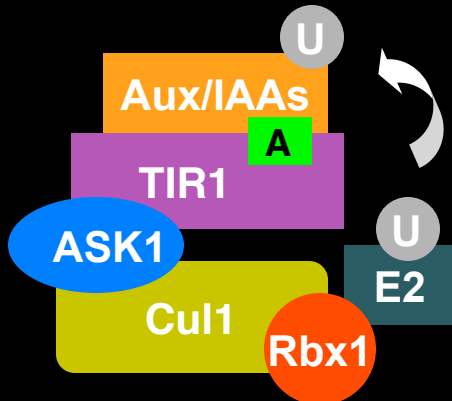


The New Paradigm Is Rapidly Expanding

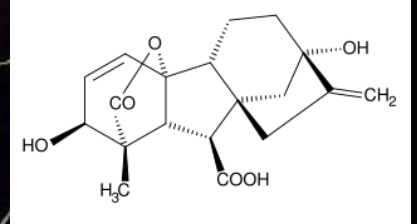
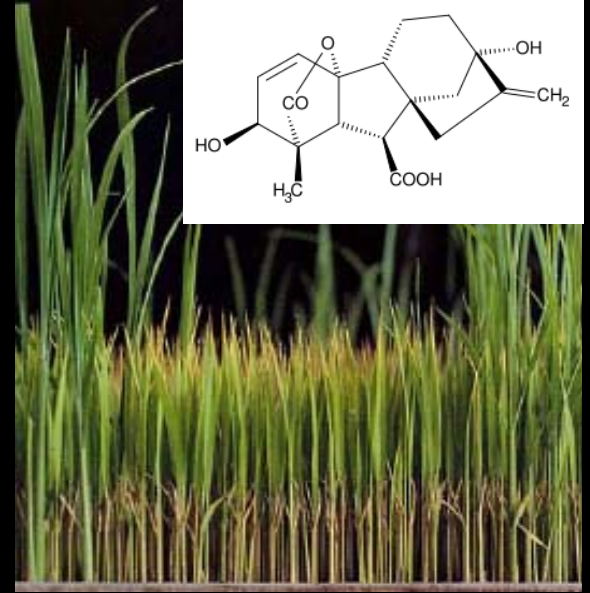
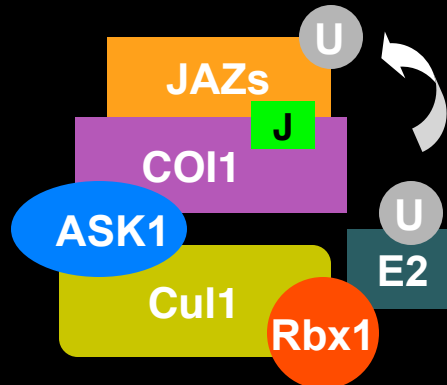
SCF Ubiquitin Ligases as Plant Hormone Receptors



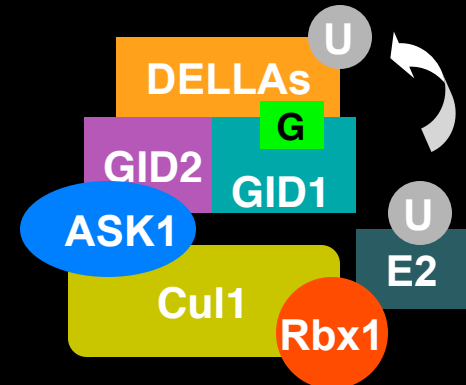
Auxin



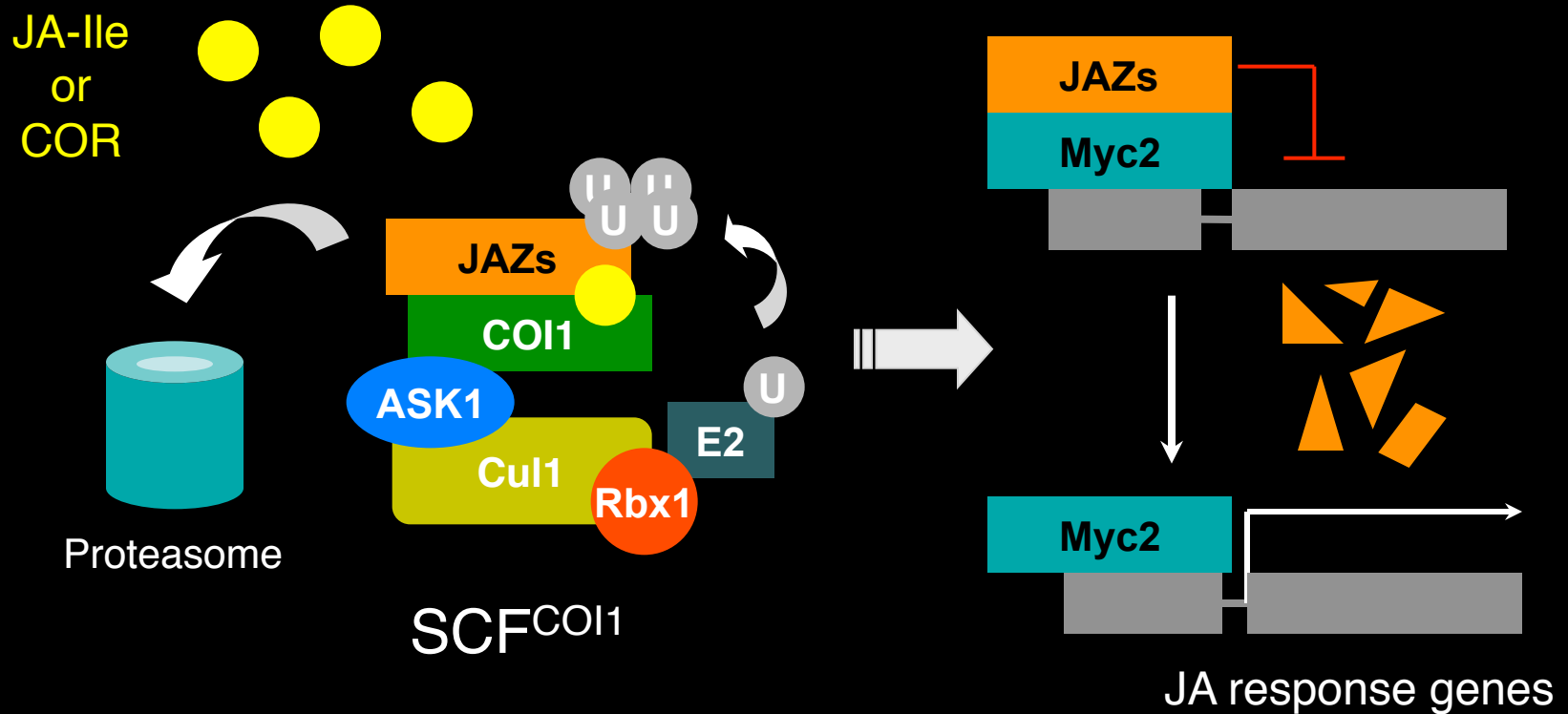
Jasmonic Acid



Gibberellin

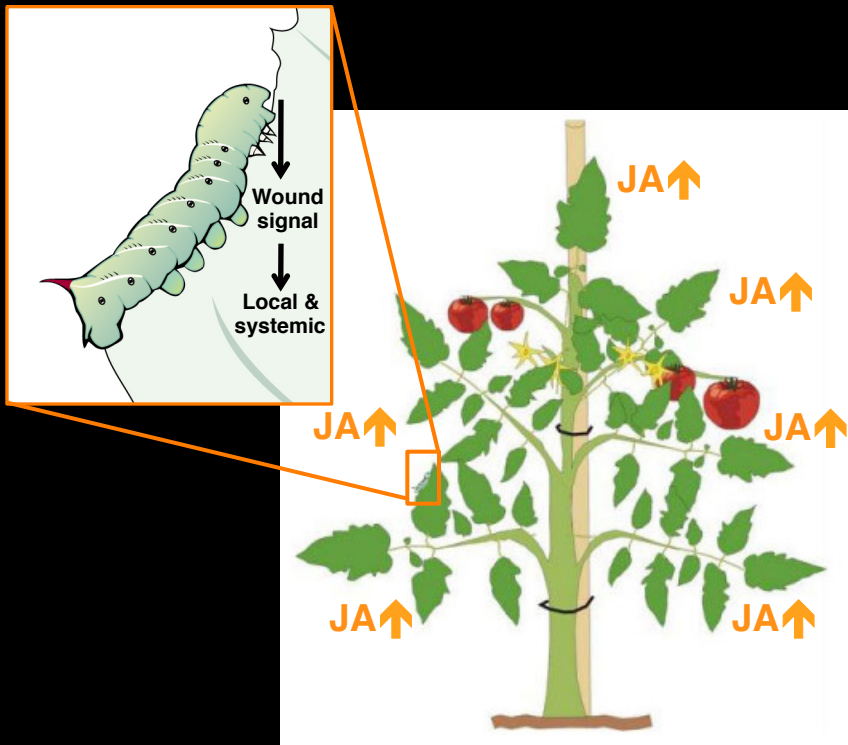


Jasmonate Signaling Pathway

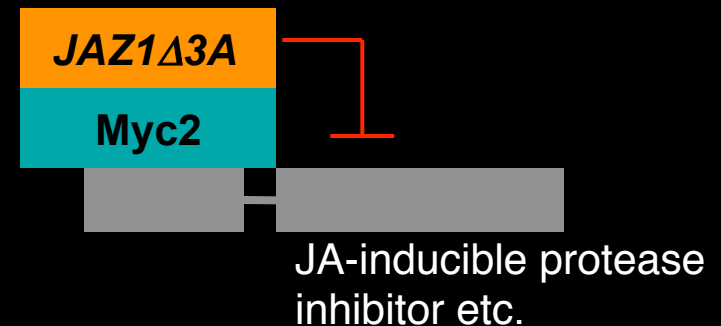
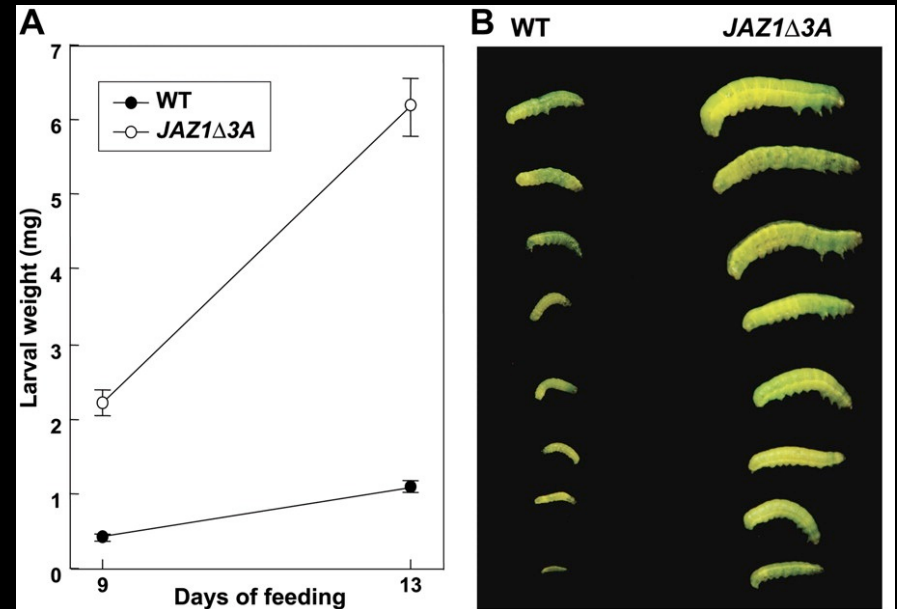


JA Signaling Regulates Wound Responses in Plants

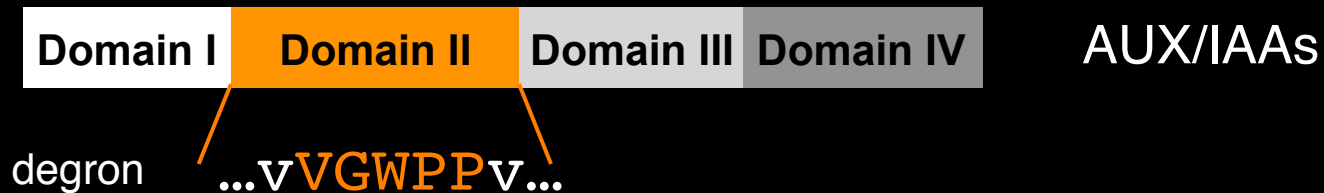
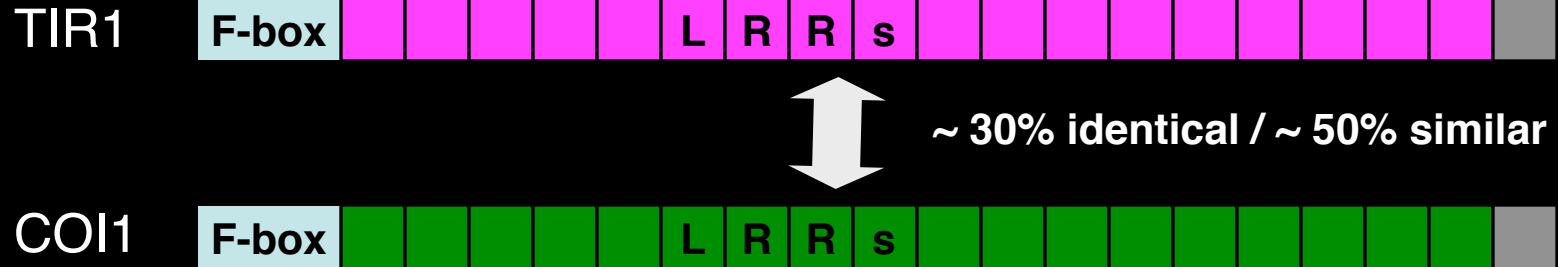
Tomato Under Herbivore Attack



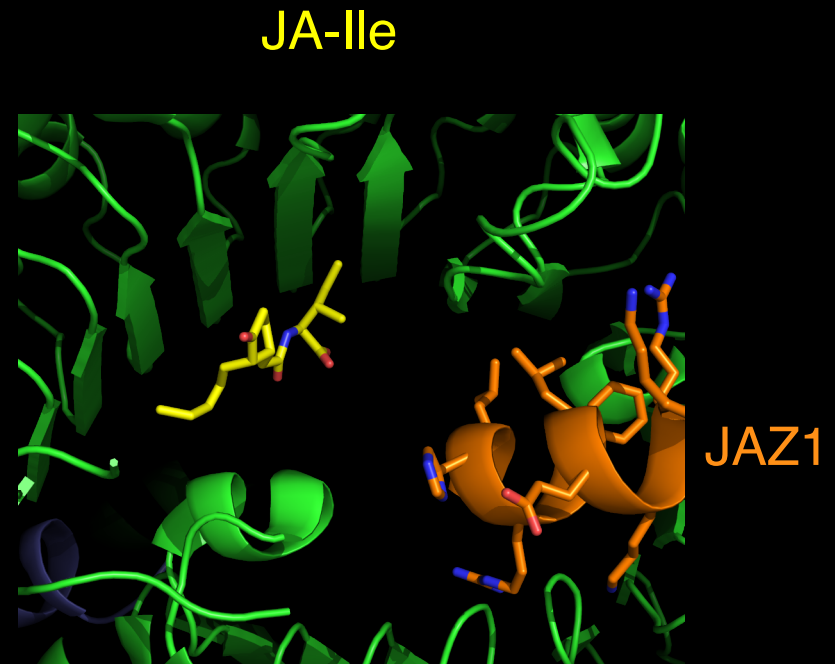
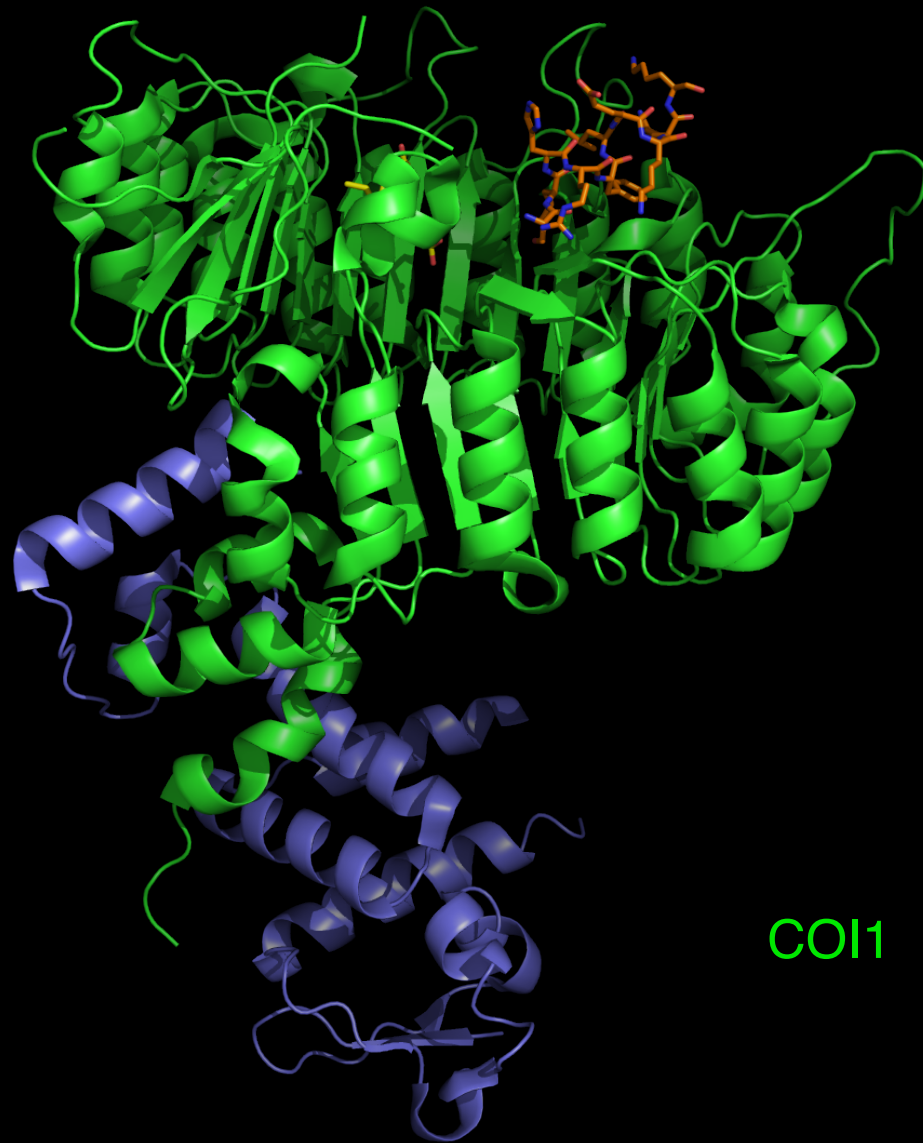
Arabidopsis JAZ1 Δ 3A mutant loses part of its defensor and fails to self-defend.



Sequence Comparison of TIR1 and COI1

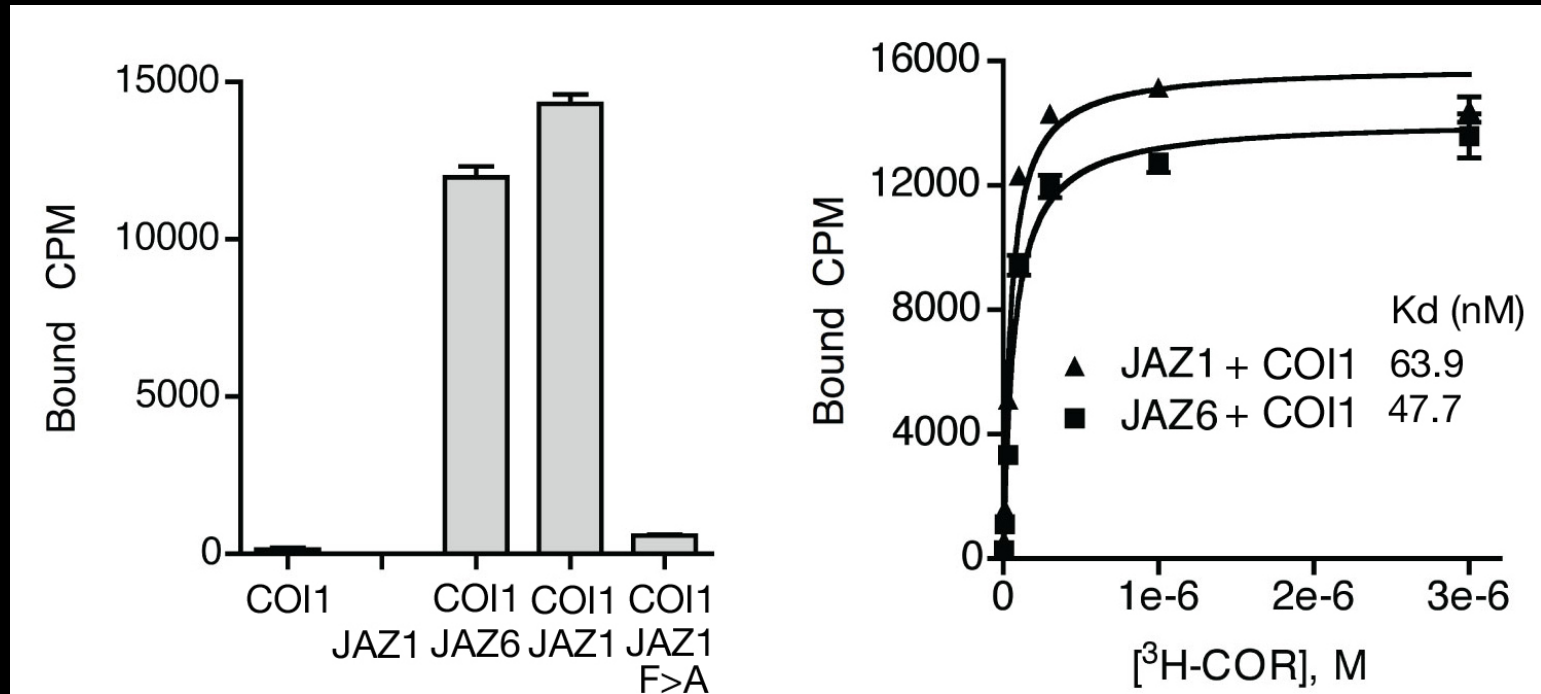


A Different Mechanism?



The True Receptor of JA Is A COI1-JAZ Complex

^3H Coronatine Binding Assay



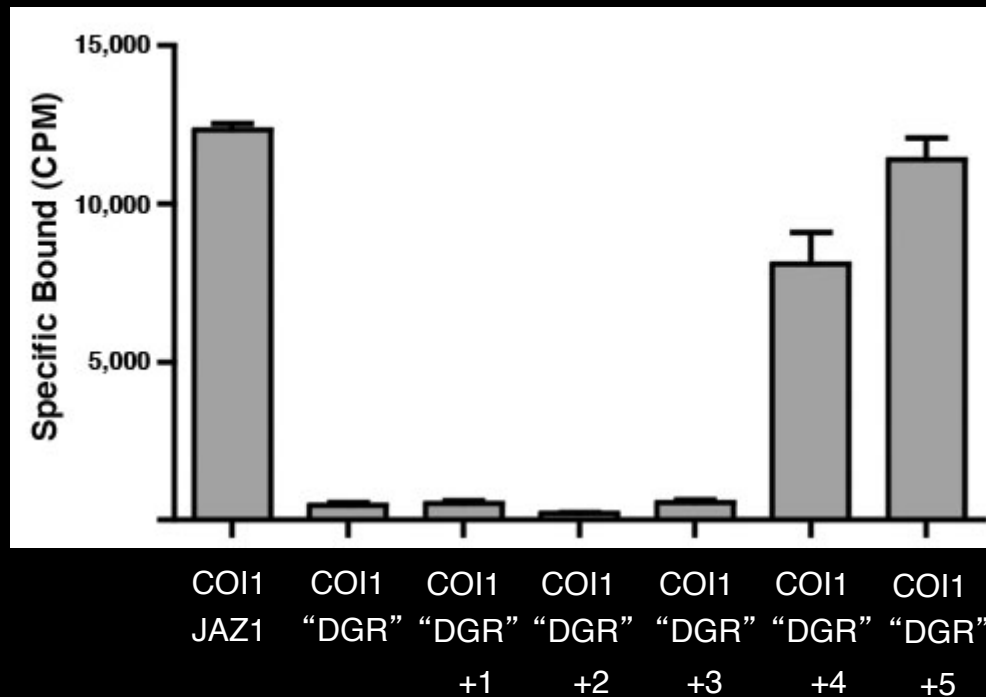
JAZ1



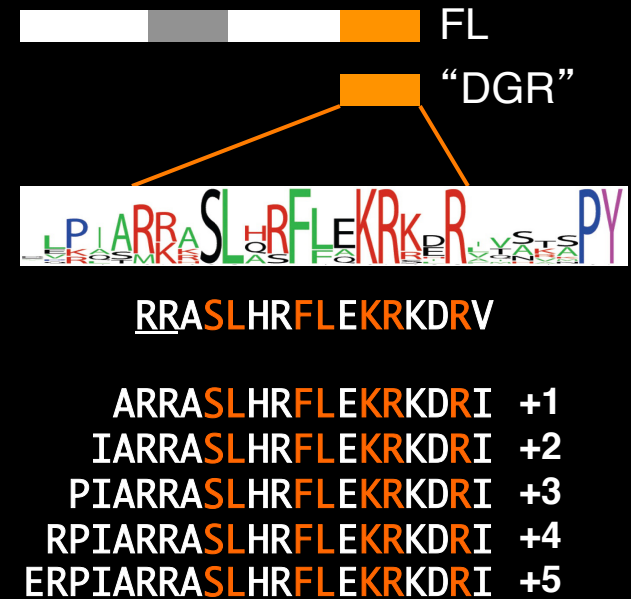
*

Redefine JAZ Degron

^3H Coronatine Binding Assay



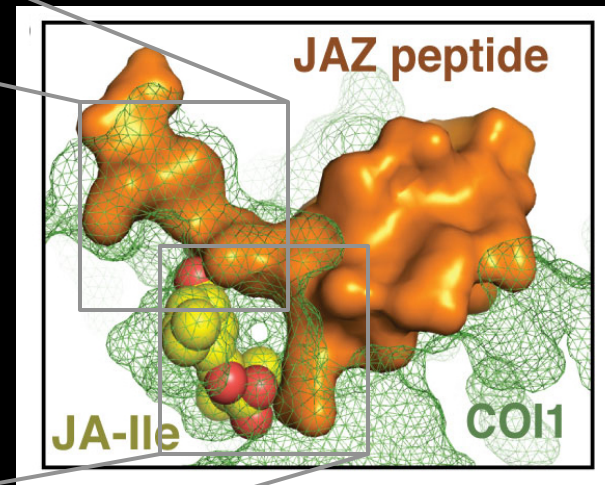
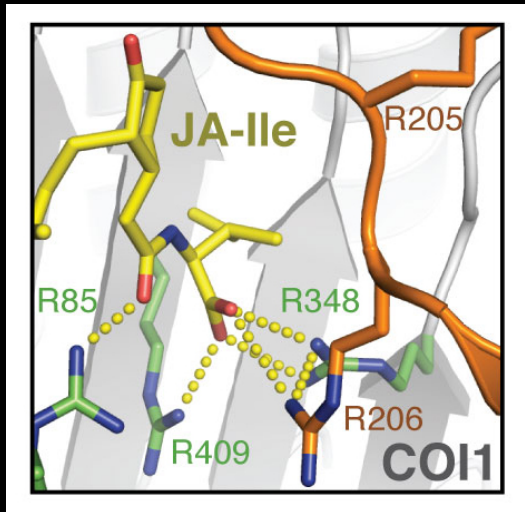
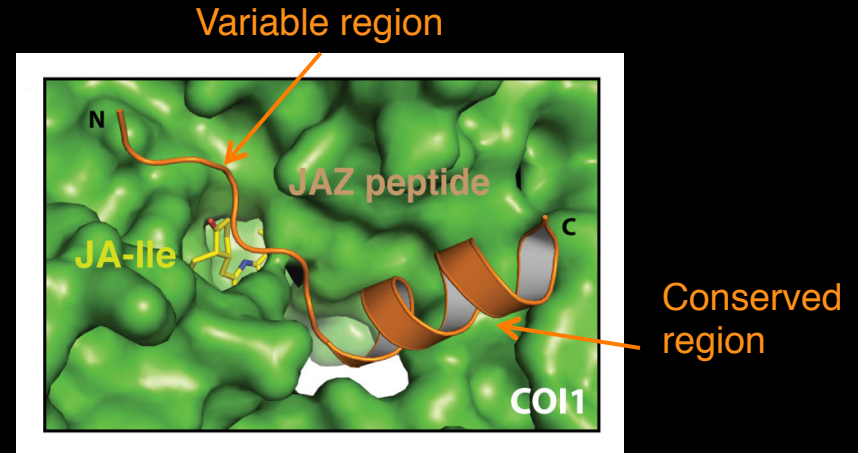
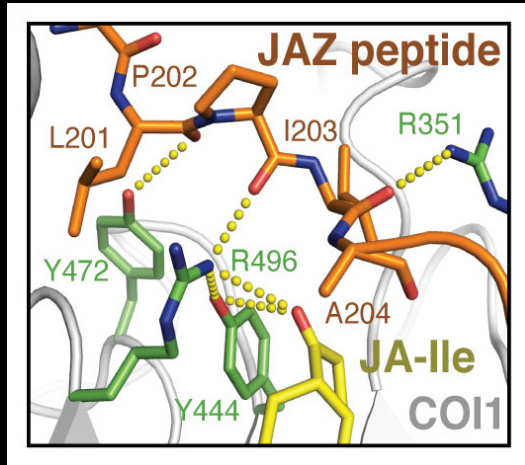
JAZ1



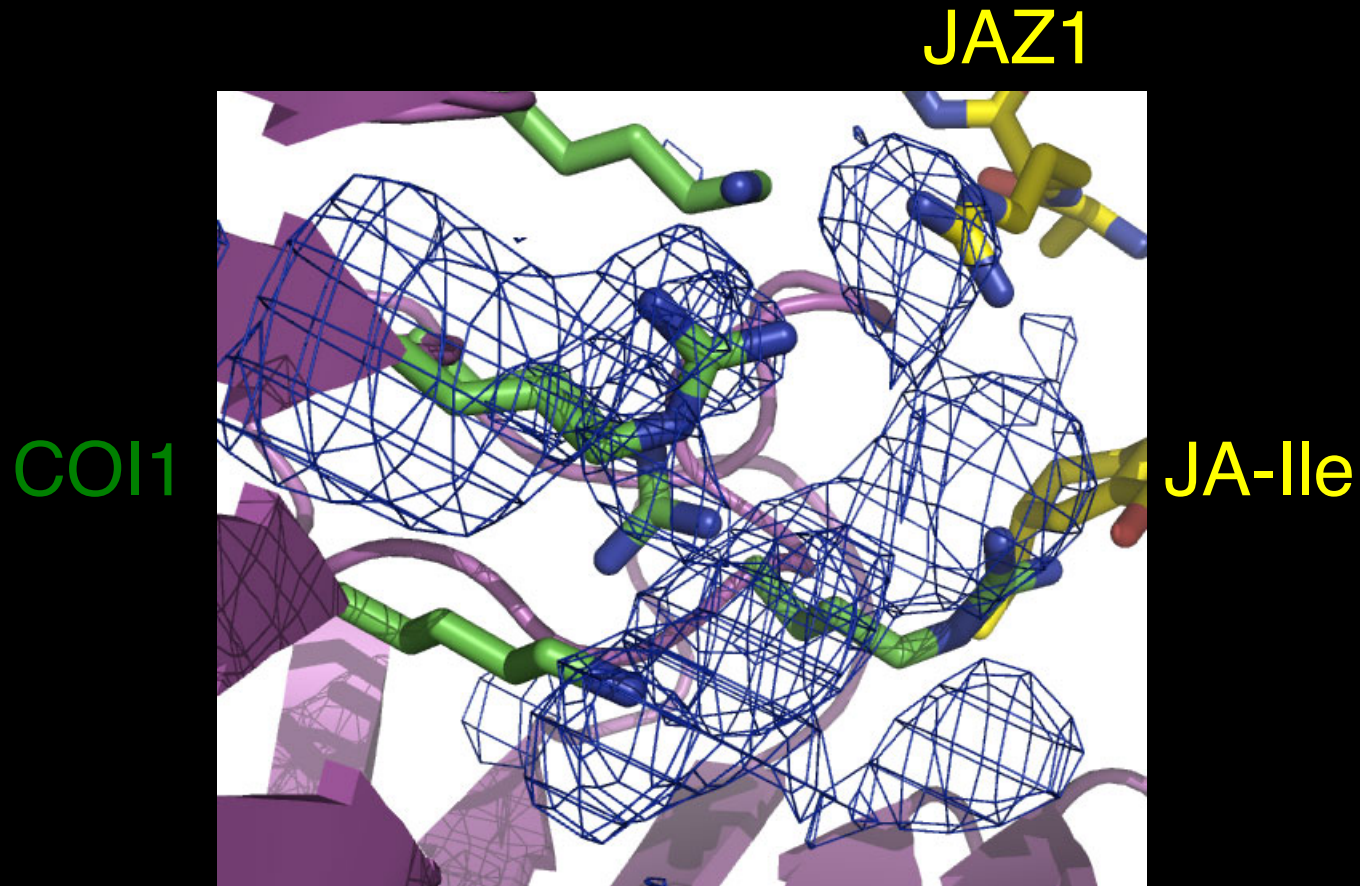
Whew!



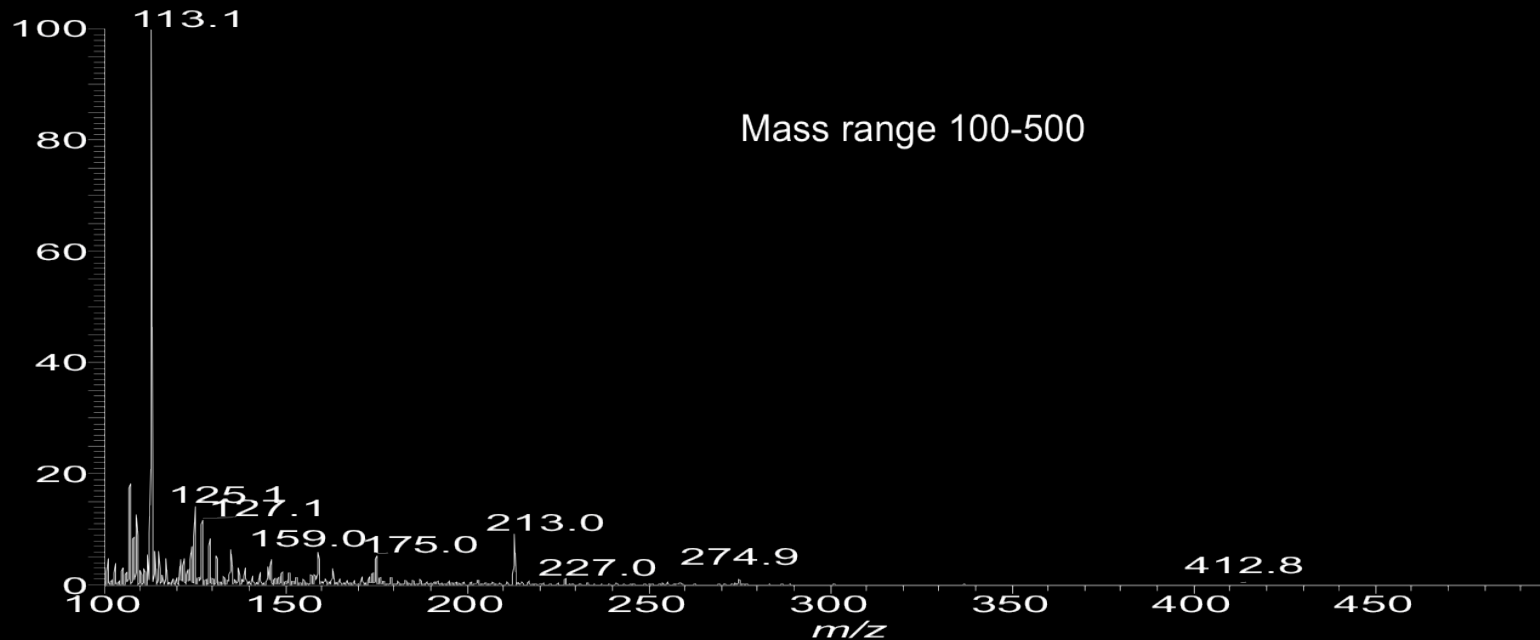
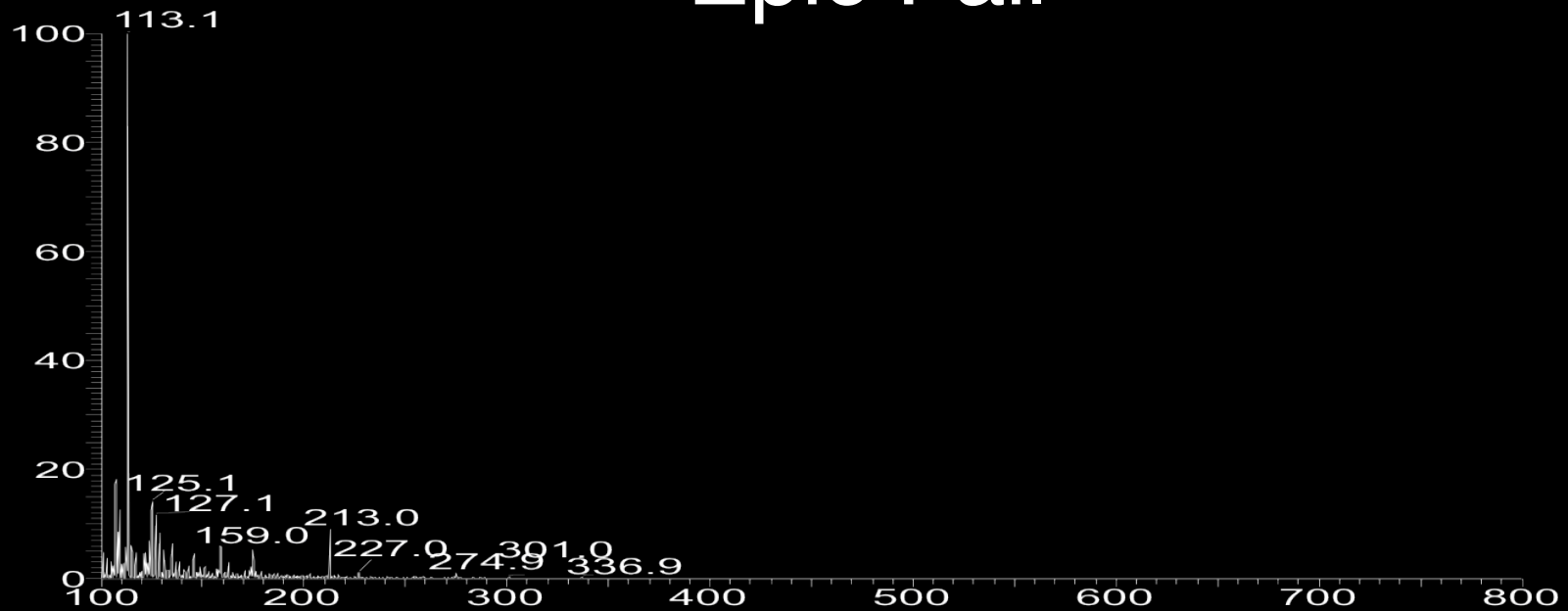
A Bipartite JAZ Degron



Does COI1 Also Have An InsP₆ Co-factor?

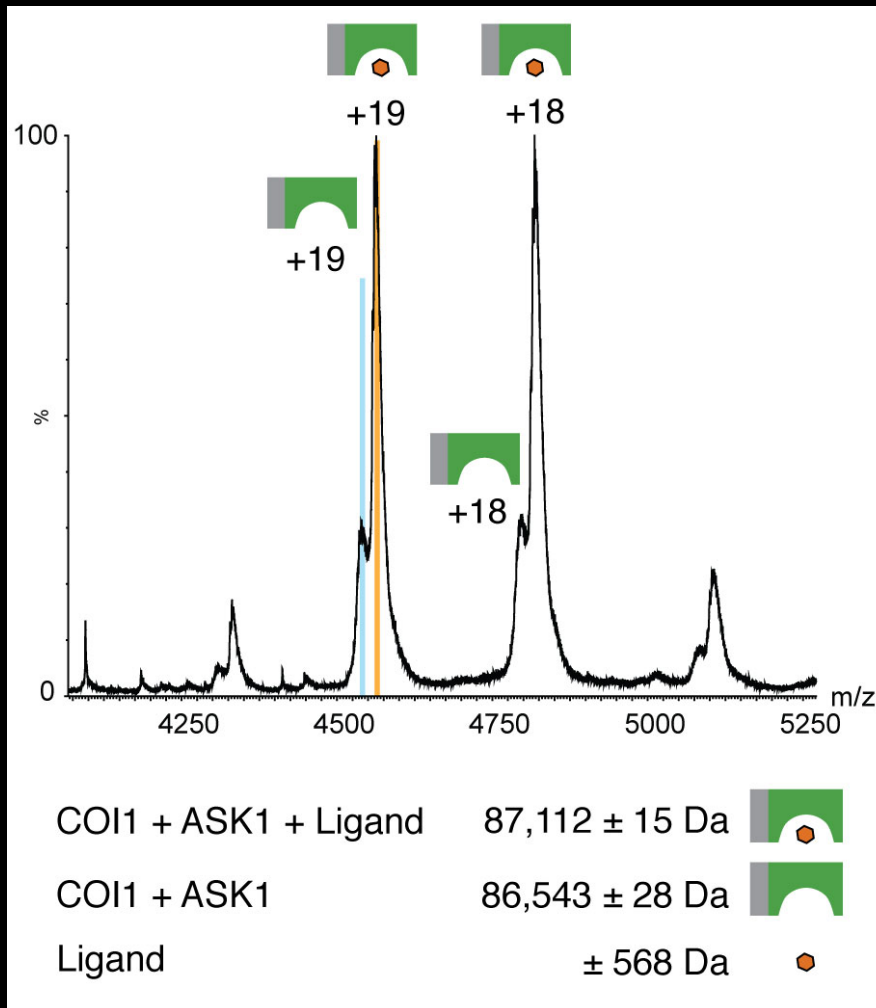


Epic Fail



COI1 Does Have A Co-factor!

Native protein mass spectrometry



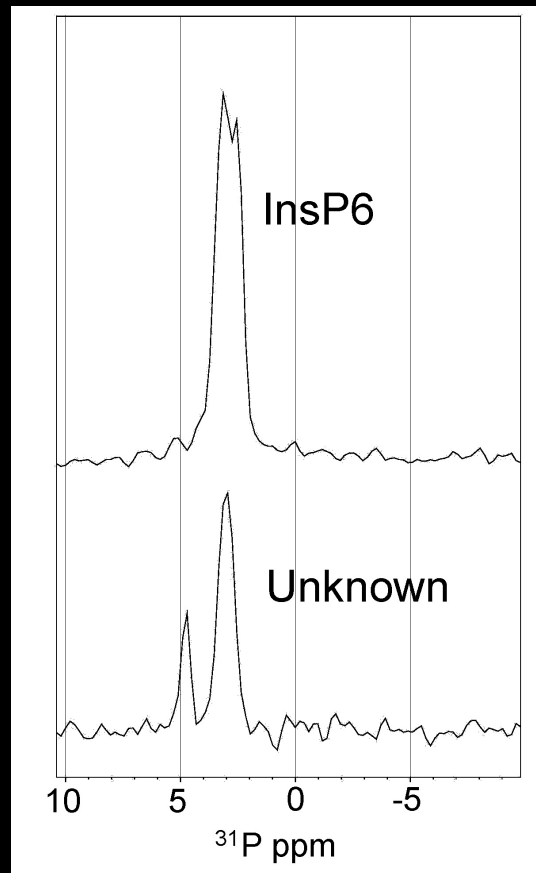
But it is NOT InP6.

InP6 MW = 660

What could it be???

The Co-factor Also Has Phosphate

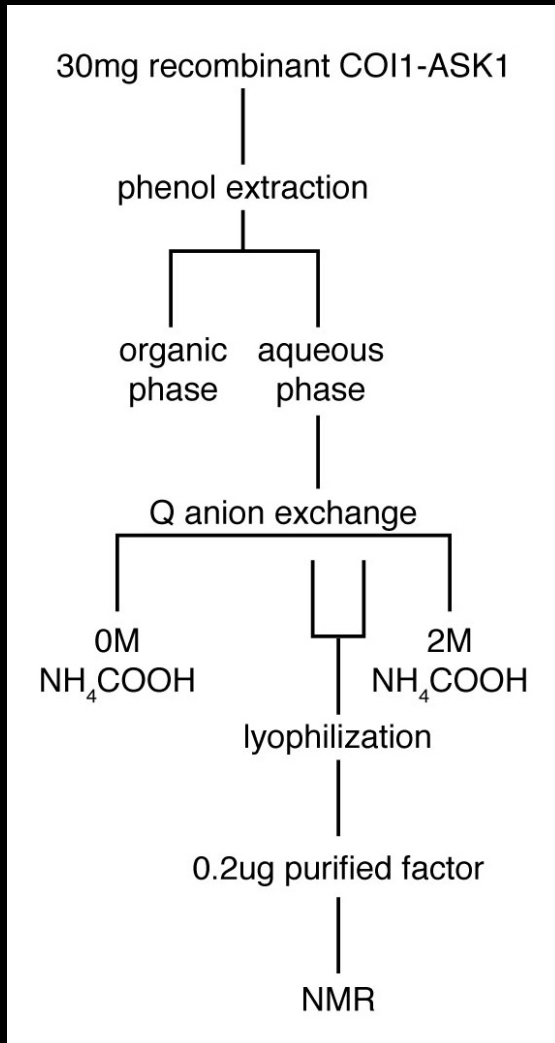
1D ^{31}P NMR



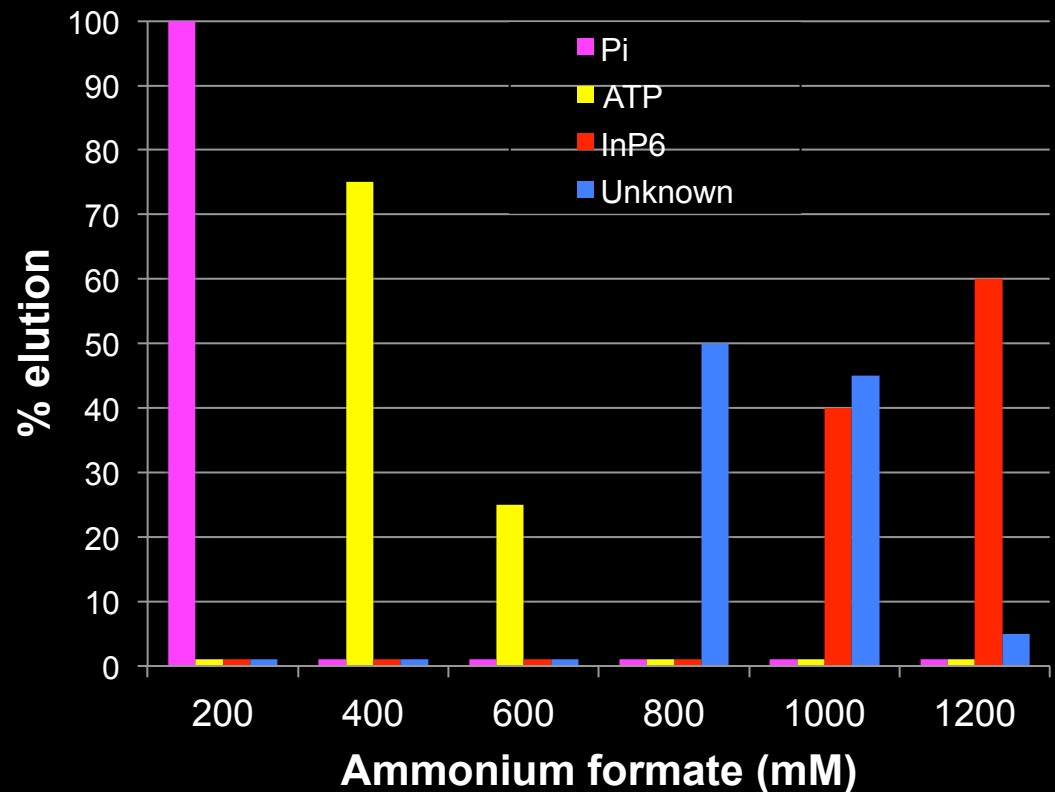
Standard
InsP₆

Trypsinized
COI1-ASK1

Purification of The Unknown Cofactor

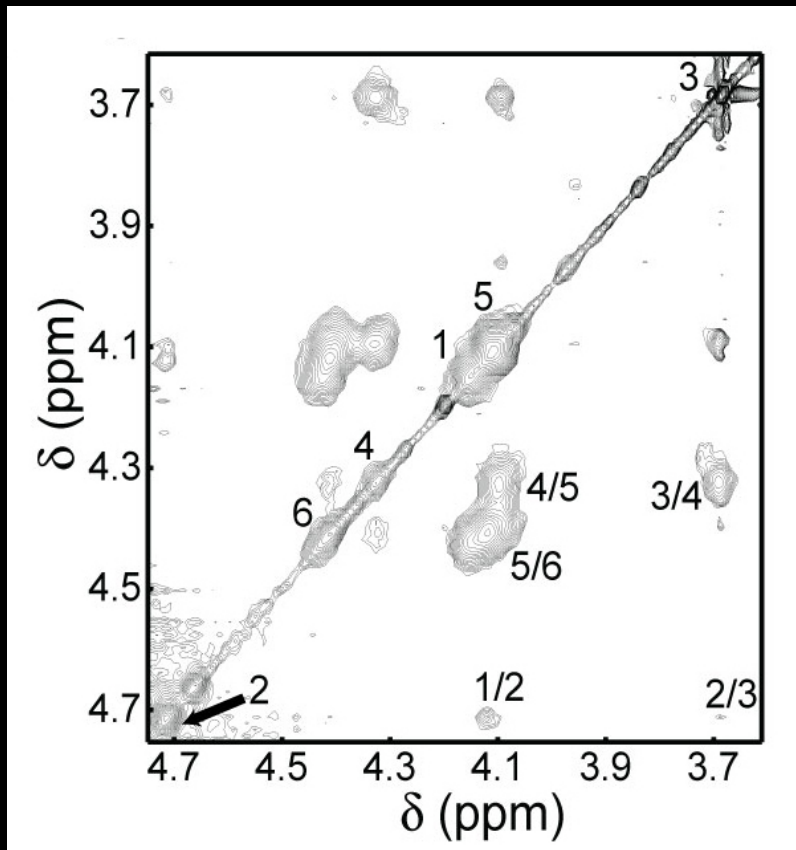


Q anion exchange chromatography
Detection: wet-ashing phosphorus assay



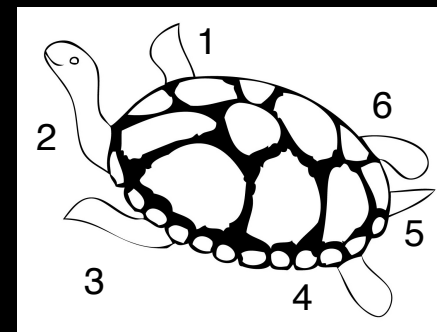
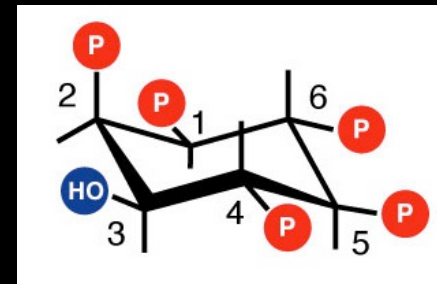
The Unknown Co-factor = Inositol (1,2,4,5,6) Pentakisphosphate

^1H TOCSY NMR Spectrum

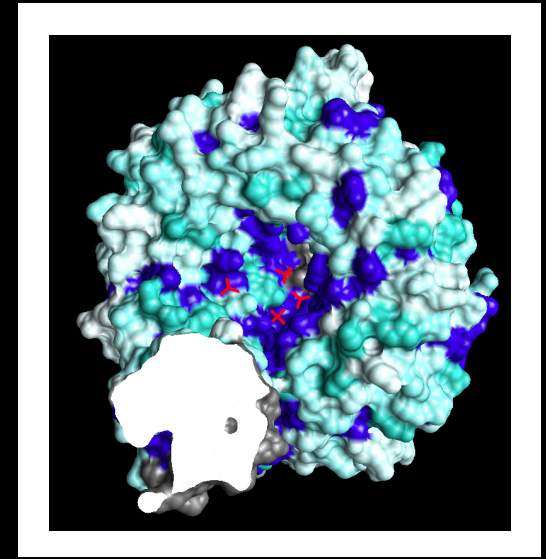
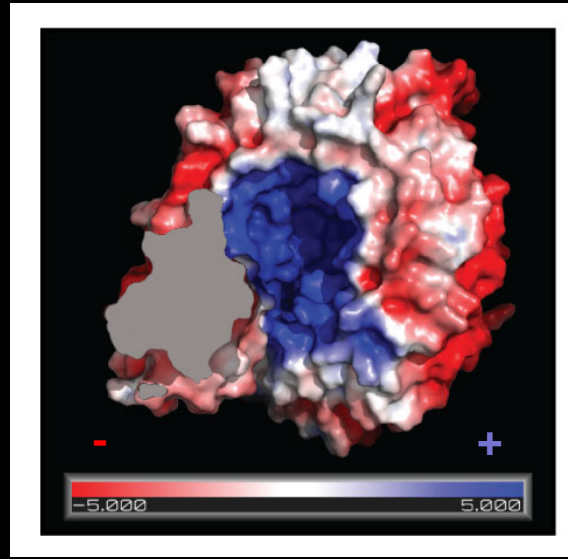
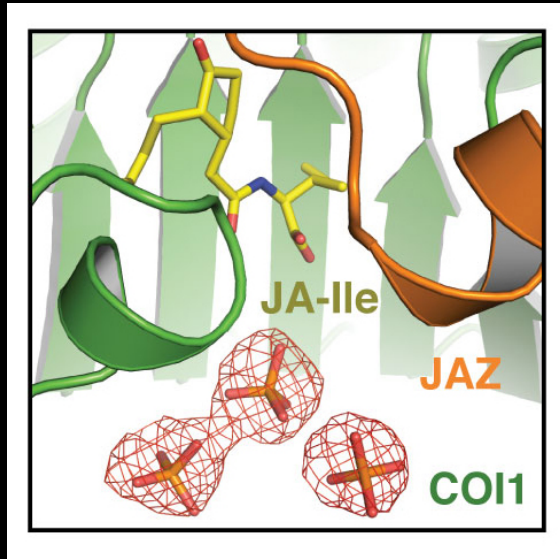


Inositol (1,2,4,5,6) pentakisphosphate

Ins (1,2,4,5,6) P_5



The InsP_5 Binding Site Is Occupied by Free Phosphates in the COI1-ASK1-JAZ1-JA Crystal



100% conserved

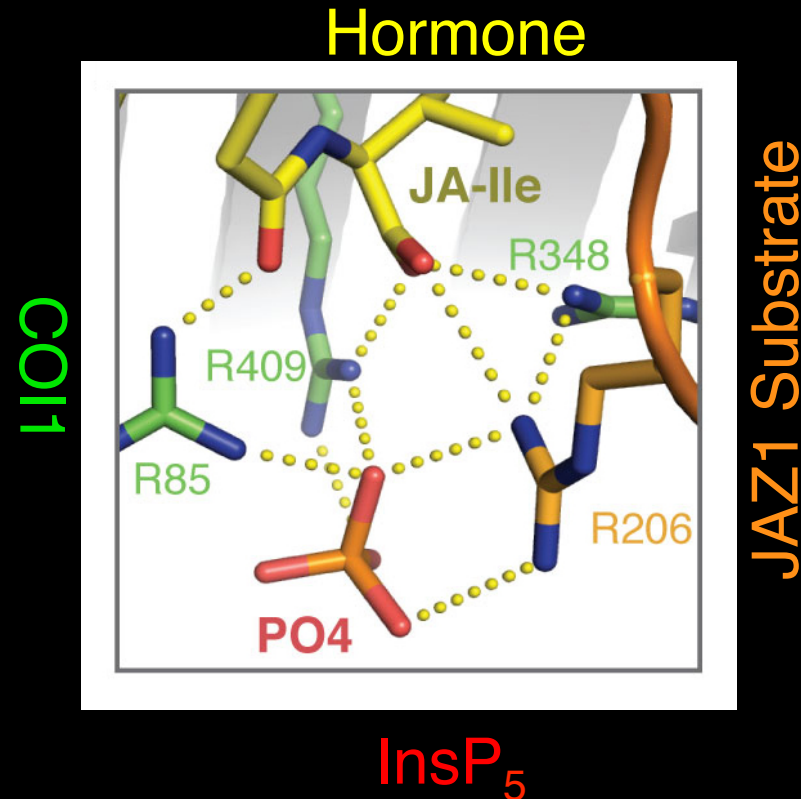


Crystallization Condition

1.5M Ammonium Phosphate

Phosphate at high concentration has replaced InsP_5 in the crystal.

The Binding Mode of the Phosphates Suggests An Critical Role of InsP₅

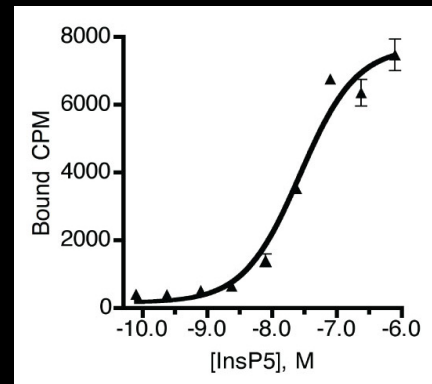
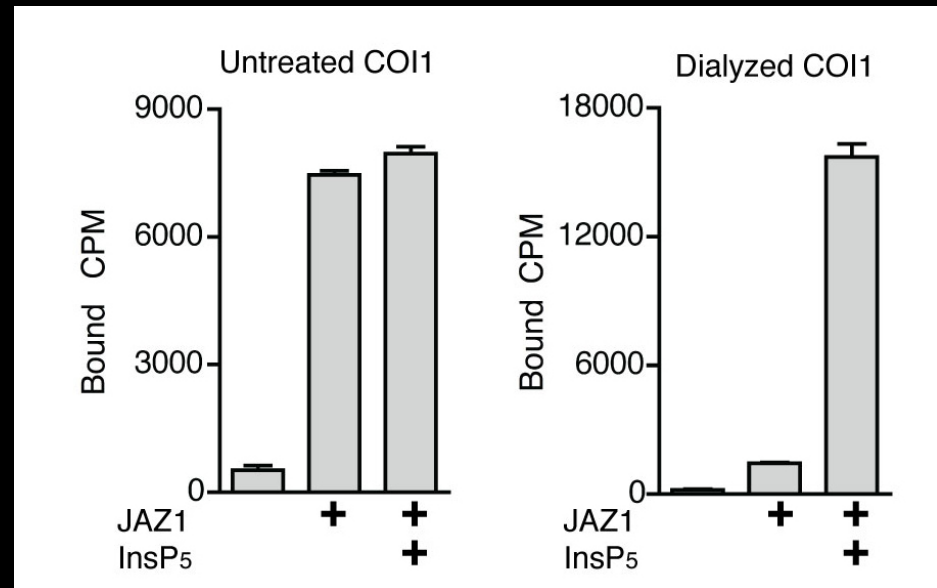
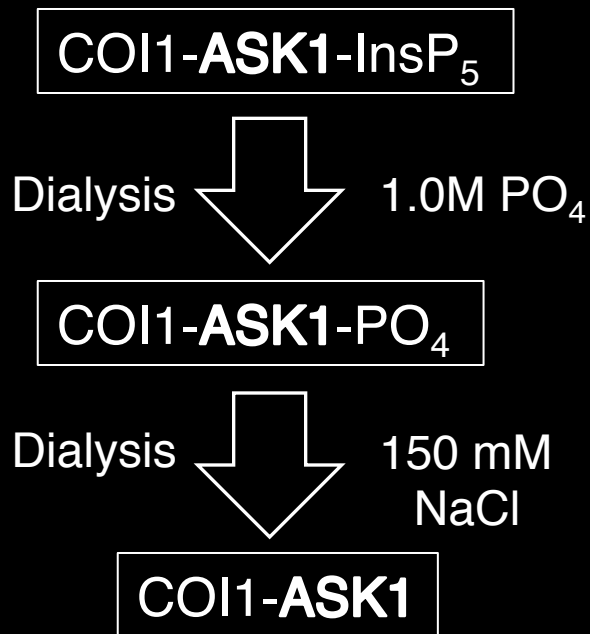


A Tetragonal Bipyrimidal Interaction Network

InsP₅ Is Crucial for the Binding Activity of the JA Co-receptor

³H Coronatine Binding Assay

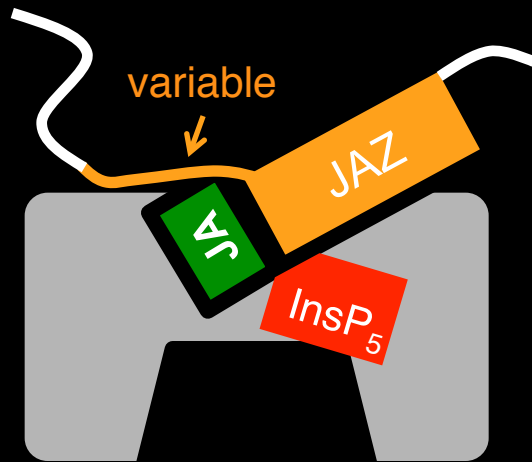
Removal of InsP₅ from COI1



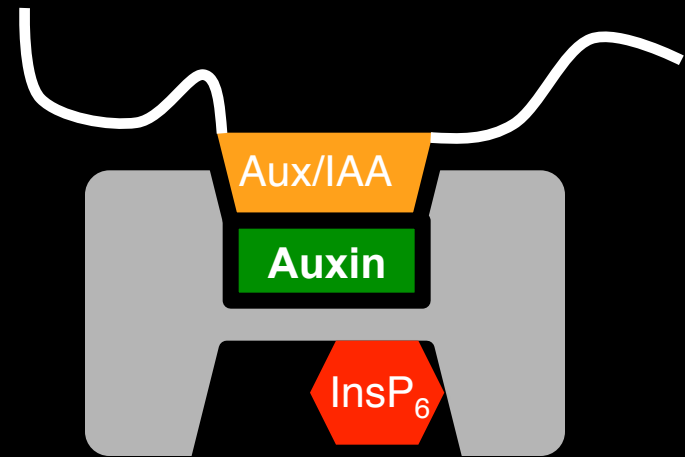
InsP₅ rescues the dialyzed JA co-receptor with an

EC₅₀ = 26.8 nM

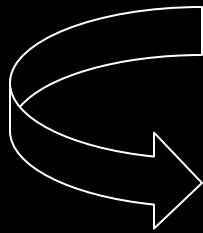
Ubiquitin Ligase-based Tri-molecular Co-receptors of Two Important Plant Hormones



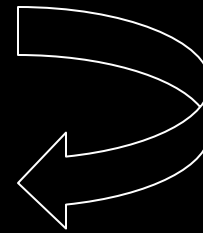
COI1



TIR1



Cross Talk?



Acknowledgement (Auxin)



Xu Tan
(Univ of Washington)

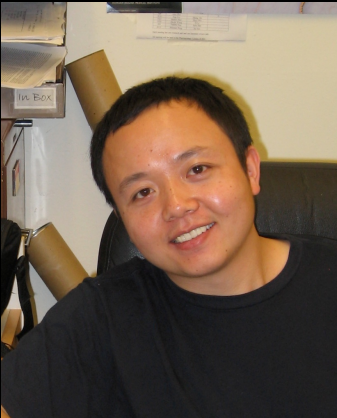


Mark Estelle
Luz Irina A. Calderon V.
(Indiana Univ)



Carol Robinson
Michal Sharon
(Univ of Cambridge)

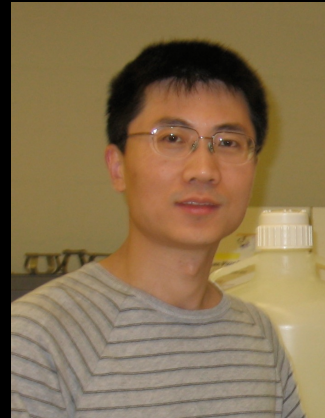
Acknowledgement (Jasmonate)



Xu Tan
(Univ of Wash)



Laura Sheard
(Univ of Wash)



Haibin Mao
(Univ of Wash)



Michal Sharon
(Weizmann Inst)



Rachel Klevit
(Univ of Wash)



Jose Rizo-Rey
(UTSW)



Gregg Howe
(MSU)



Sheng Yang He
(MSU)



John Browse
(WSU)