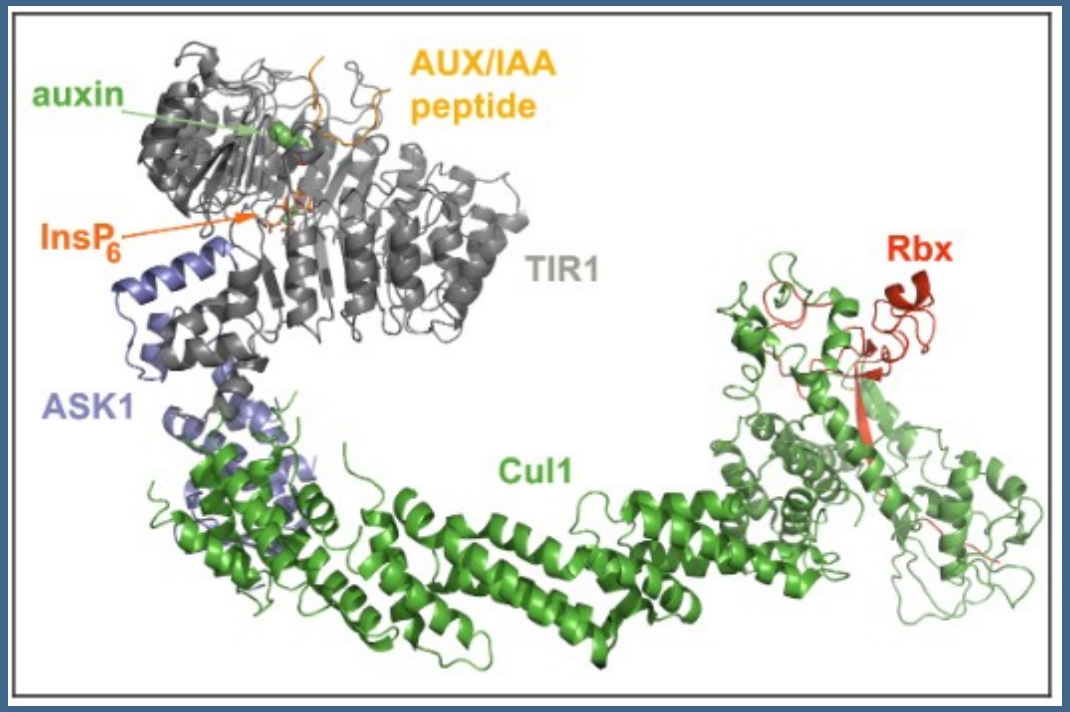
Molekulare Mechanismen der Signaltransduktion



12 – Mechanism of auxin perception
slides:
http://tinyurl.com/Modul-MMS

doi: 10.1038/nature05731

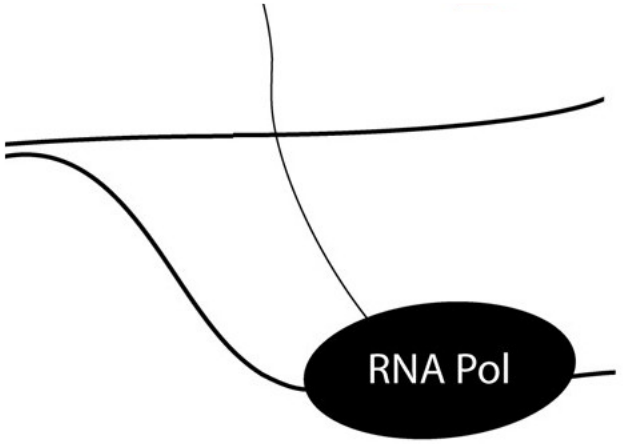
previous model

auxin

Aux/IAAs

auxin-induced
gene expression

auxin responses



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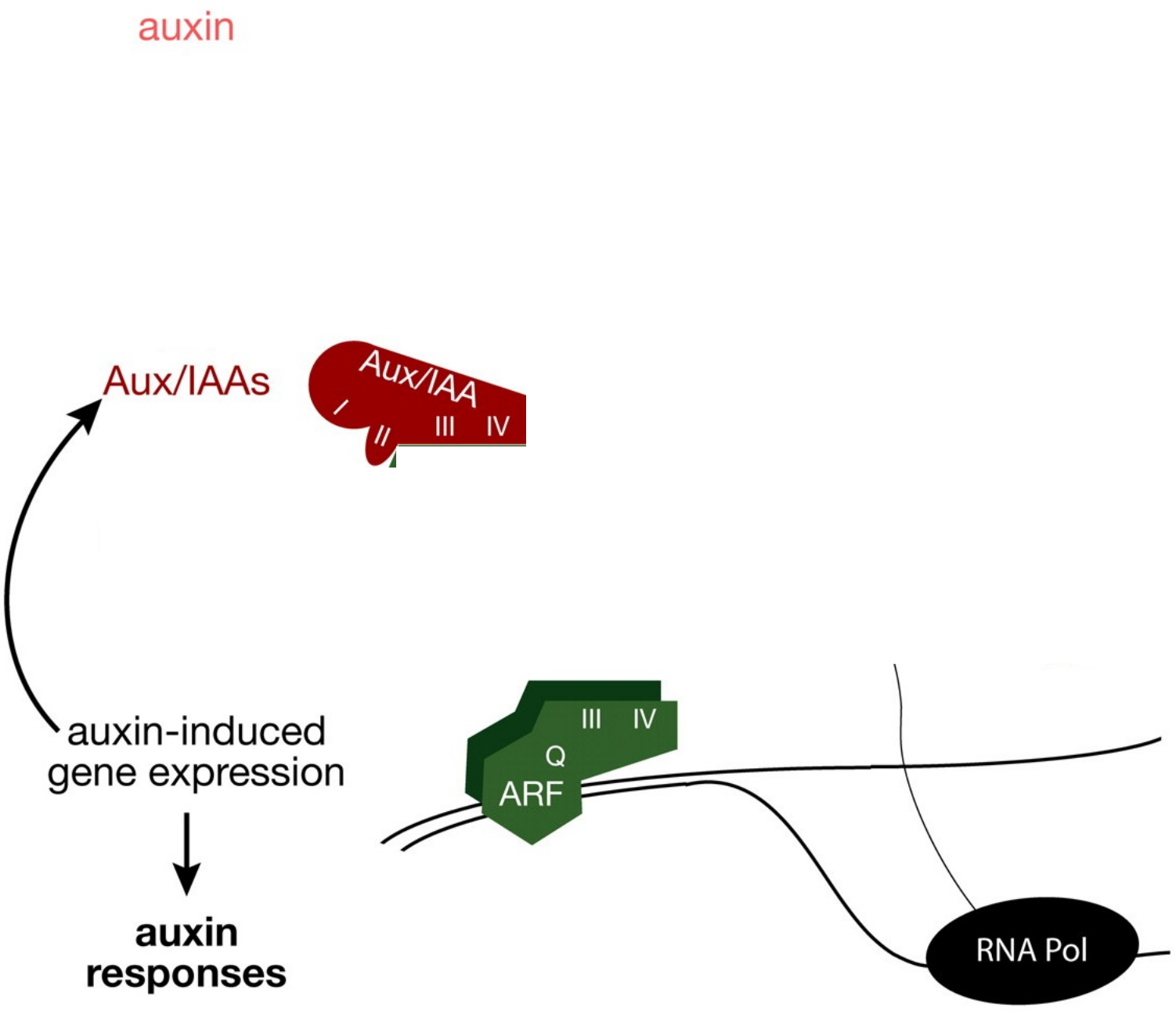
previous model



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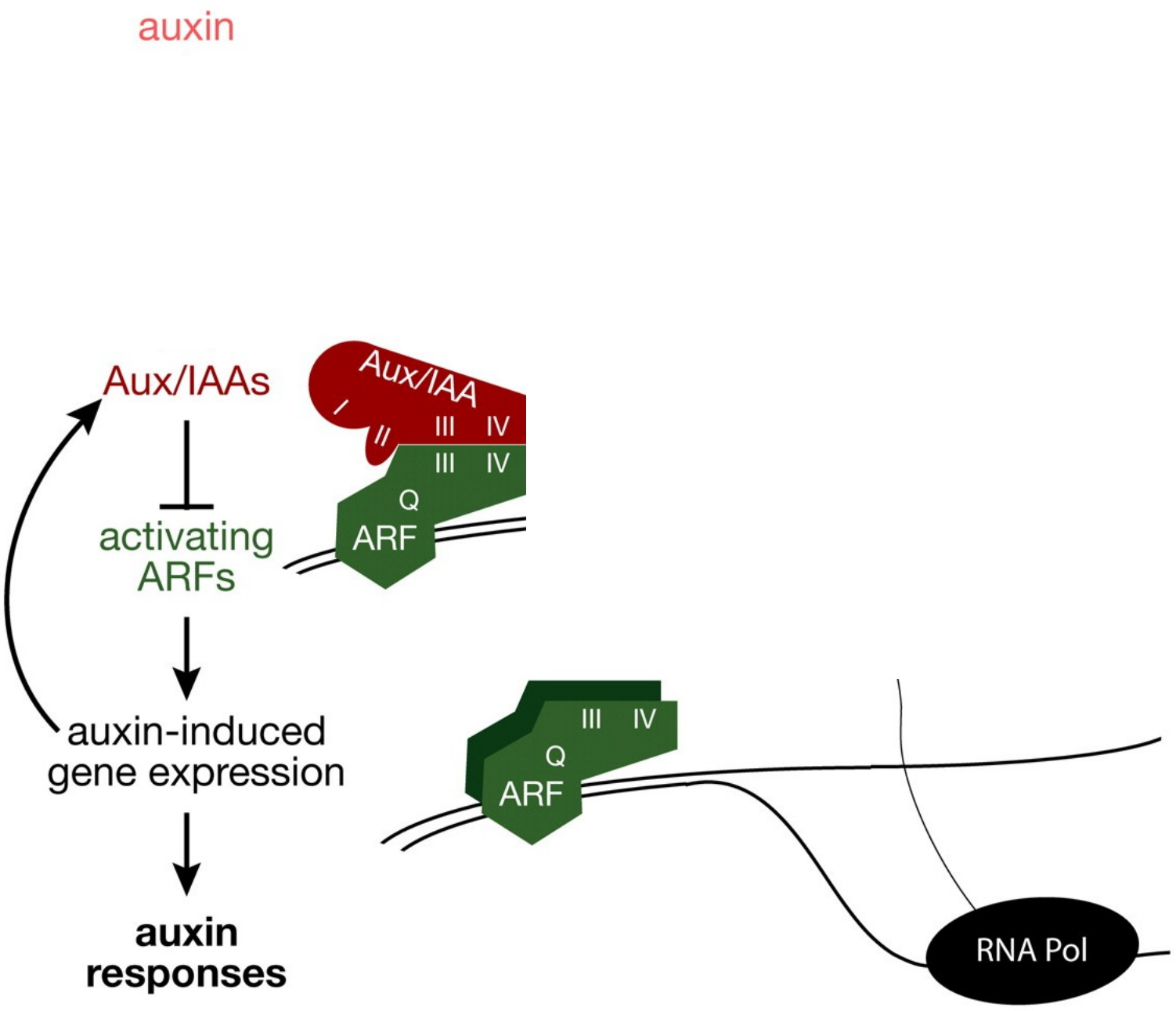
previous model



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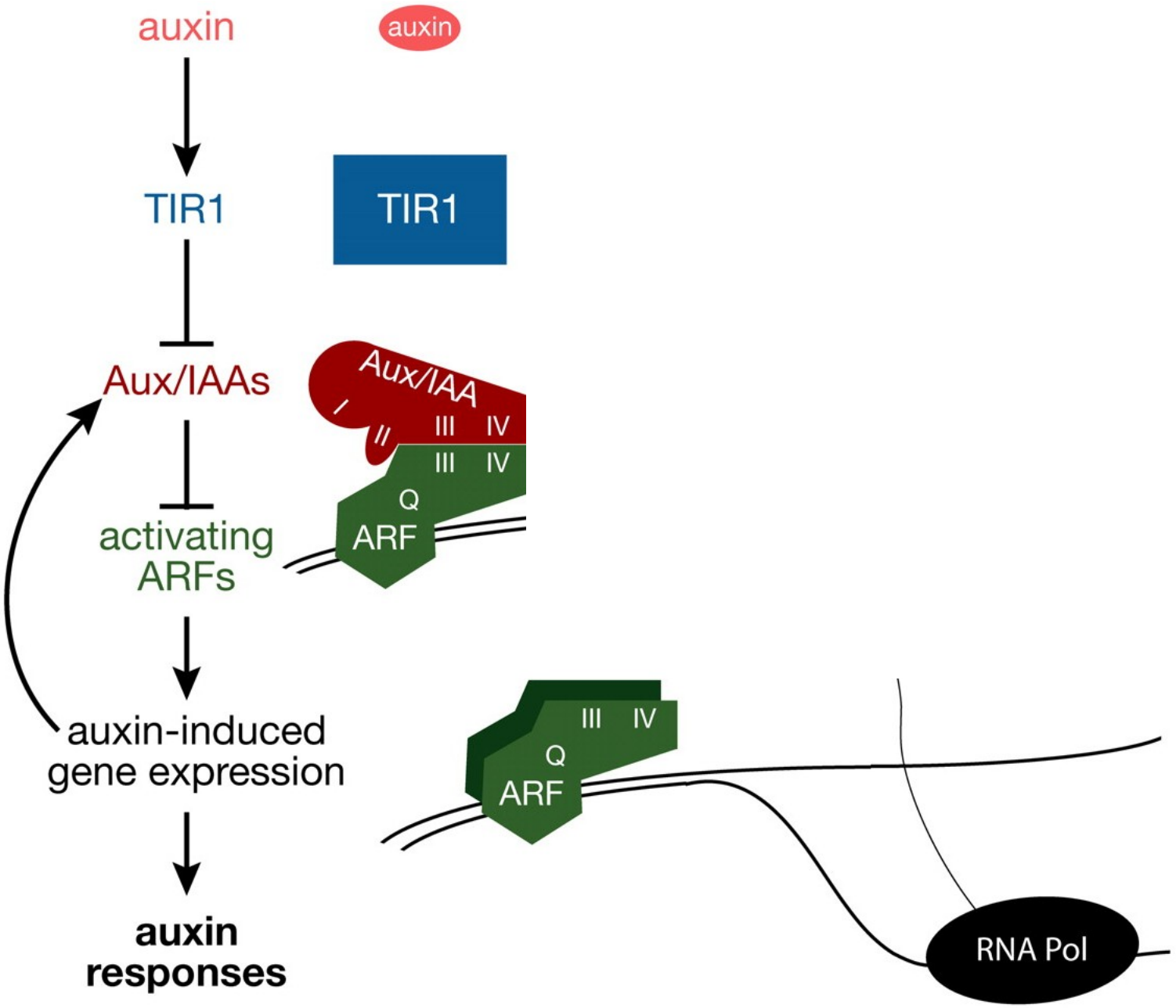
previous model



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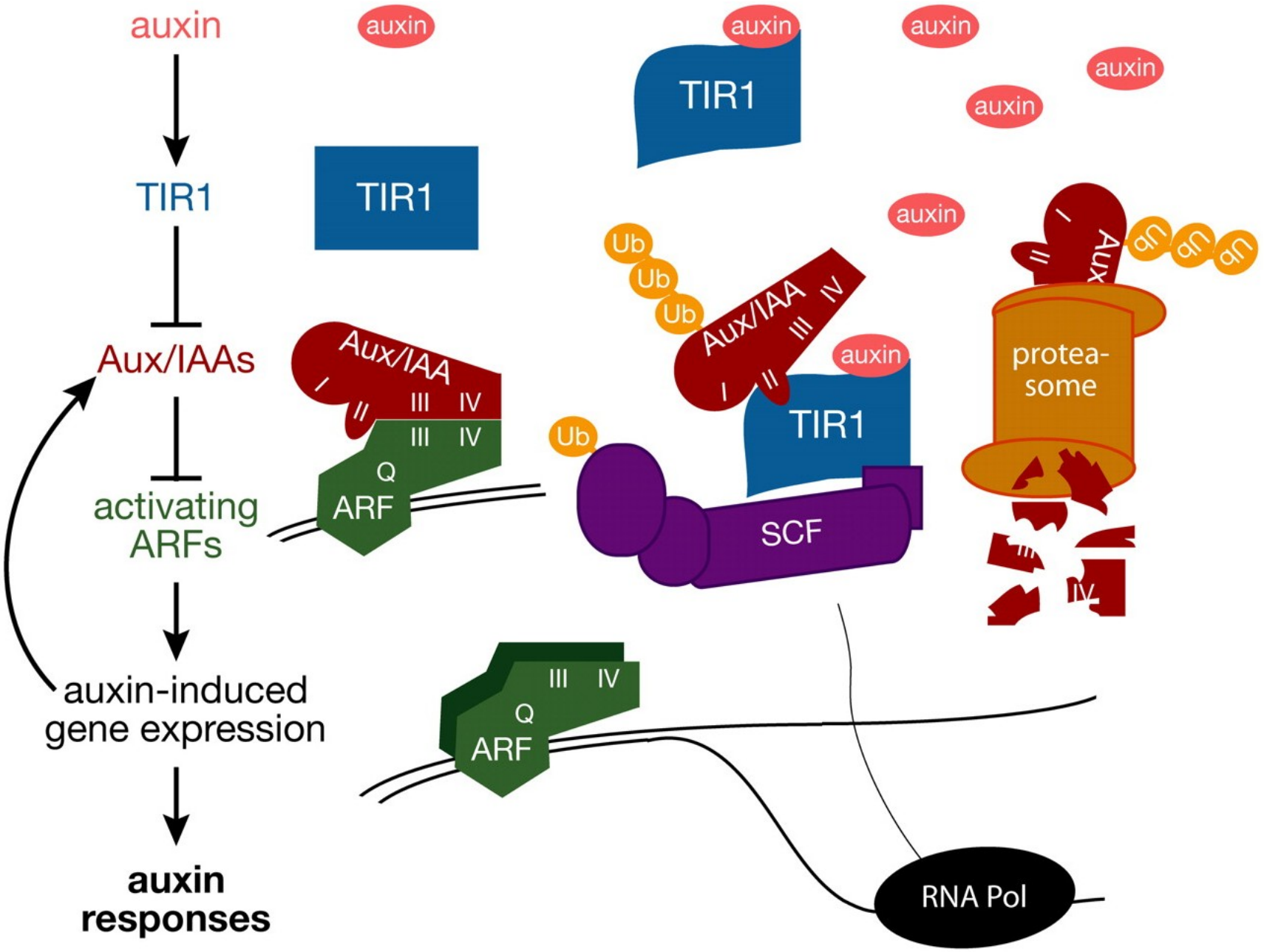

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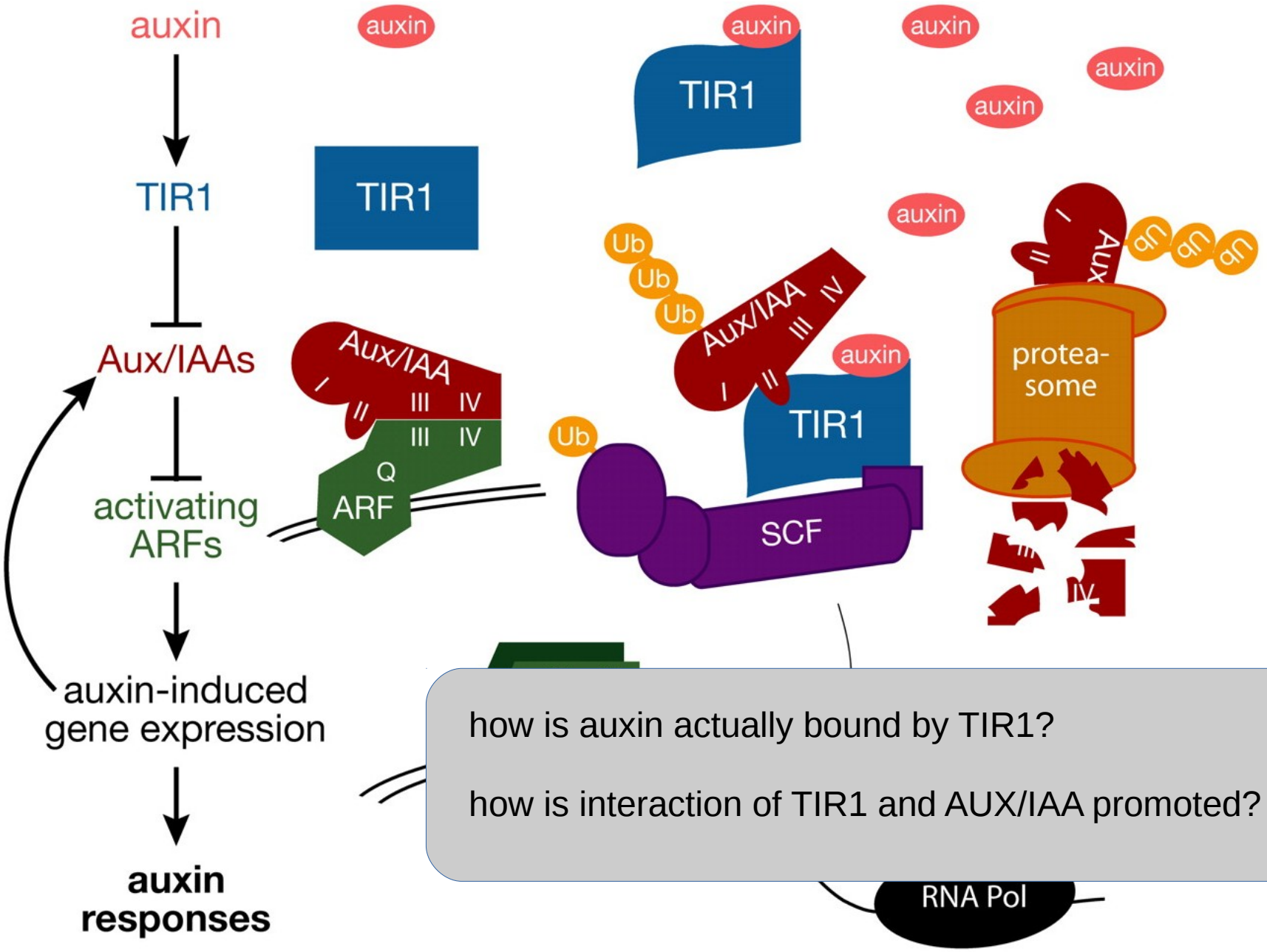


how is auxin actually bound by TIR1?

how is interaction of TIR1 and AUX/IAA promoted?

http://www.plantcell.org/content/17/9/2425/F1.large.jpg

today's paper

nature Vol 446|5 April 2007|doi:10.1038/nature05731

ARTICLES

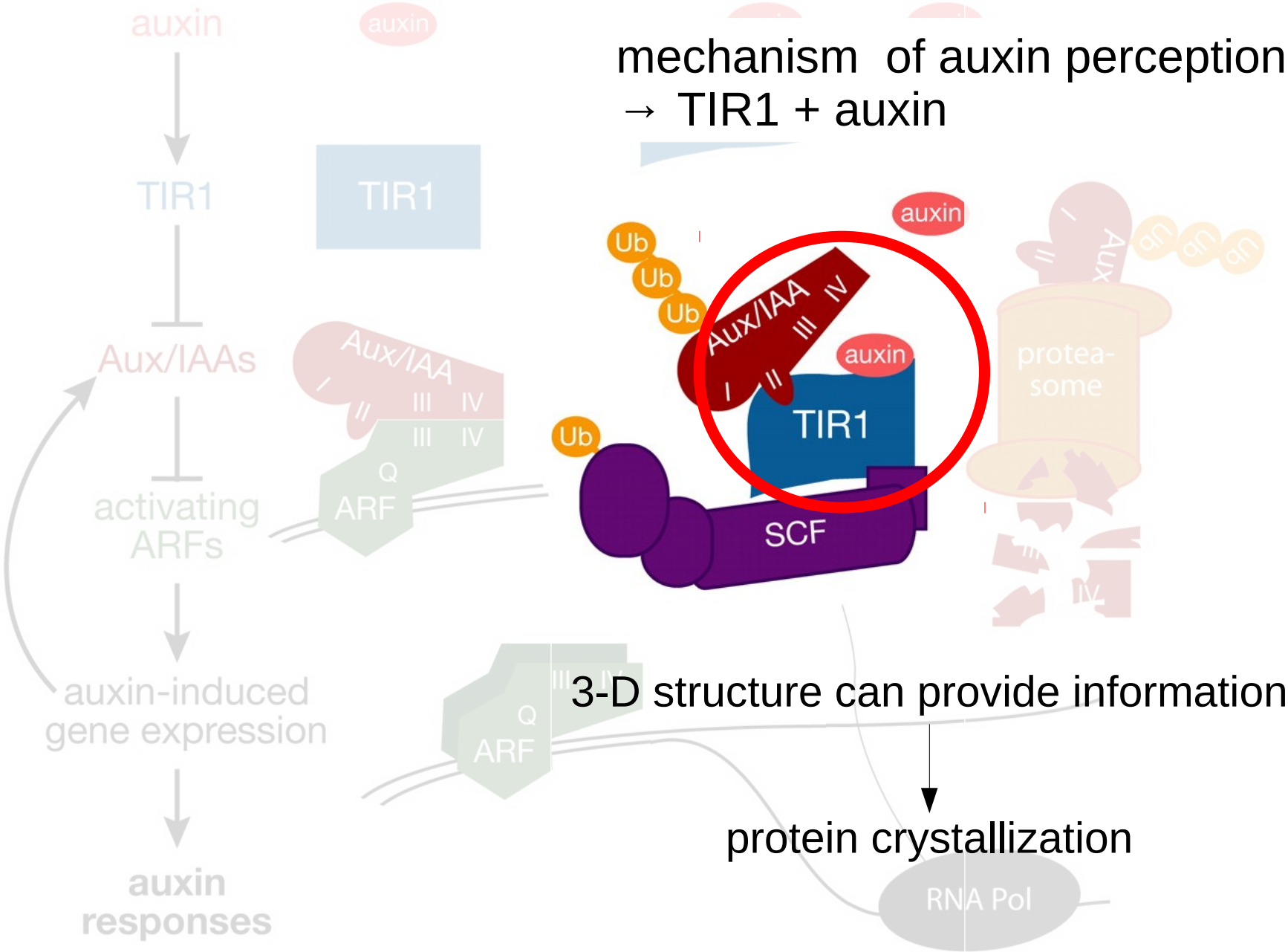
Mechanism of auxin perception by the TIR1 ubiquitin ligase

Xu Tan[1], Luz Irina A. Calderon-Villalobos[2], Michal Sharon[3], Changxue Zheng[1], Carol V. Robinson[3], Mark Estelle[2] & Ning Zheng[1]

aim:
elucidation of auxin perception mechanism by analysis of protein crystal structure

approach

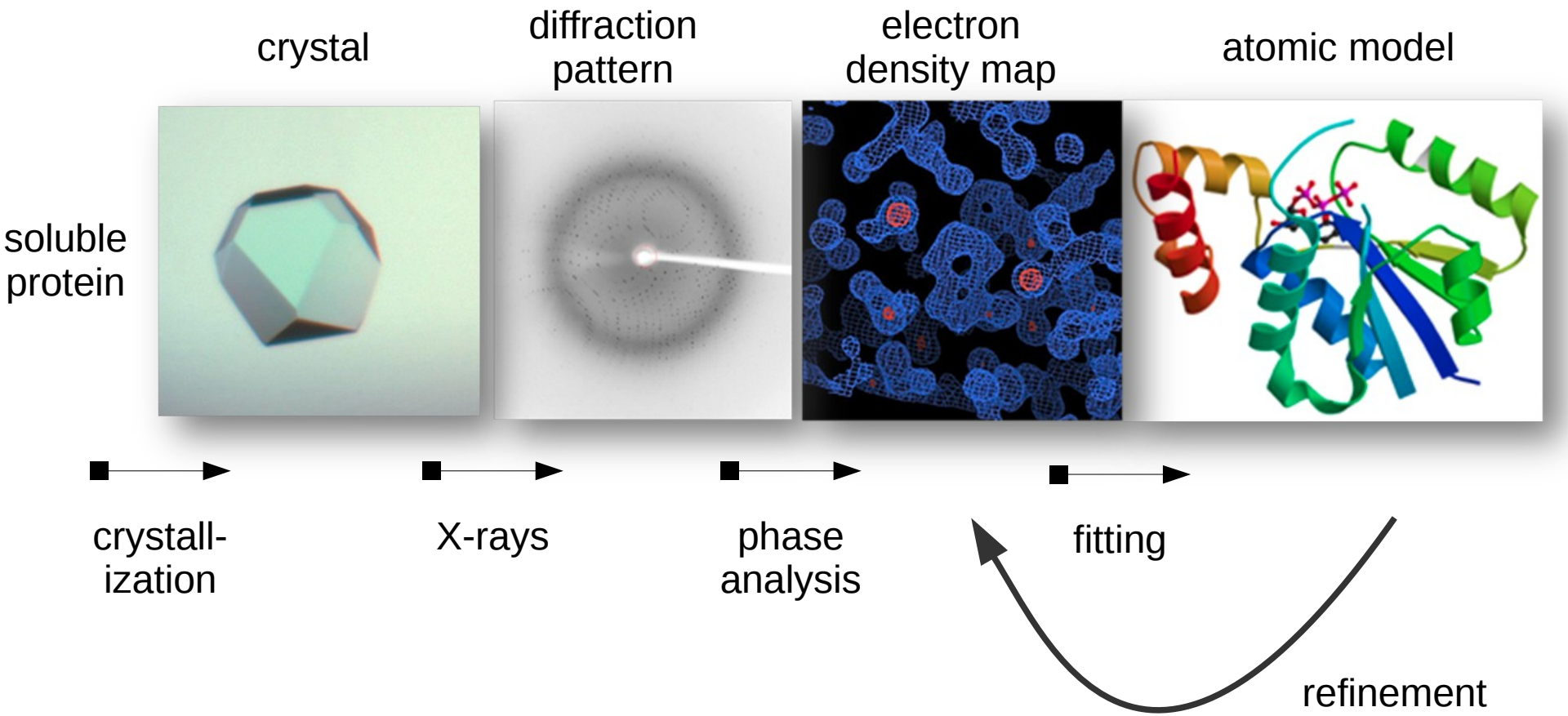
mechanism of auxin perception:
→ TIR1 + auxin



3-D structure can provide information

protein crystallization

crystallization and structure elucidation



crystallization and structure elucidation

soluble protein

crystal → diffraction pattern → electron density map → atomic model

heterologous expression
of (tagged) proteins in:
E. coli
yeast
insect cells
etc...

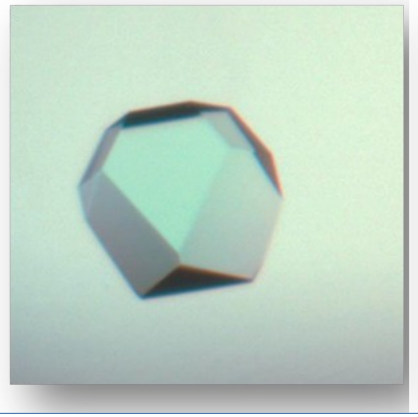
extraction + purification:
antibodies
dialysis
buffer conditions!
re-folding?

http://crystal.csiro.au/en/User-Guide/%7E/media/FFBDBC5367A94A5DA01ED509EE25DFD5.ashx

crystallization and structure elucidation

soluble protein

crystal



diffraction pattern

electron density map

atomic model

→protein requirements:

- high concentration + amount of protein
- purity (>95%)
- stable + soluble!

se
sis

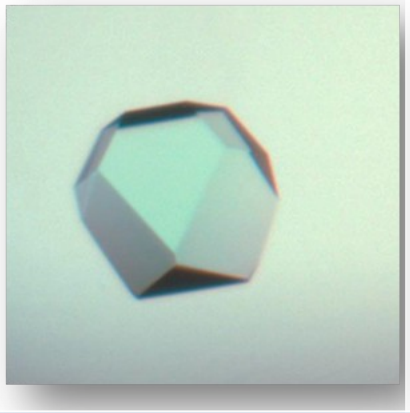
fitting

http://crystal.csiro.au/en/User-Guide/%7E/media/FFBDBC5367A94A5DA01ED509EE25DFD5.ashx

crystallization and structure elucidation

soluble protein

crystal



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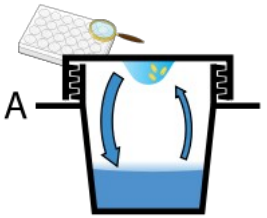
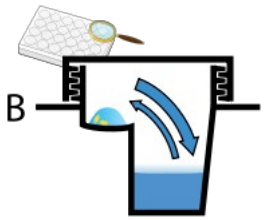
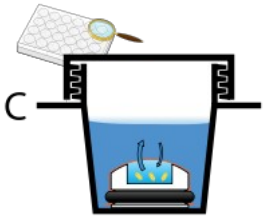


hanging drop



sitting drop



microdialysis

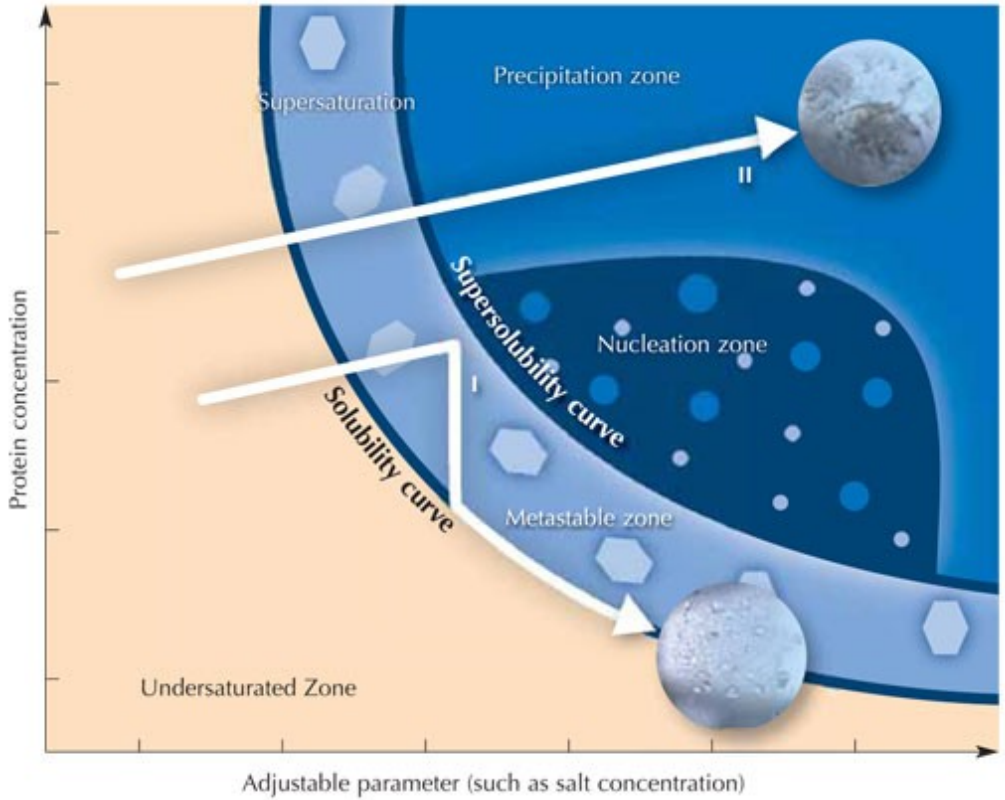
A + B: Vapor diffusion = a drop of protein solution equilibrates with a larger reservoir containing similar buffers and precipitants in higher concentration

→ as the drop and reservoir equilibrate, the precipitant and protein concentrations increase in the drop → crystallization

→ gentle and gradual changes aid in the growth of large and well-ordered crystals

http://crystal.csiro.au/en/User-Guide/%7E/media/FFBDBC5367A94A5DA01ED509EE25DFD5.ashx

factors that influence crystallization



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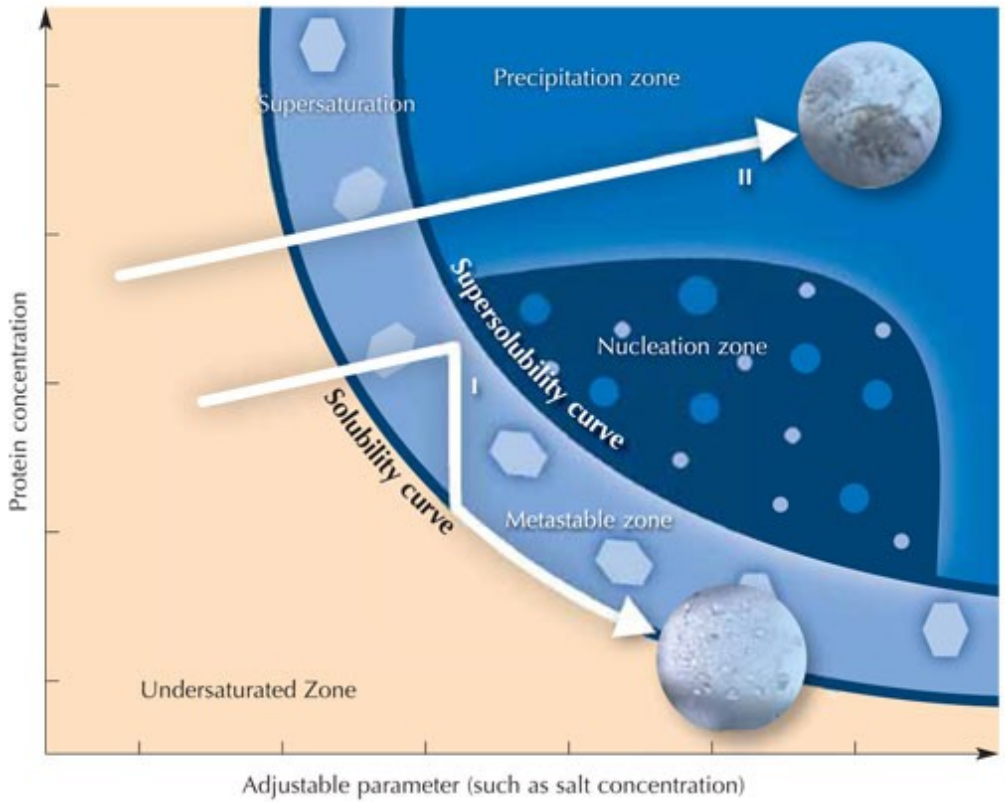
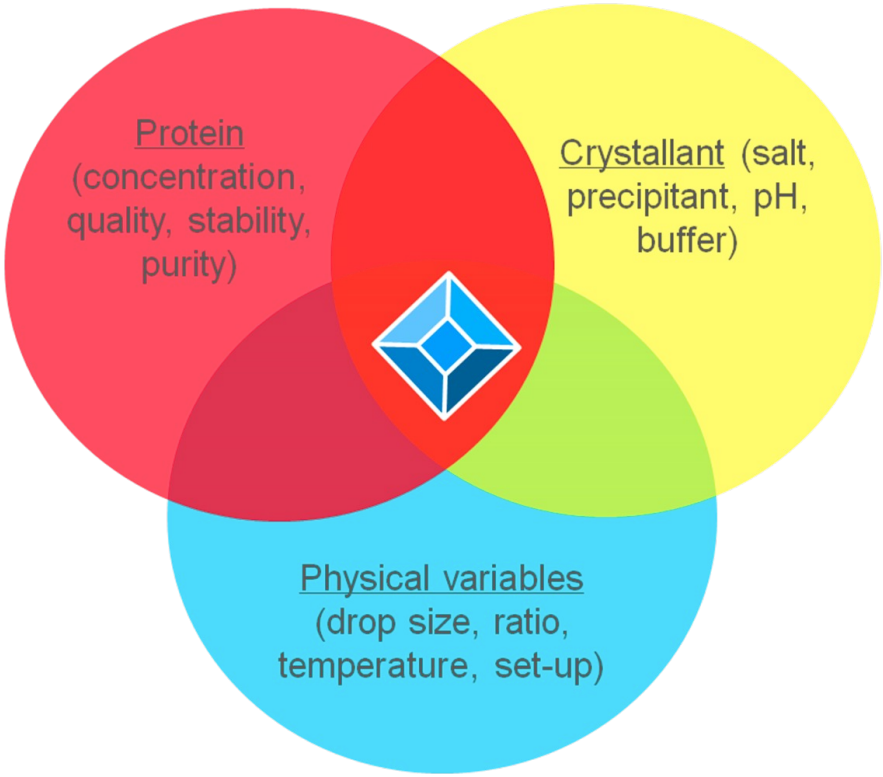
factors that influence crystallization





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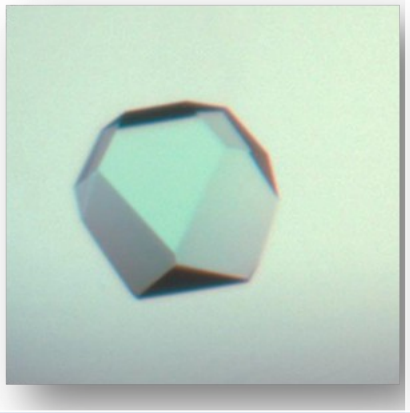
http://www.scienceinschool.org/sites/default/files/articleContentImages/11/lysozyme/issue11lysozyme1_l.jpg

crystallization and structure elucidation

soluble protein

crystal



diffraction pattern

electron density map

atomic model

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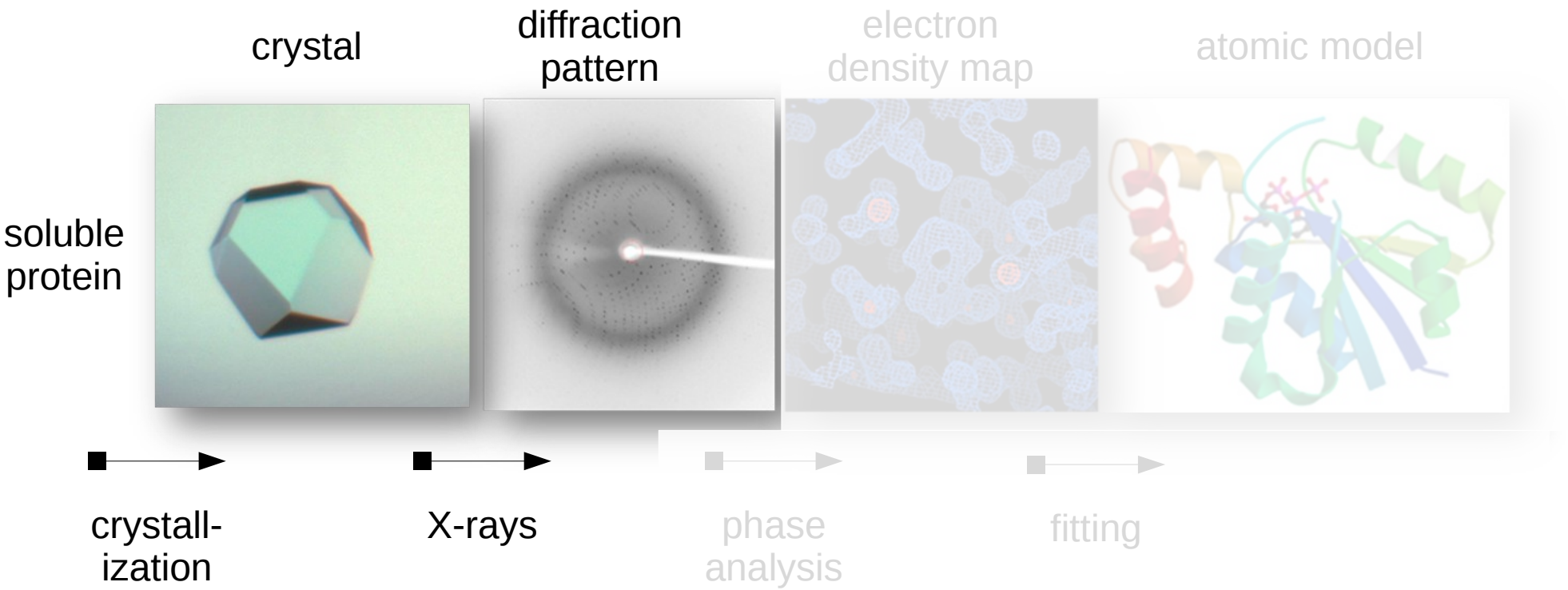
se
sis

fitting

https://www.youtube.com/embed/8WOPLUBq4l0?feature=player_detailpage

http://crystal.csiro.au/en/User-Guide/%7E/media/FFBDBC5367A94A5DA01ED509EE25DFD5.ashx

crystallization and structure elucidation



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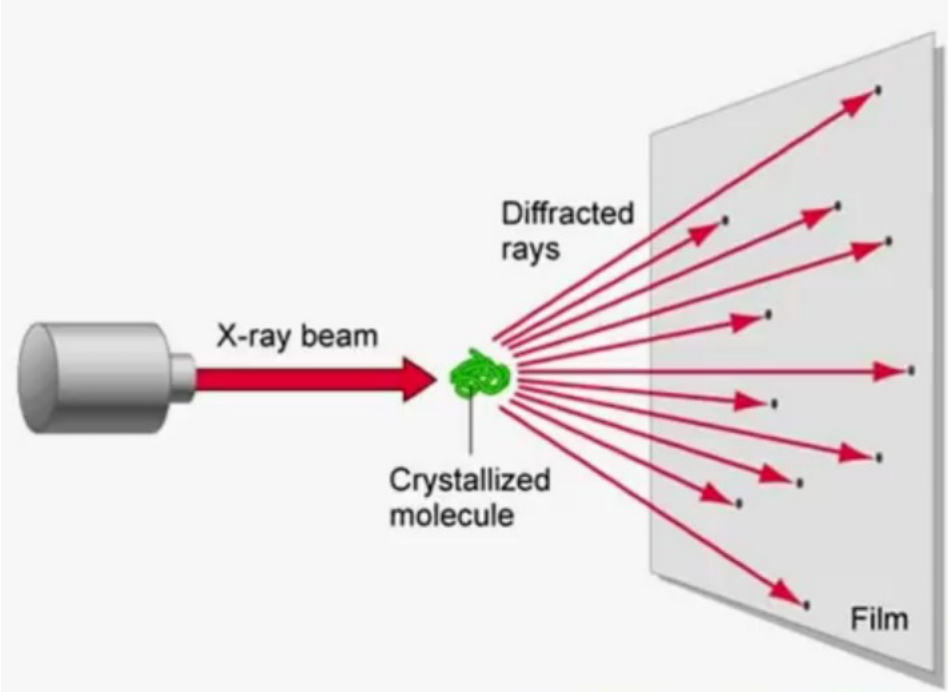
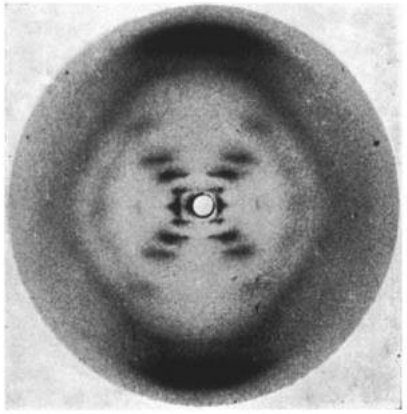
x-ray and diffraction pattern analysis





http://blogbiosyn.files.wordpress.com/2013/04/dna-figure-1953.jpg

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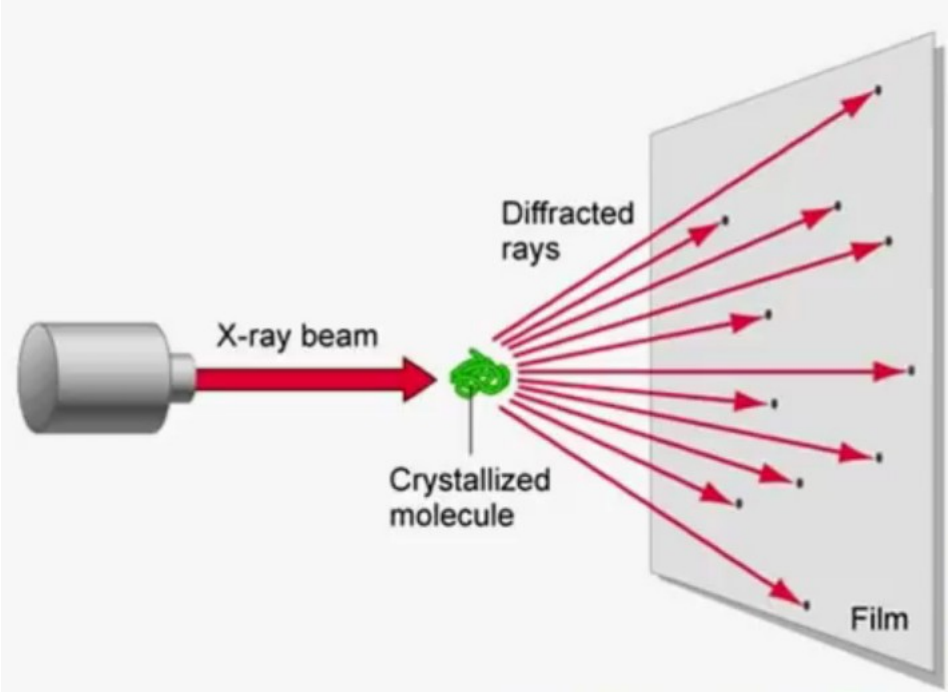
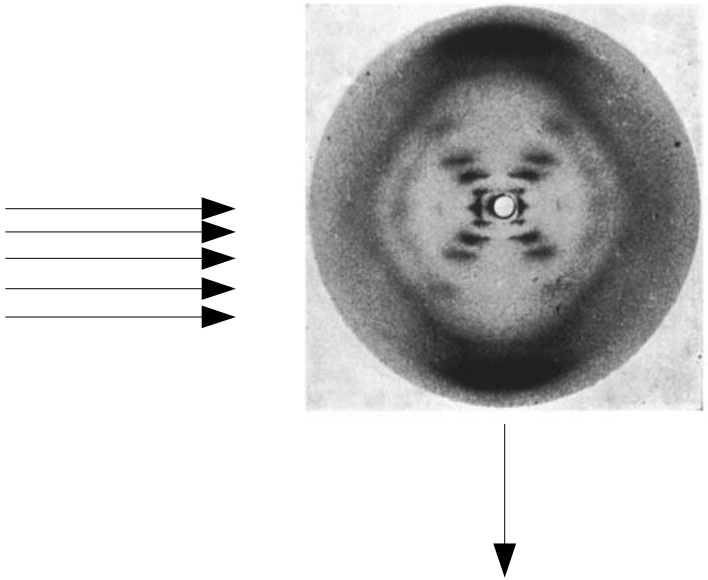
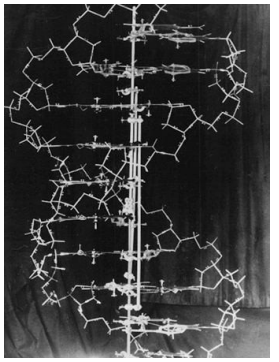
x-ray and diffraction pattern analysis





Watson, Crick, Franklin



https://teamforensics.wikispaces.com/file/view/Watson%20crick%20franklin.jpg/377895562/318x226/Watson%20crick%20franklin.jpg

http://biology.kenyon.edu/courses/biol114/Chap01/dna-model.jpg

http://blogbiosyn.files.wordpress.com/2013/04/dna-figure-1953.jpg

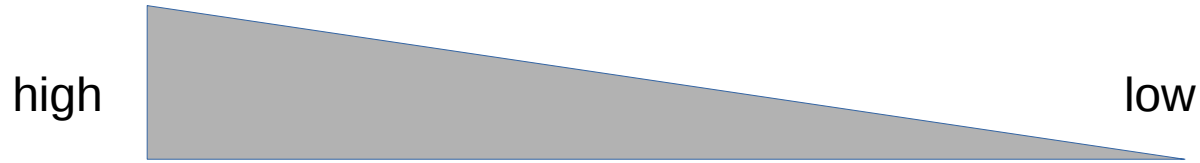
https://upload.wikimedia.org/wikipedia/en/b/b2/Photo_51_x-ray_diffraction_image.jpg

factors that influence resolution

very high purity
well ordered

high purity
poorly ordered

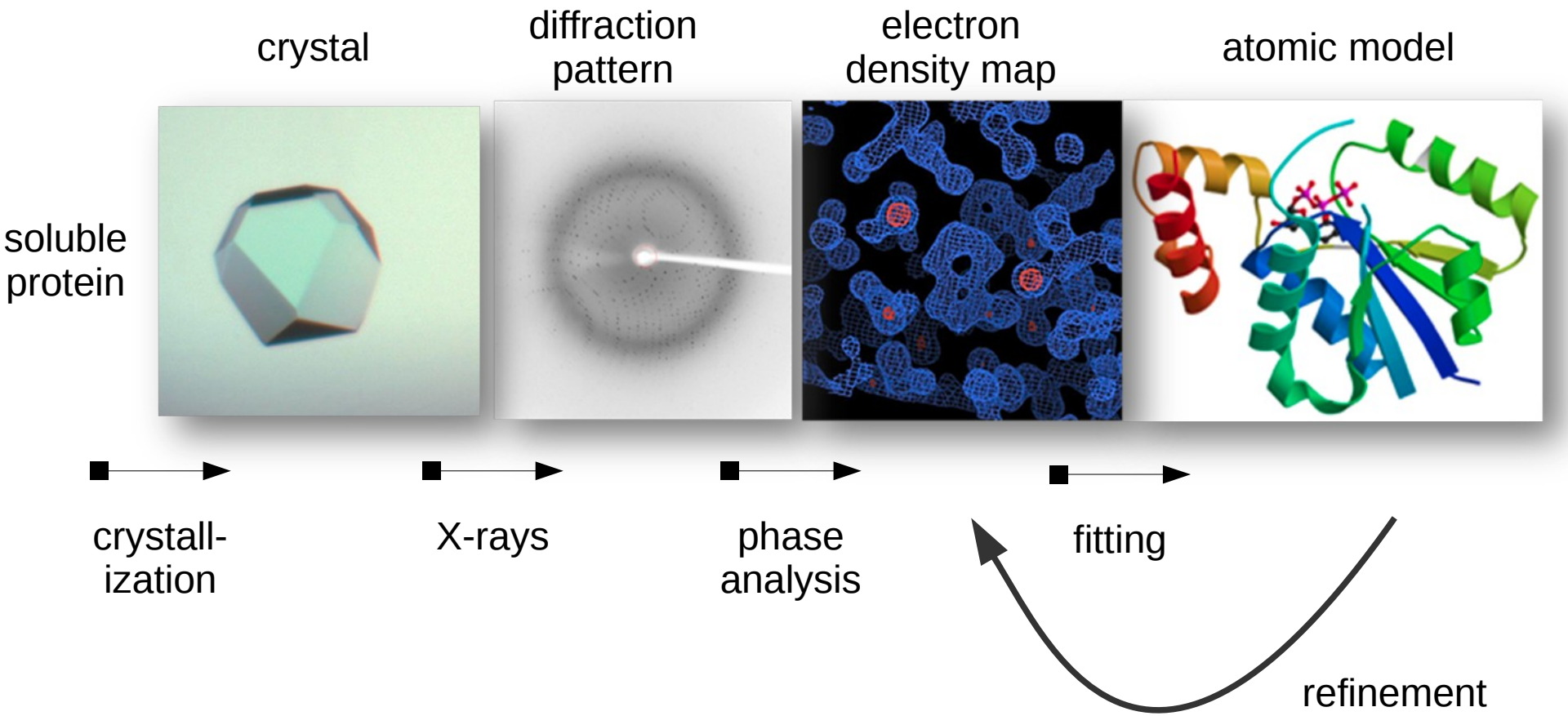
heterogenous
poorly ordered



resolution of diffraction pattern

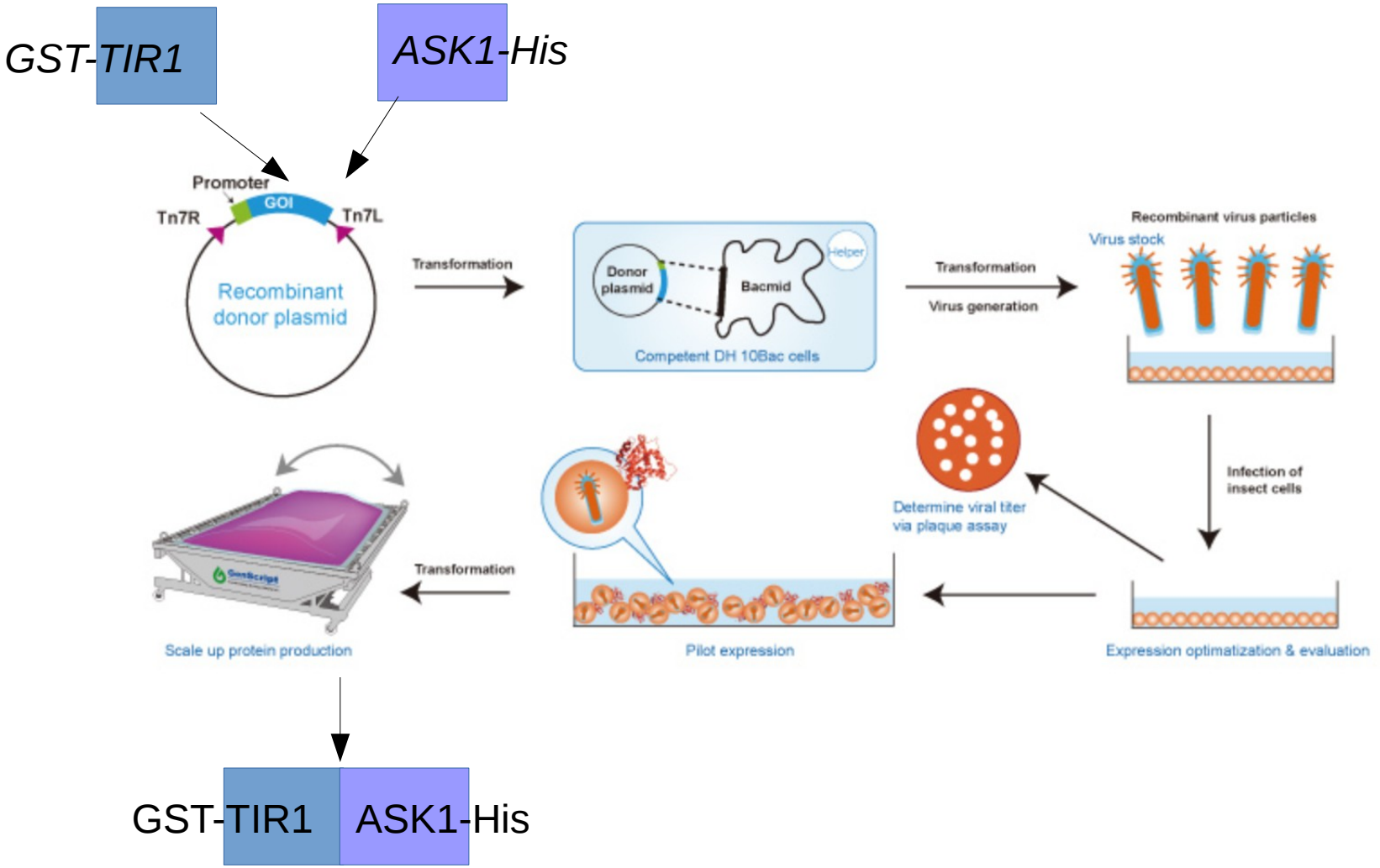
http://crystal.csiro.au/en/User-Guide/~/media/Images/About%20C3/Mis-packing.ashx

crystallization and structure elucidation



crystallization of TIR1

1. co-expression of TIR1-GST and ASK1-His proteins in insect cells and purification of the TIR1-ASK1 complex



http://www.genscript.com/gsimages/genscript/Protein_Insect_system.jpg

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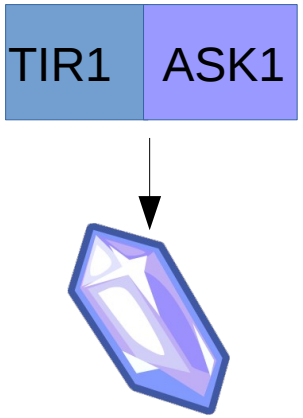
2. TEV-cleavage of tags and crystalization

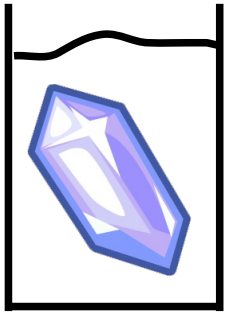


crystallization of TIR1

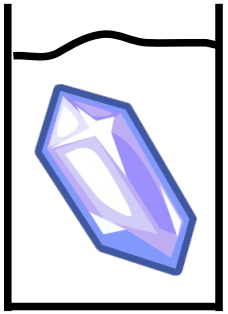
1. co-expression of TIR1-GST and ASK1-His proteins in insect cells and purification of the TIR1-ASK1 complex

2. TEV-cleavage of tags and crystallization

3. soaking of crystals with additional compounds

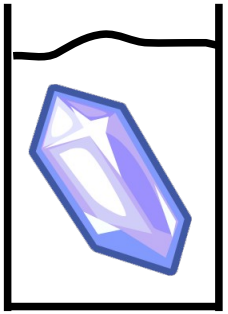


TIR1 + ASK1

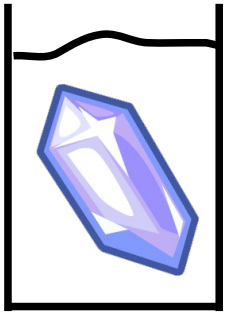


TIR1 + ASK1
+IAA

TIR1 + ASK1
+2,4-D

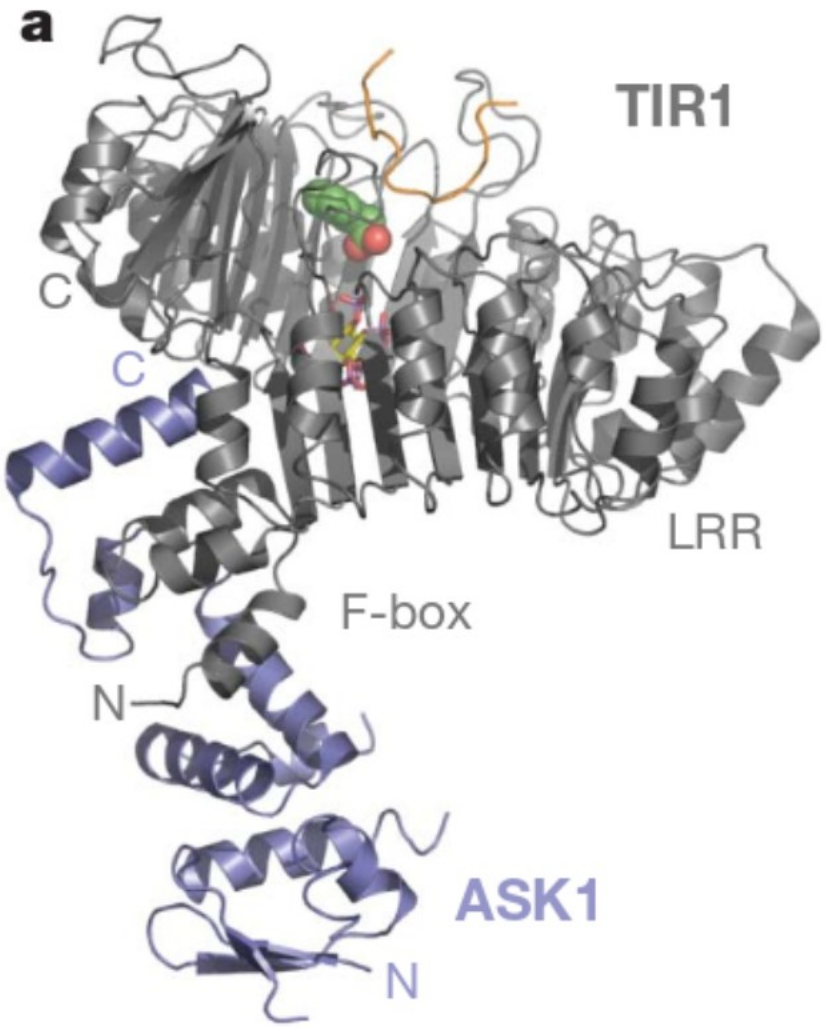


TIR1 + ASK1
+NAA



TIR1 + ASK1
+IAA
+ IAA-DII peptide

TIR1-ASK1-DII-IAA structure

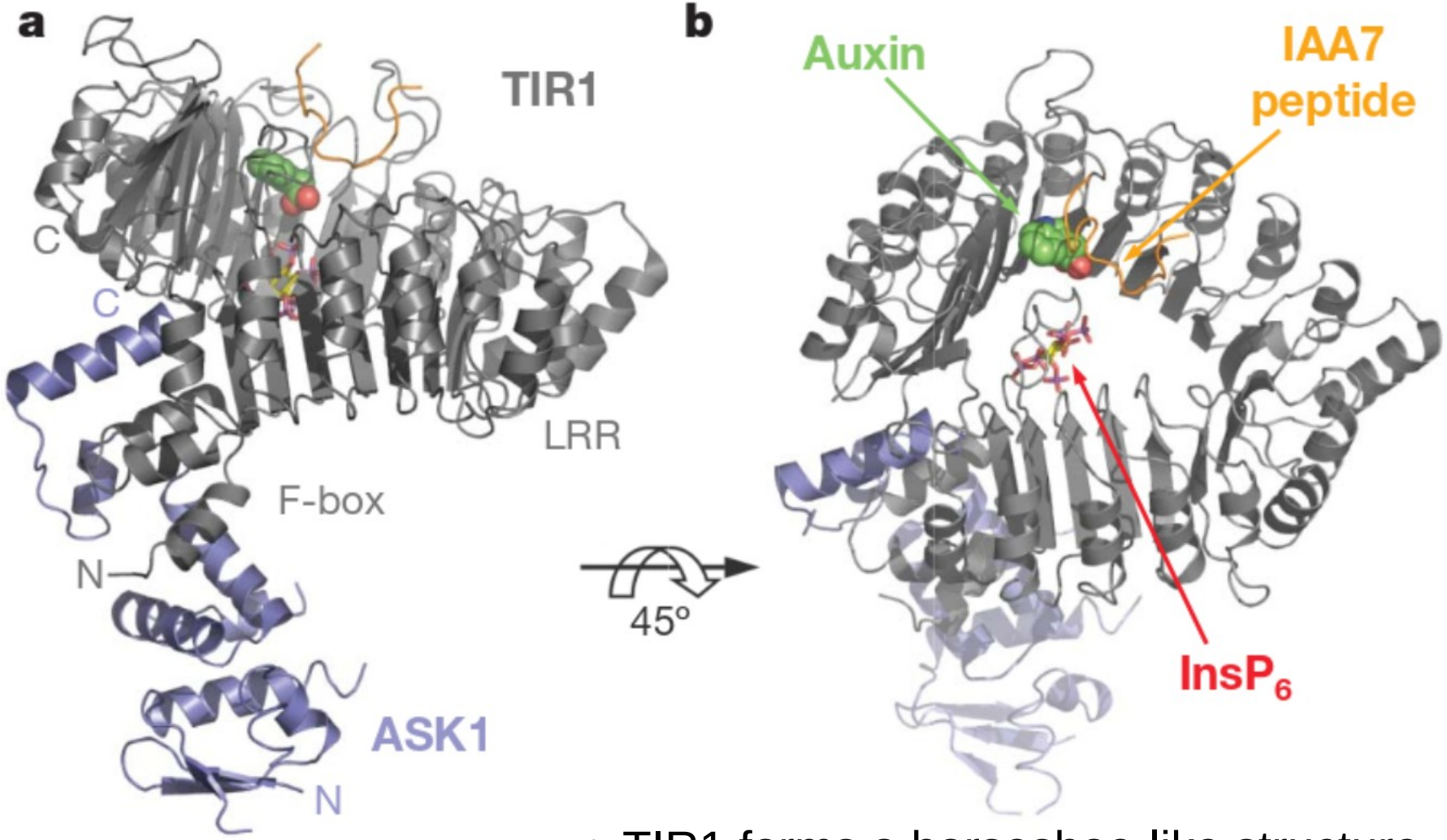


complex forms a mushroom-like
structure:

ASK1+ TIR1 F-box = stem

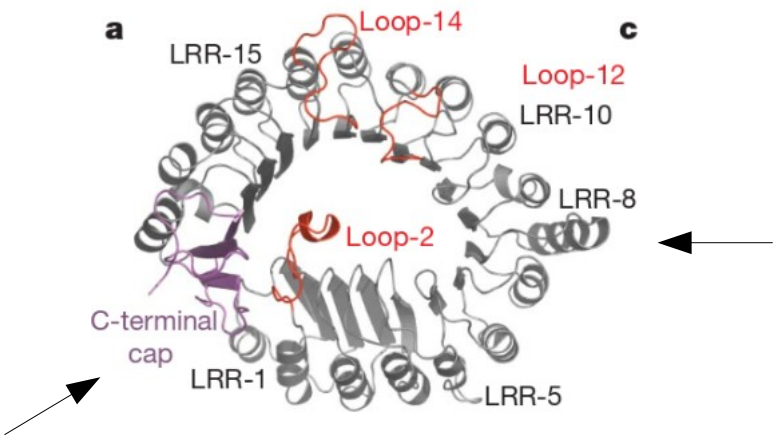
TIR1 LRRs = cap

TIR1-ASK1-DII-IAA structure



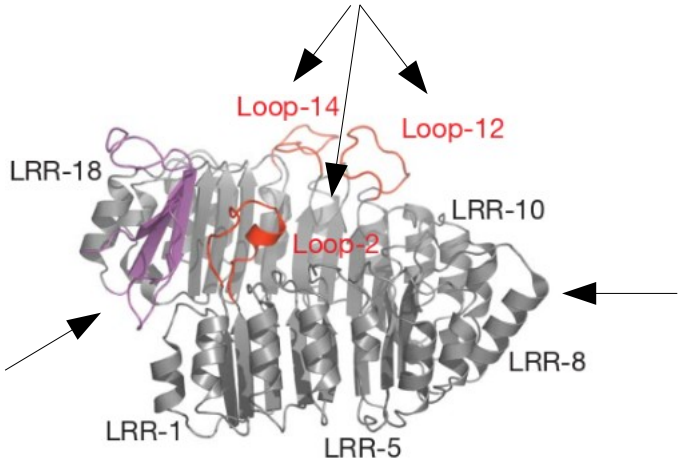
- TIR1 forms a horseshoe-like structure
- auxin and IAA7-DII bind at the same site
- unexpected co-factor identified

TIR1: the important loops 2, 12 and 14



TIR1 structure with and without IAA7-DII:
→ structure remains largely unchanged
= no / only minor conformational changes

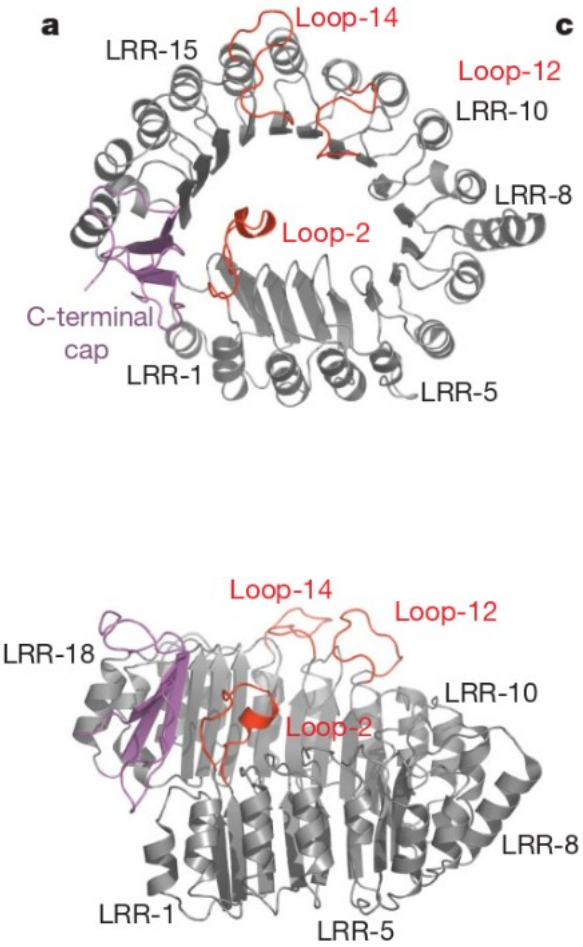
TIR1 forms unusual LRR structure
→ “kink” at LRR8

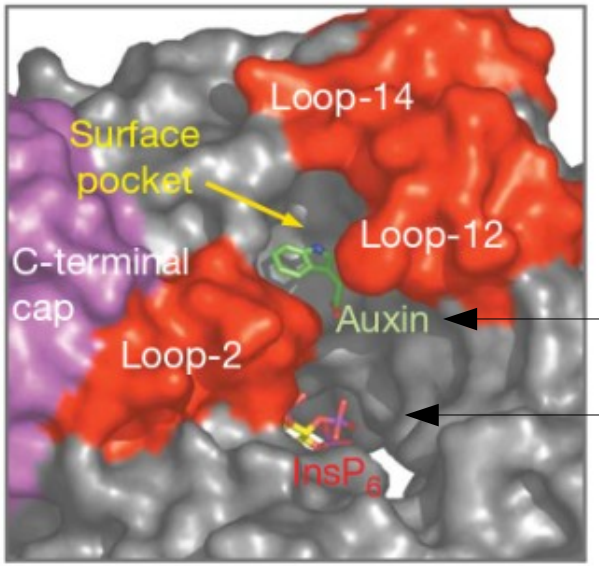


TIR1 forms unusual LRR structure
→ C-terminus is raised above the start of the LRR domain and closes to a full circle

Loops 2, 12 and 14 are located at the top surface
C-term. ß-sheets close the circle and form a “wall”

TIR1: the important loops 2, 12 and 14





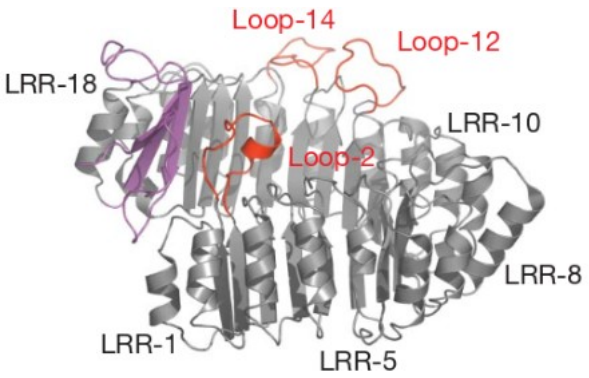
auxin

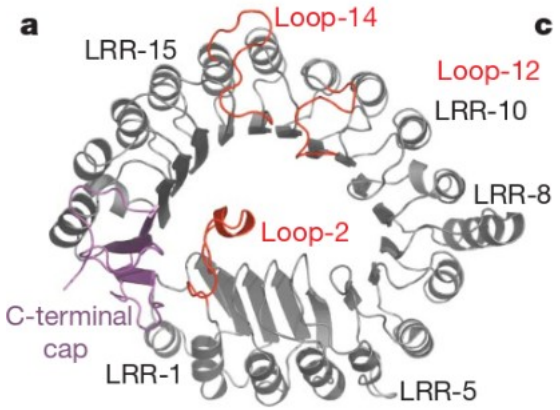
InsP6
(putative
co-factor)

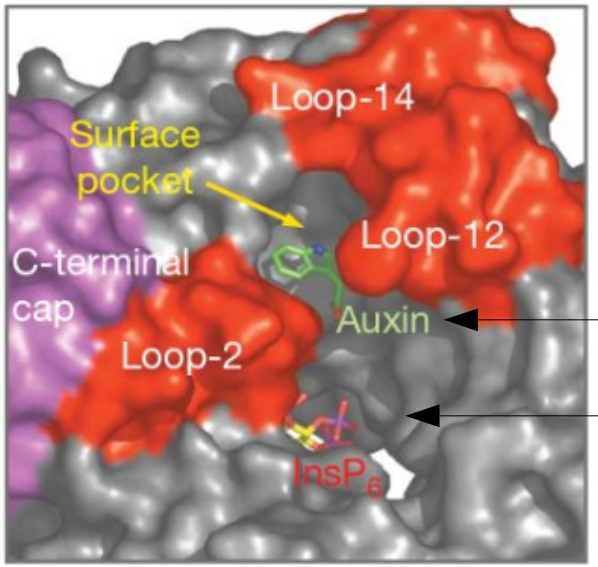
loops 2, 12 and 14

- are unusually long
- located at the top of the TIR1 surface
- are critical in forming the auxin binding pocket

TIR1: the important loops 2, 12 and 14







auxin

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(putative
co-factor)

loops 2, 12 and 14

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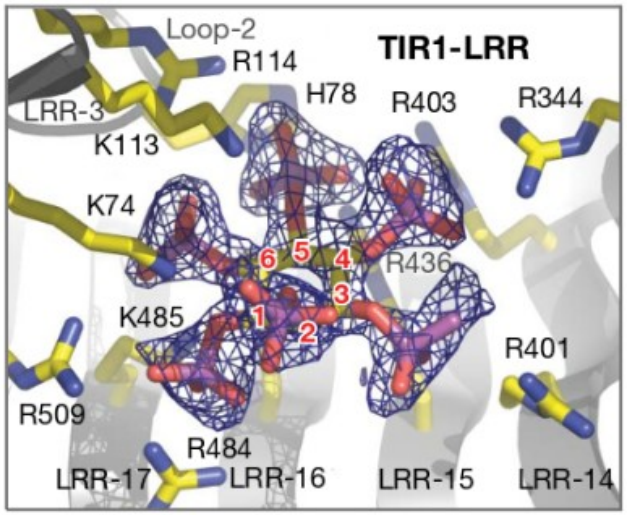
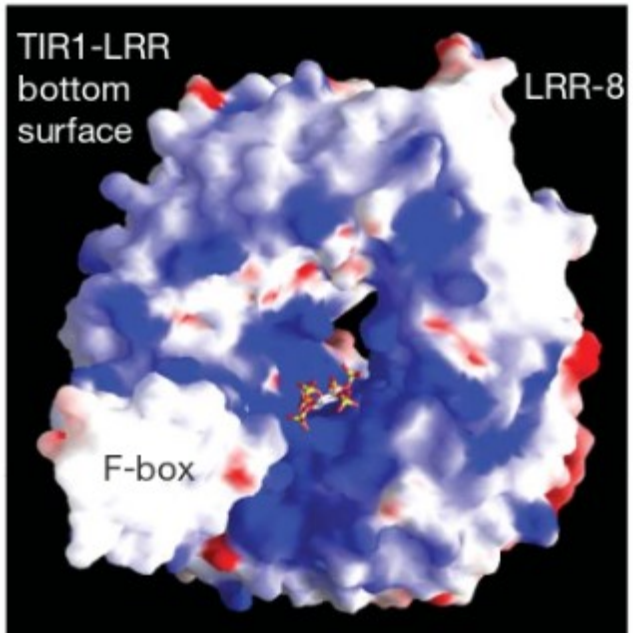
- are critical in forming the auxin binding pocket

is InsP6 a co-factor of the auxin receptor?



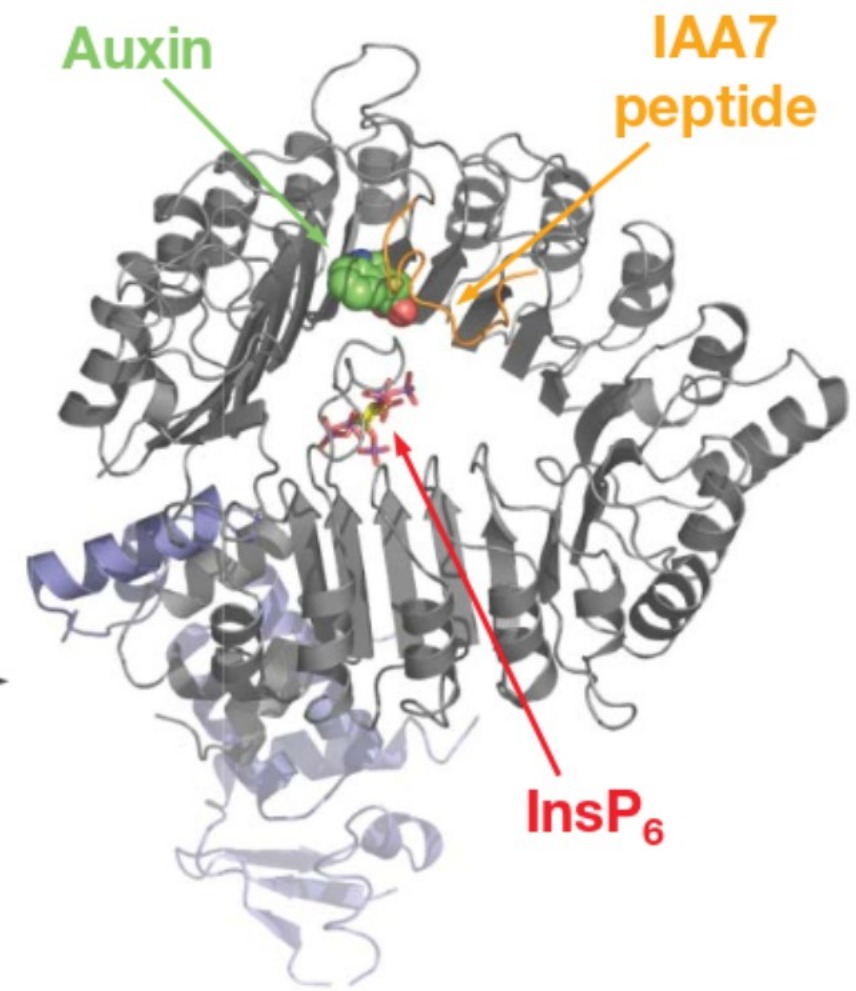
crystallography shows island of high electron density in TIR1 structure that fits to InsP6 structure (confirmed by GS-MS)

→ tightly bound and fits to surrounding AA-residues



bottom view in TIR1 structure (electrostatic surface potential representation)
→ positive charge (blue) would promote strong interaction with negatively charged InsP6

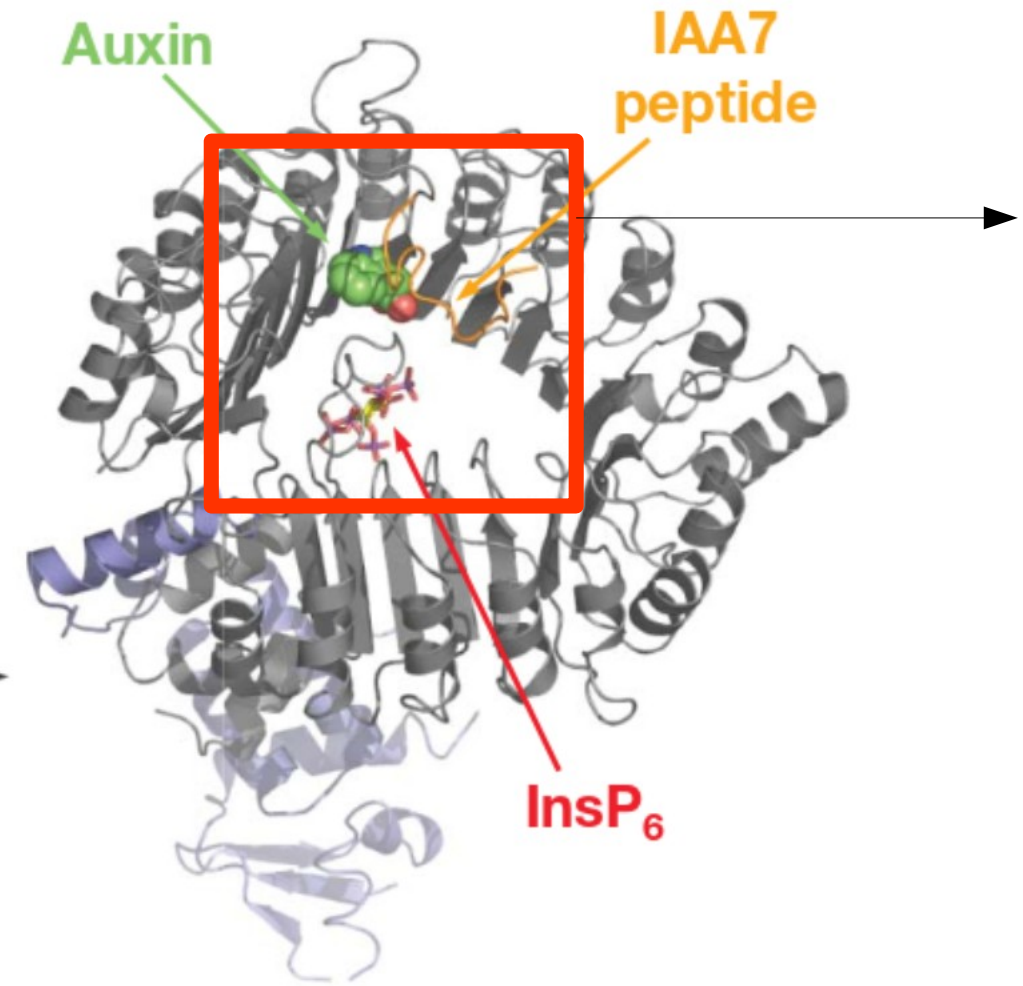
is InsP6 a co-factor of the auxin receptor?

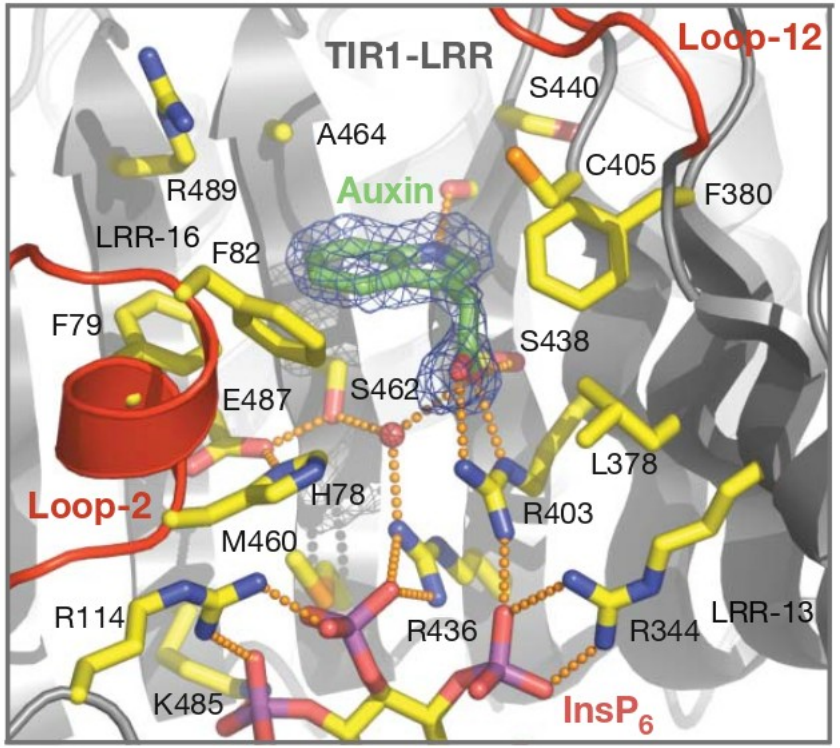


InsP6 likely acts as a co-factor of TIR1-auxin interaction

→ stabilization of the auxin binding pocket

the auxin binding pocket

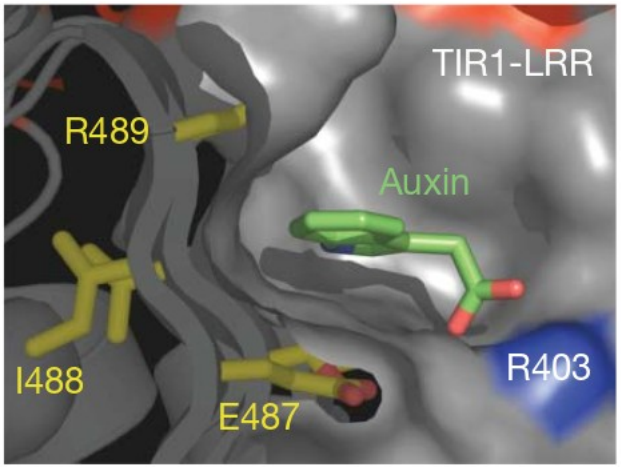


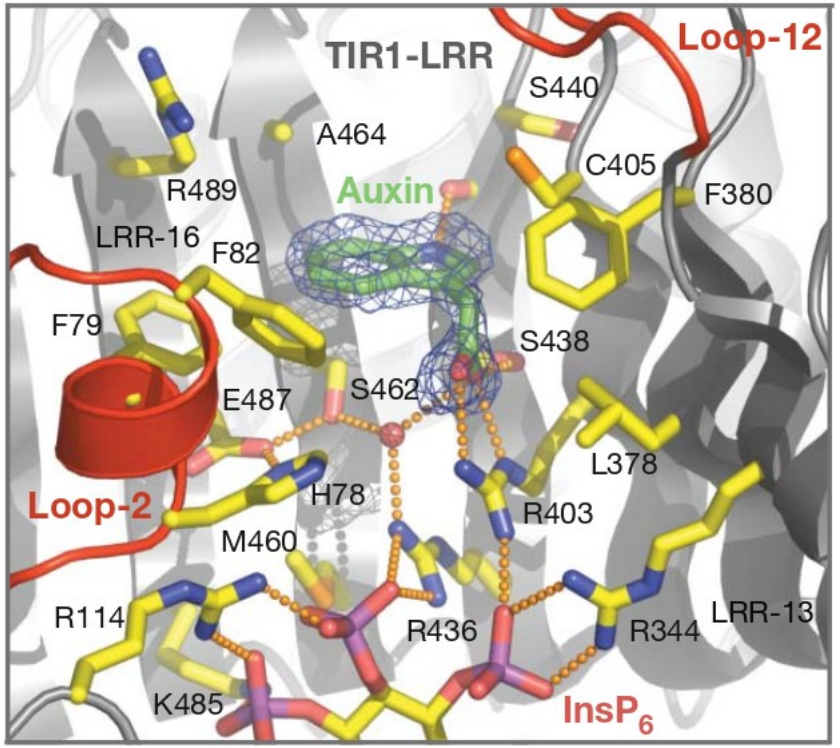


the auxin binding pocket

IAA carboxyl side chain interacts with TIR1 amino acids at the bottom of the pocket

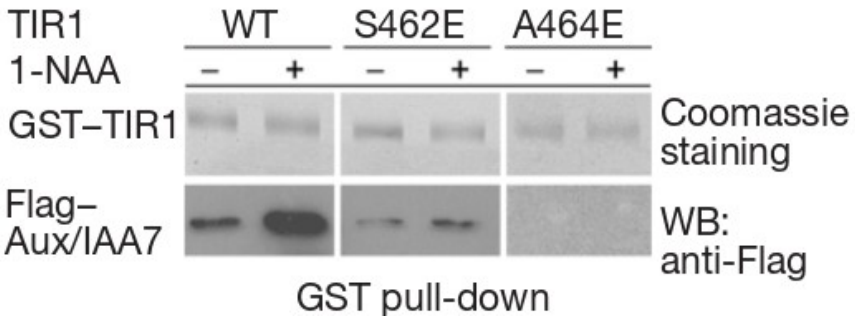
planar indole moiety interacts with hydrophobic residues (“sandwiched”)

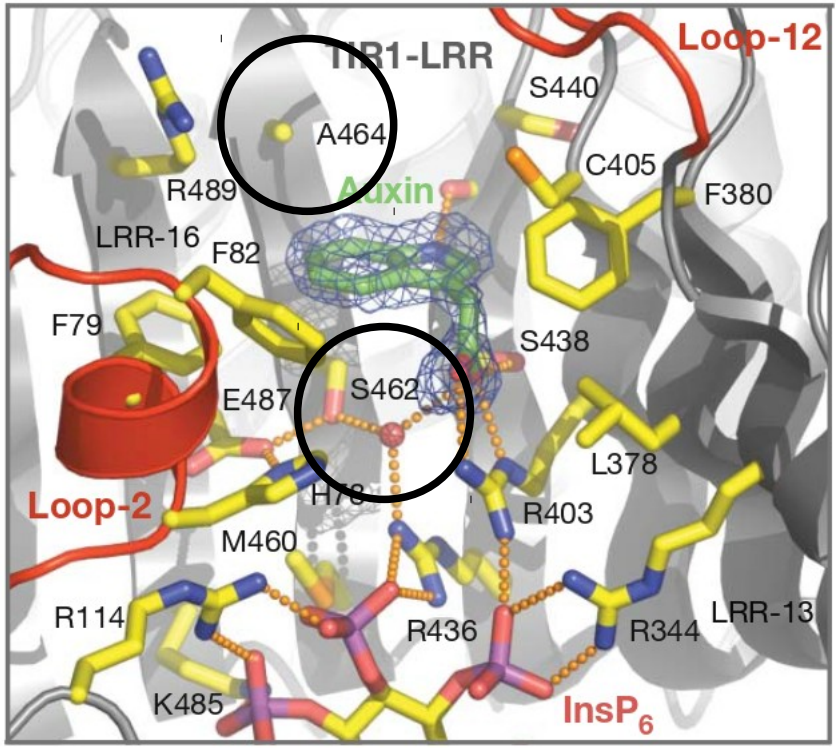




site-directed mutagenesis affects Aux/IAA interaction

if structure correct → mutation of critical AAs should affect auxin binding and/or Aux/IAA interaction

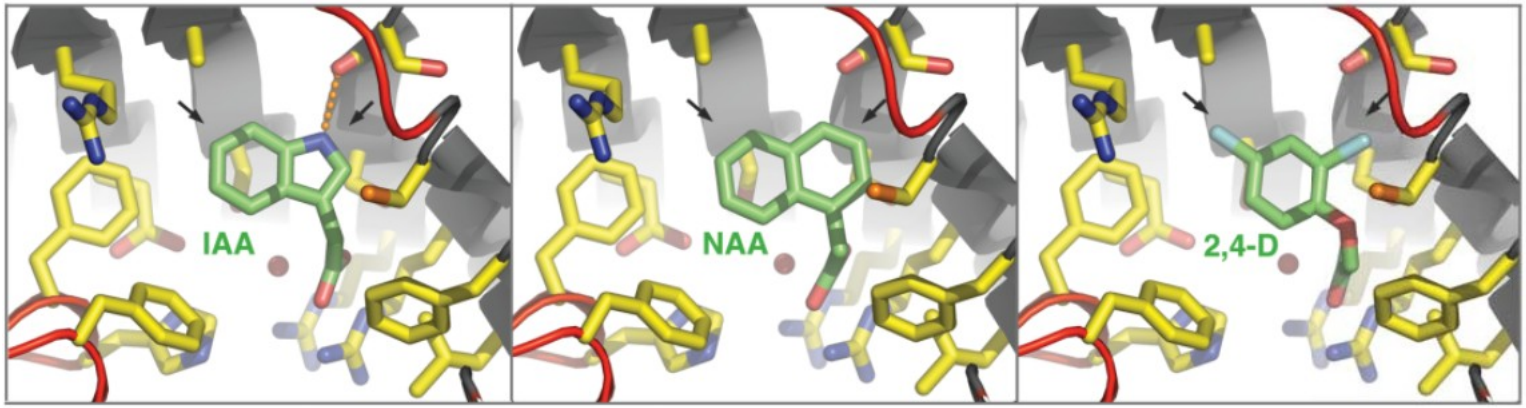




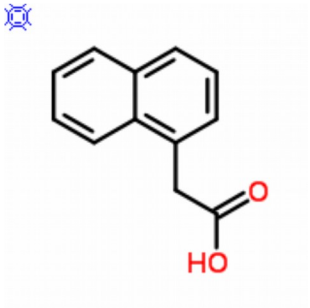
S462E: basal TIR1-Aux/IAA7 interaction but no promotion by auxin
→ integrety of the “floor” essential for auxin binding
A464E: no interaction

binding properties of different auxins

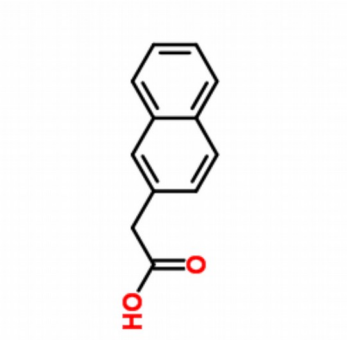
top view into binding pocket with different auxin compounds:



- carboxyl side chain anchors all three auxin analogs in the pocket
- although different in identity, the dimensions of planar ring structure differs only slightly → all three fit into pocket

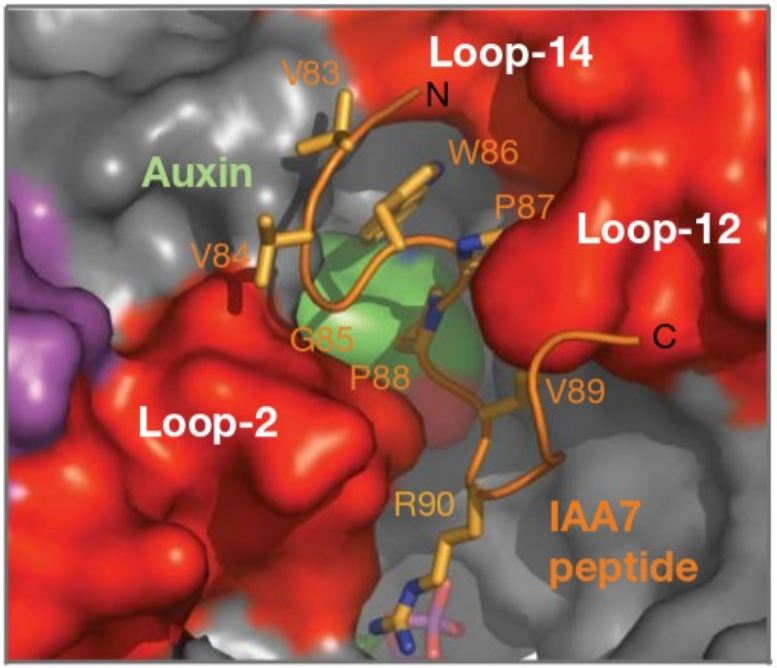


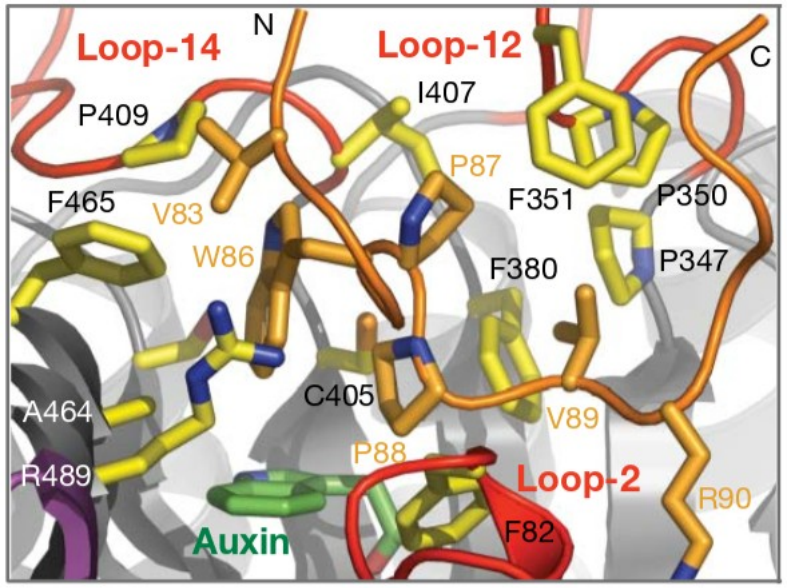
1-NAA
(active)



2-NAA
(inactive)

TIR1 – AUX/IAA interaction





Top view into the pocket:

Aux/IAA peptide interacts with surface of TIR1 + auxin

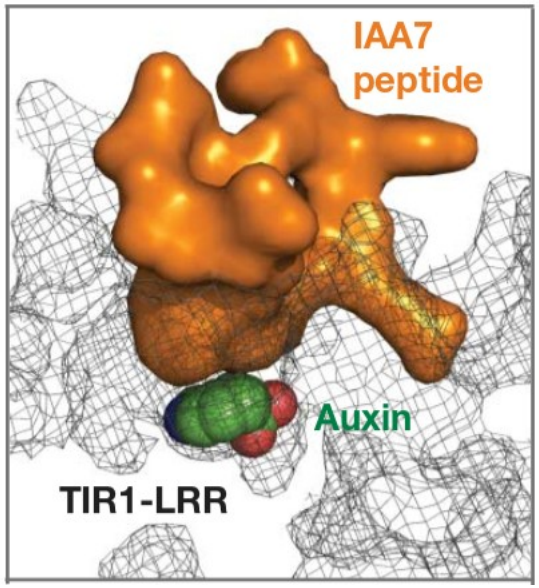
13 amino acids of IAA7 form a highly coiled structure

central hydrophobic motif GWPPV reaches into the binding pocket

flanking Aux/IAA amino acids interact with surface of TIR1 (loops 2, 12, 14)

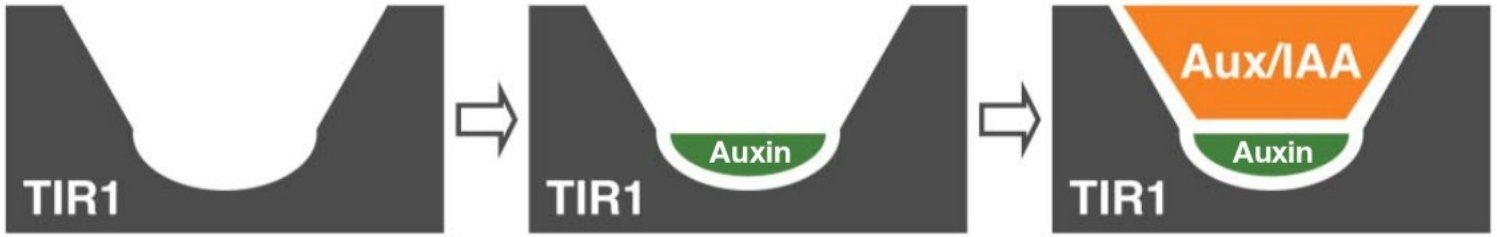
GWPPV = core degron

TIR1 – AUX/IAA interaction



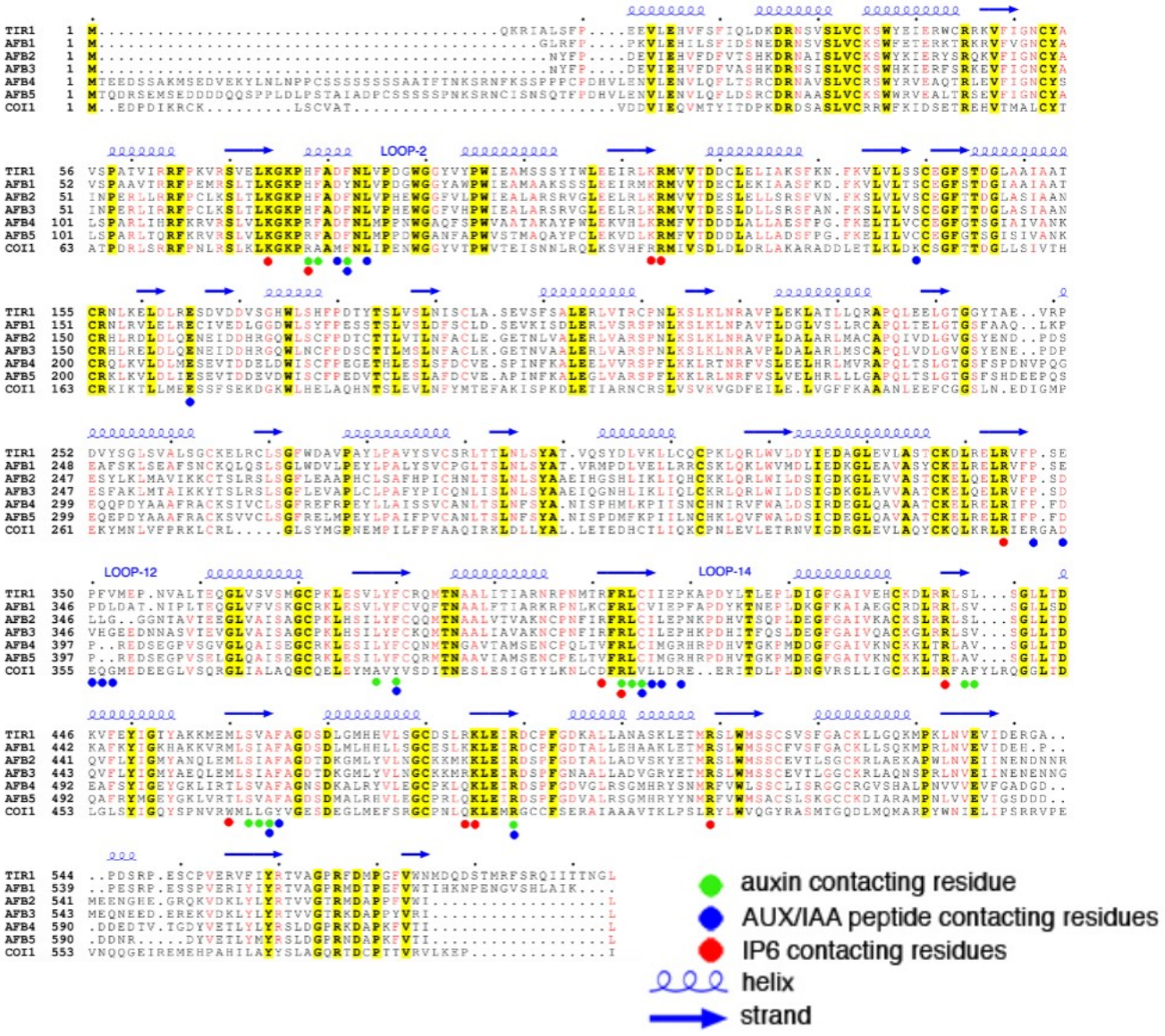
auxin molecule fills a cavity in the AUX/IAA binding site of TIR1
→ increases hydrophobic surface of the interaction surface

“molecular glue” model of auxin binding (no allosteric switch):



TIR1 + AUX/IAA form an auxin co-receptor complex

(partially) redundant functions of TIR1 homologs?



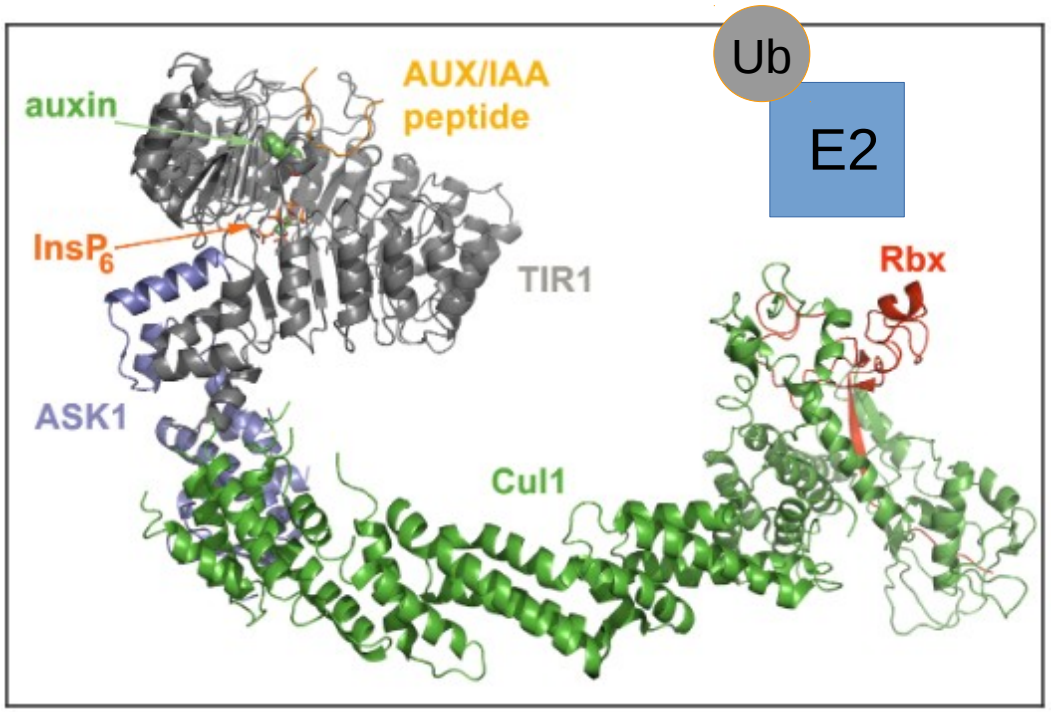
TIR1 and AFB show high degree of conservation in many critical residues

some differences (e.g. loop12) might account for partial specificities

COI1 (JA receptor) differs much more in critical residues

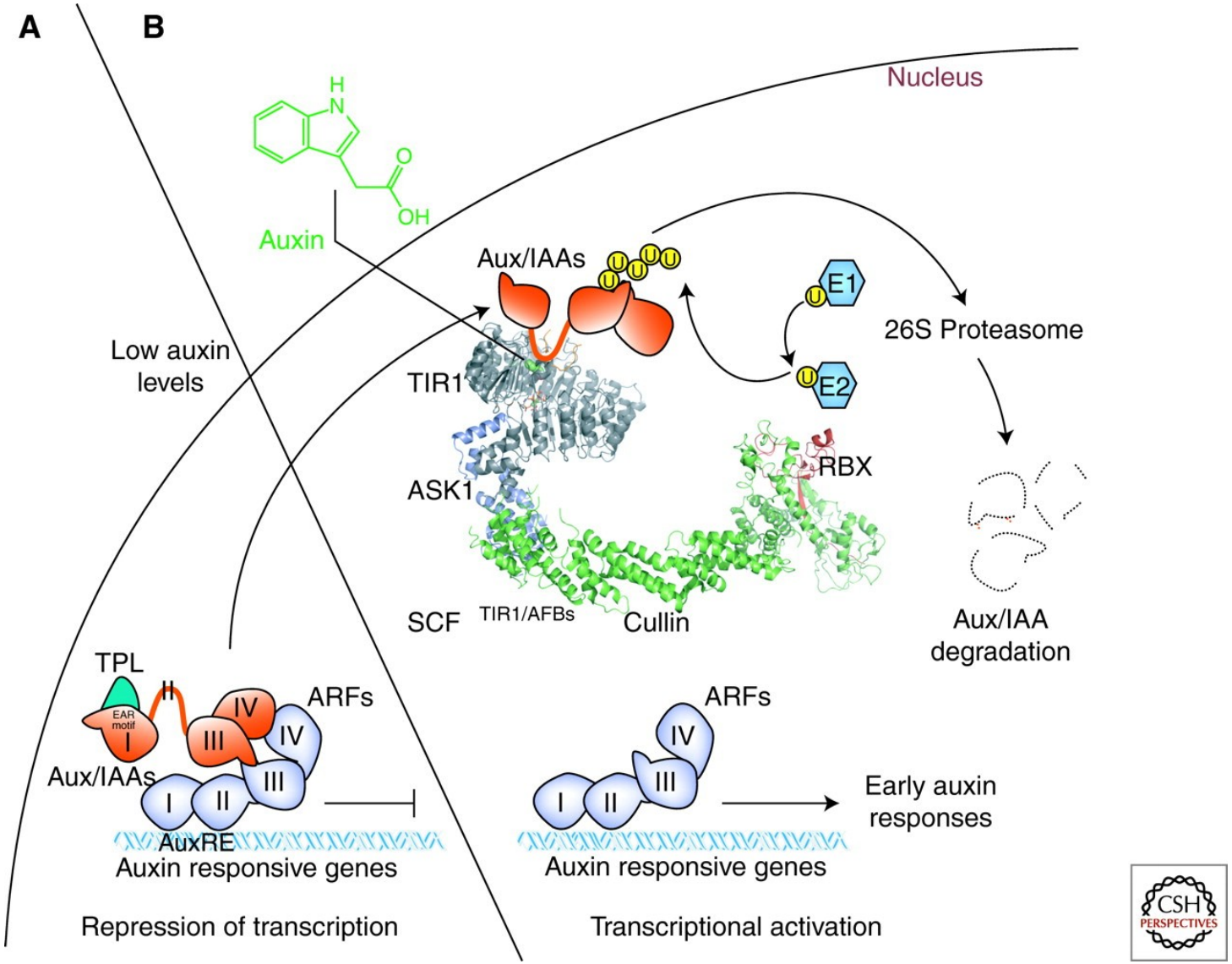
integration in the SCF complex

→ SCF-TIR1-ASK1-DII-auxin was modeled on the human SKP2 complex



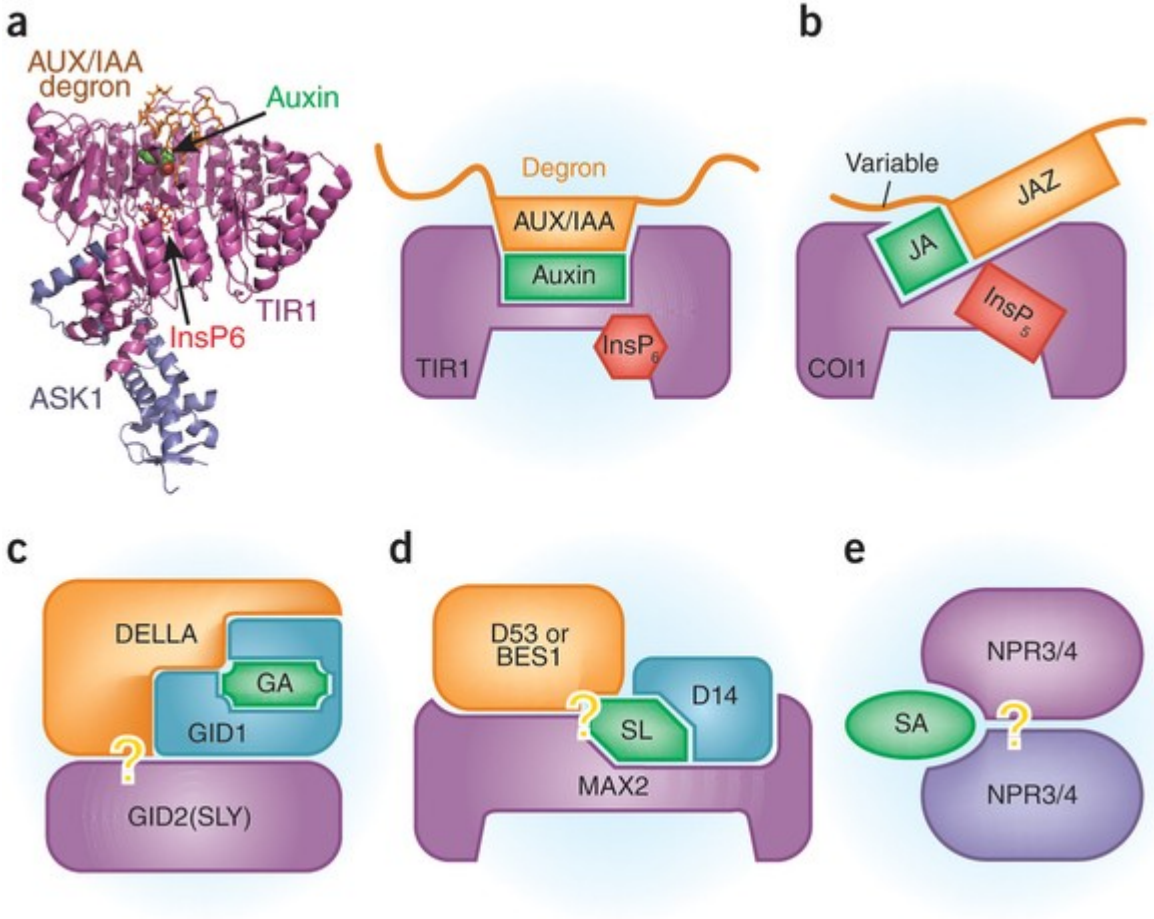
→ TIR1-ASK1 integration in complex orients top of the “mushroom cap” towards the RING subunit of the SCF-complex (→ activated E2 binding site)

new model



wider implications……

1. phytohormone biology

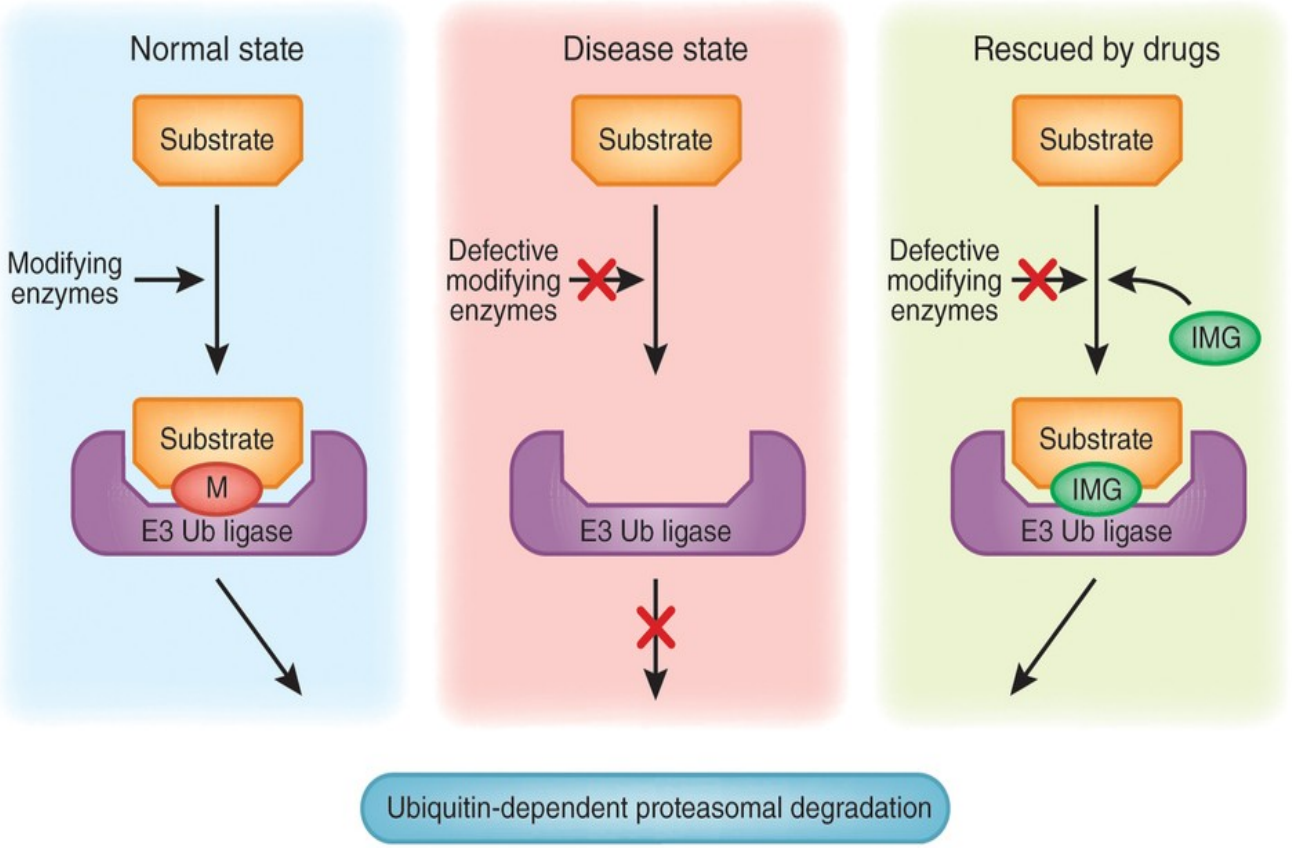


http://www.nature.com/nsmb/journal/v21/n4/images_article/nsmb.2804-F2.jpg

wider implications……

2. pharmacological approaches

cancer, neurological and immune disorders



Identification of small molecule compounds that rescue, enhance or extend SCF-substrate interaction

http://www.nature.com/nsmb/journal/v21/n4/images/nsmb.2804-F3.jpg

design of specific inhibitors or analogs

approaches:

→ modeling of chemical substances into the structure of an SCF complex (in silico approach)

→ testing of chemical components with specific properties based on a known compound (chemical screening → physiological or molecular read out required)

→ specific design / modifications of compounds

design of specific TIR1 inhibitors

Small-molecule agonists and antagonists of F-box protein–substrate interactions in auxin perception and signaling

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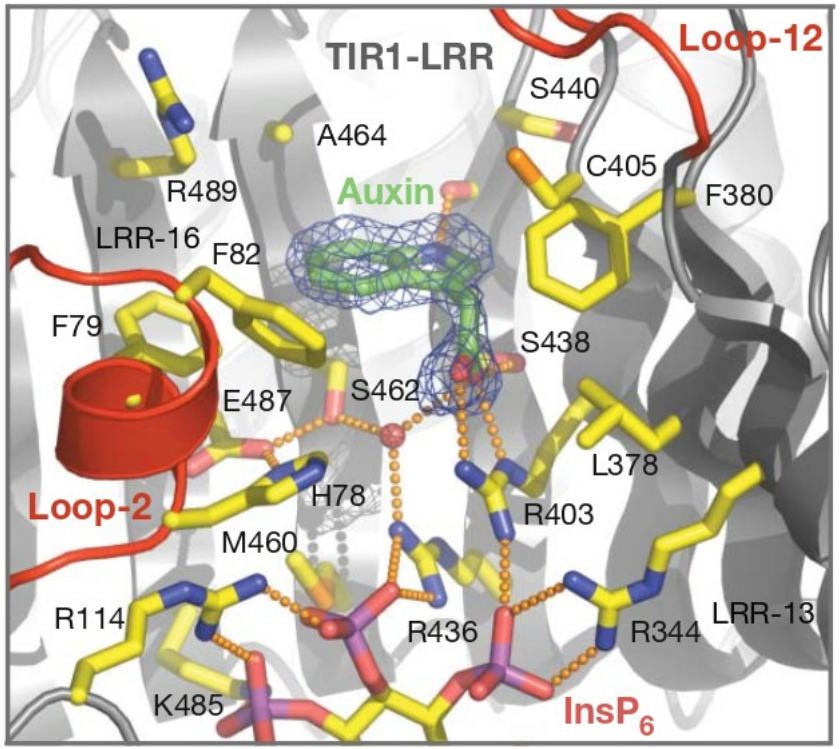
Edited by Mark Estelle, Indiana University, Bloomington, IN, and approved January 31, 2008 (received for review November 26, 2007)

→ use of structural information to design specific auxin analogs and antagonists

design of specific TIR1 inhibitors



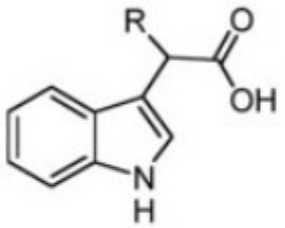
<u>agonist/analog:</u>

should have (somewhat) similar features as IAA

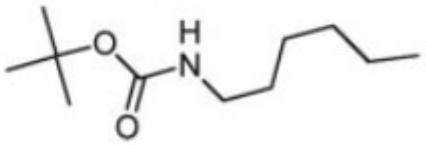
<u>antagonists/inhibitors:</u>

ideal: binds to TIR1 but inhibits AUX/IAA binding

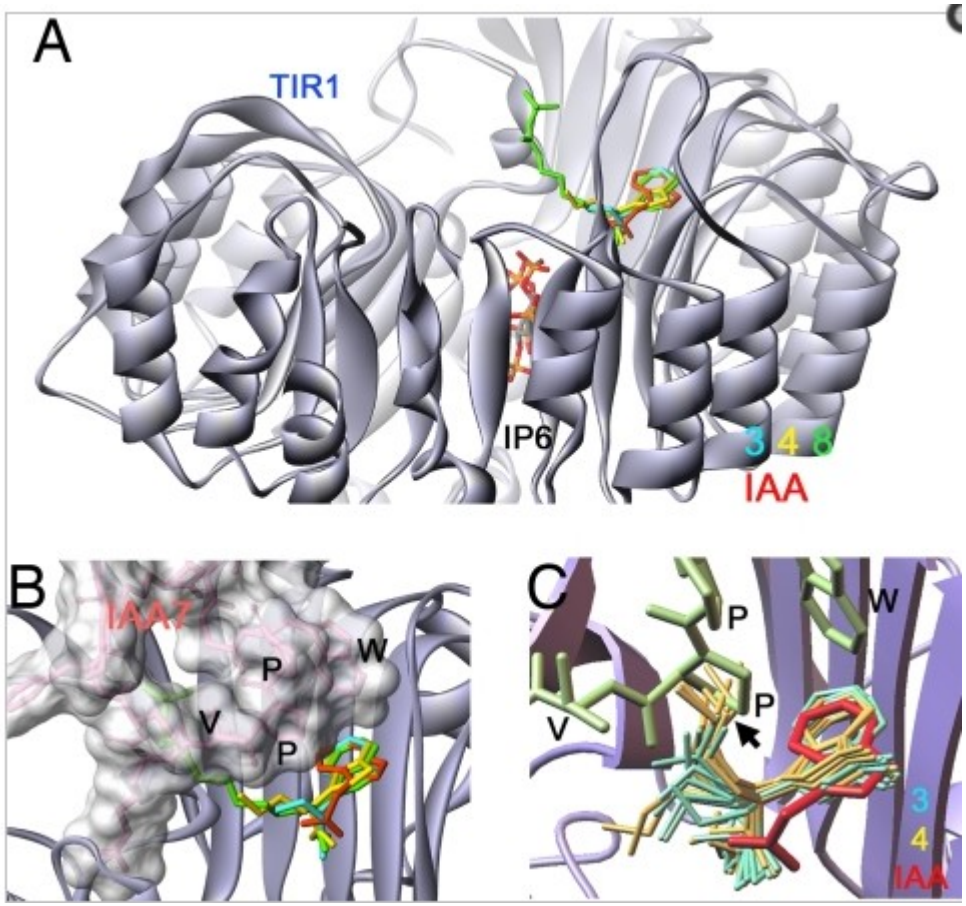
Synthesis of IAA derivatives

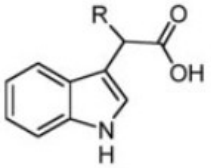


1: R=metyl **5**: R=pentyl
2: R=ethyl **6**: R=hexyl
3: R=propyl **7**: R=heptyl
4: R=butyl **8**: R=*tert*-butoxycarbonylaminohexyl



design of specific TIR1 inhibitors





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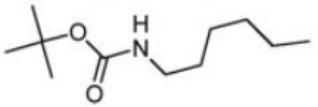
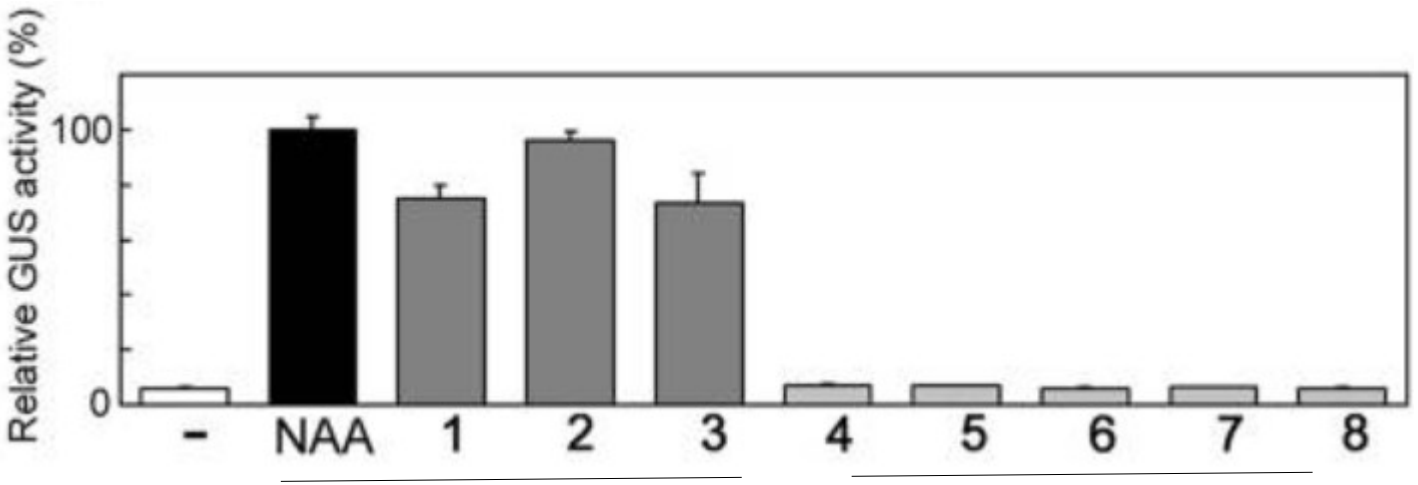


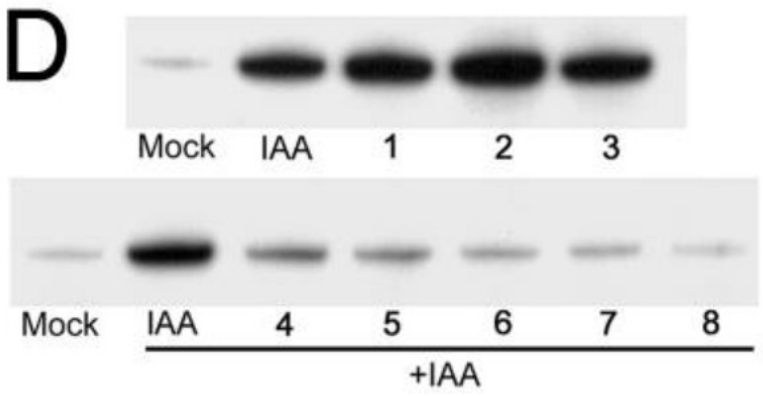
→ crystal structure shows:

probe 3 is largely like IAA
probe 4 side chain extents into AUX/IAA binding site
probe 8 occupies too much space

physiological validation

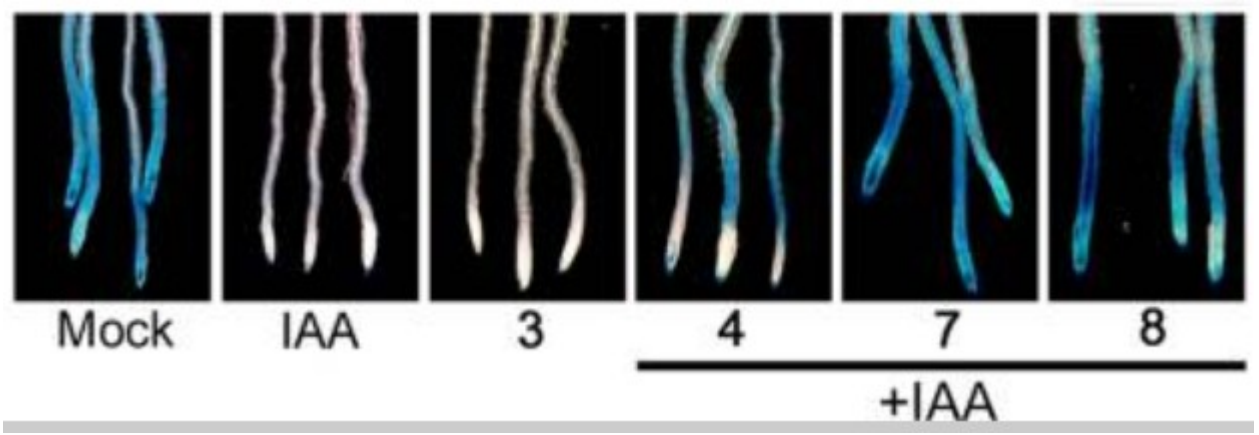
activation of DR5:GUS reporter:





physiological validation

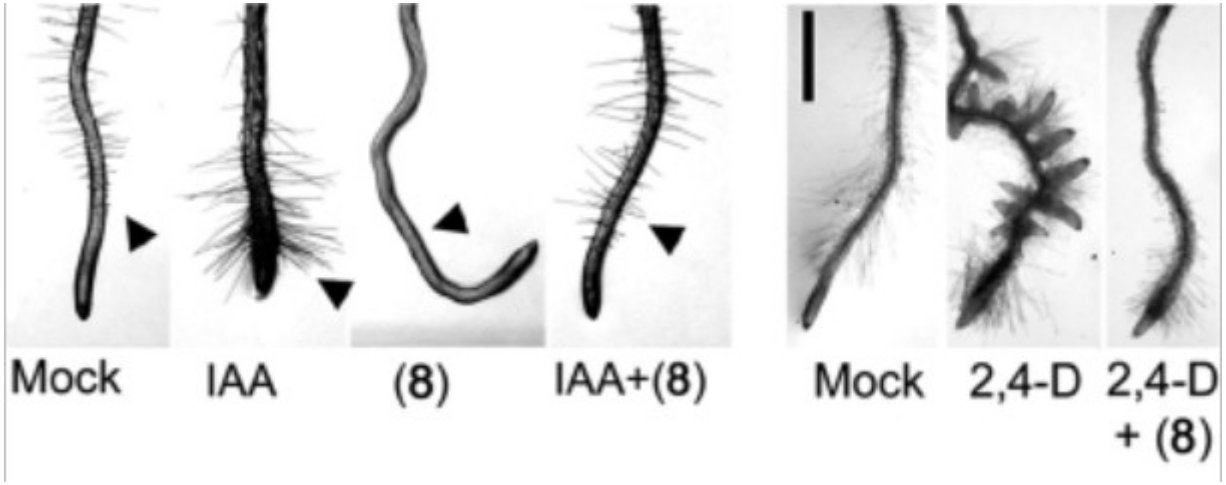
HS:AXR3NT-GUS



IAA + #3 promote degradation, >#4 inhibits degradation even in the presence of IAA

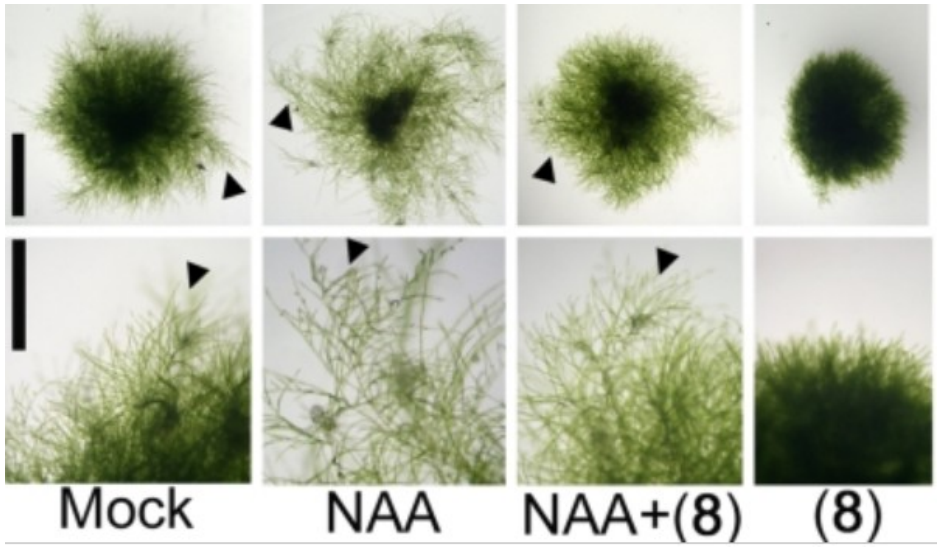
→ dominant inhibition of auxin signaling

antagonist action of #8



in
Arabidopsis

effects of high auxin are counteracted by #8 (evol. conserved!)



in
moss

new model