

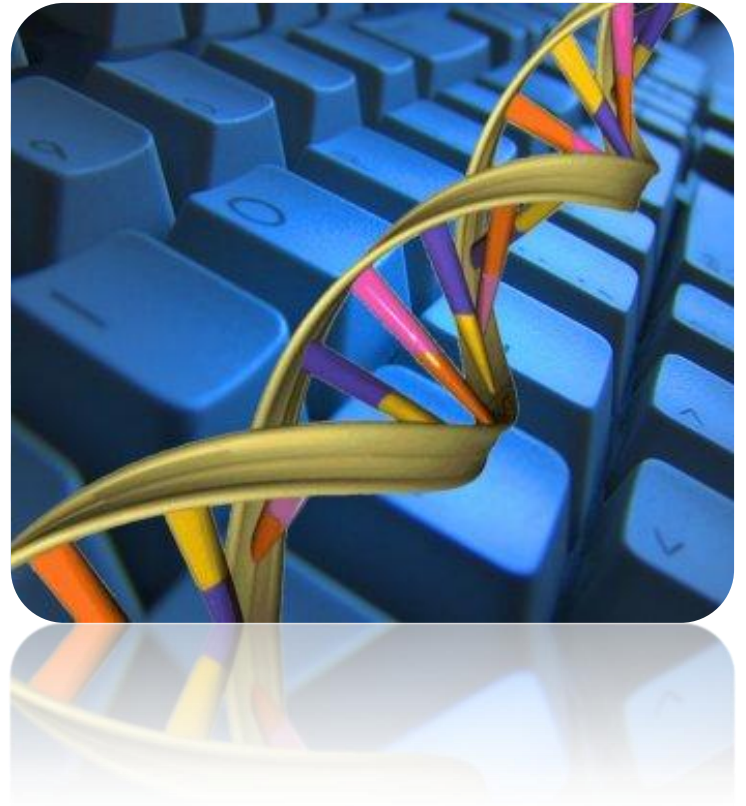
Structural Prediction of SCF^{EBF1/2} & substrate EIN3 in *Arabidopsis*

Xing Zhang

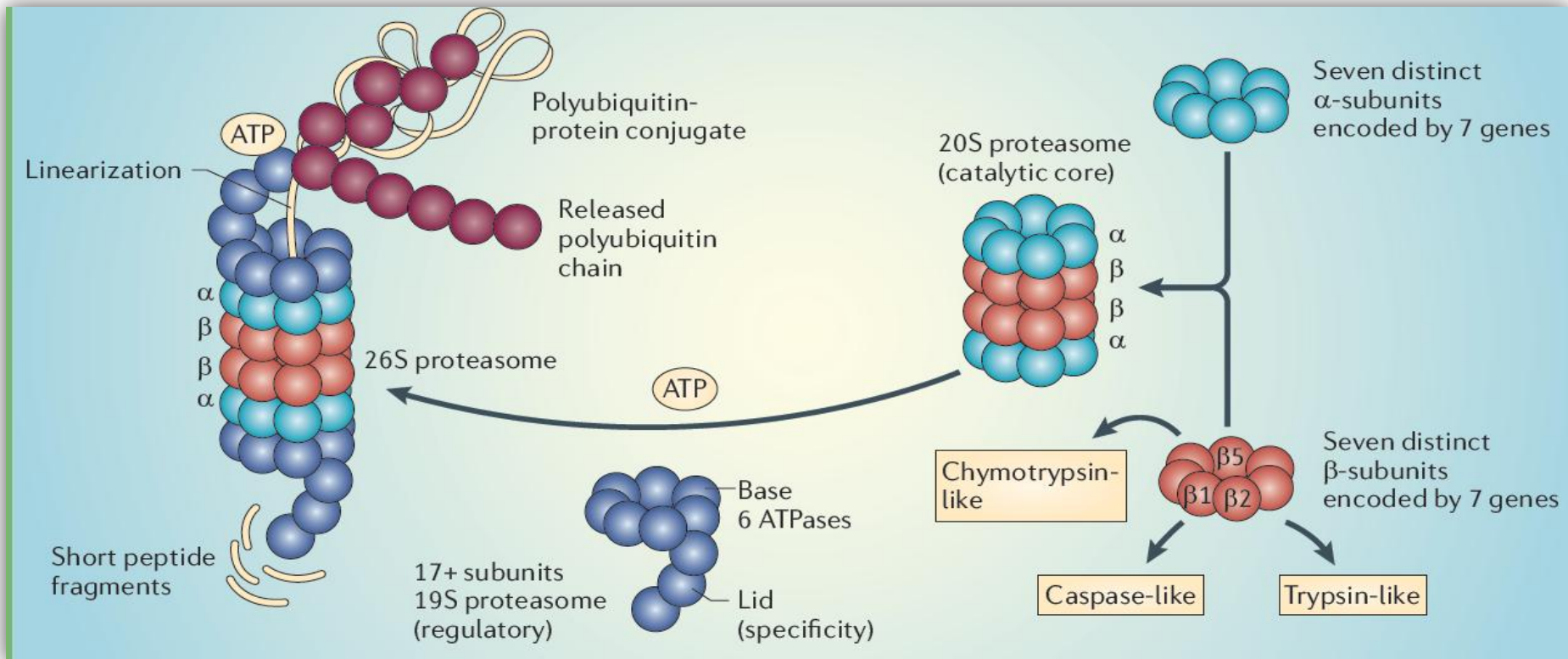
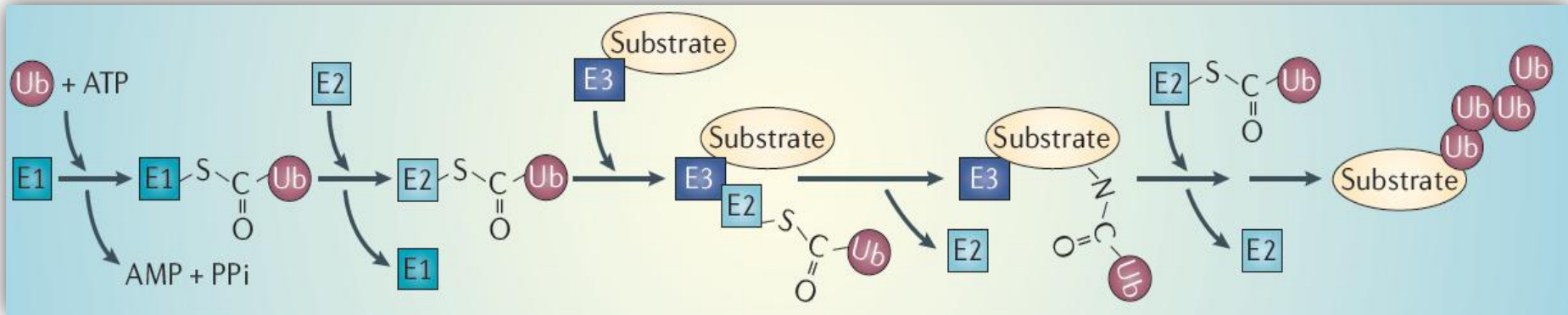
2011-12-23

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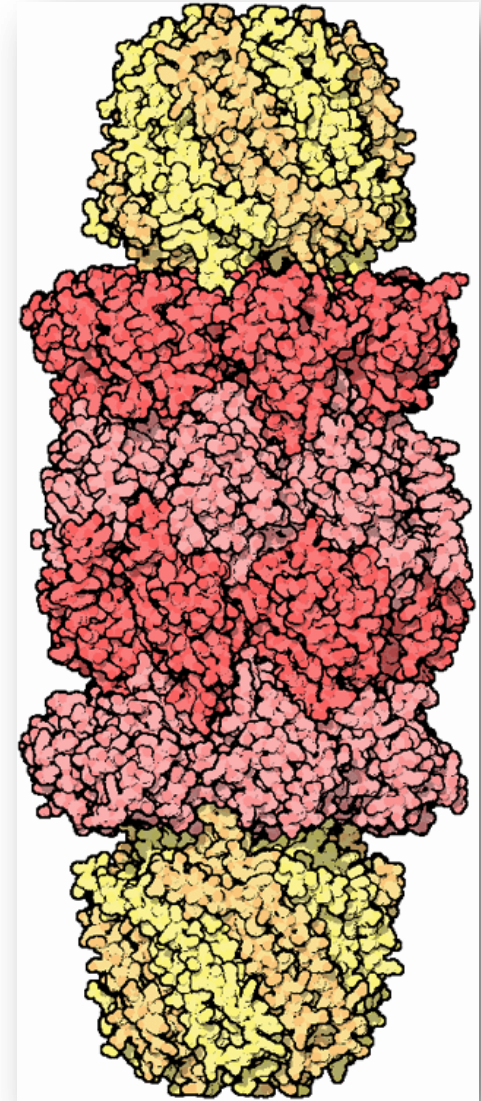
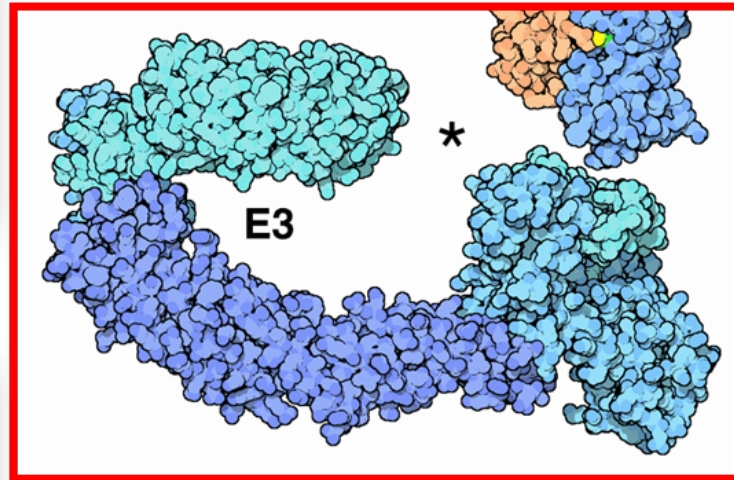
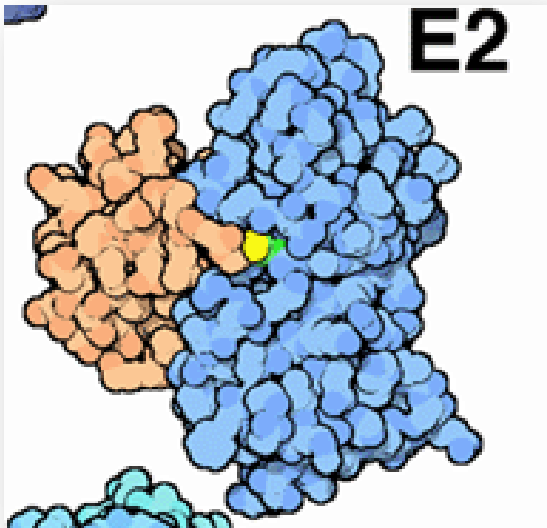
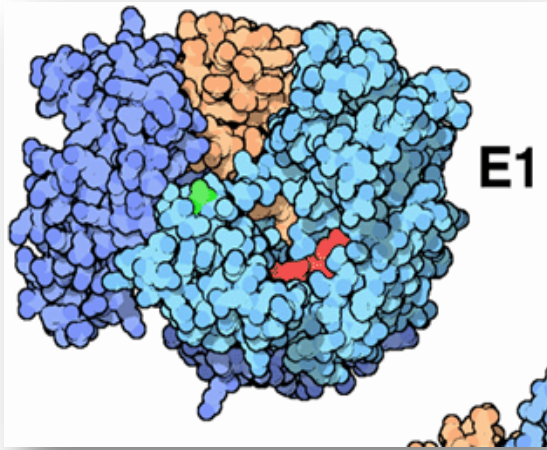


Ubiquitin-Proteasome System(UPS)



What Determines the Specificity of UPS?

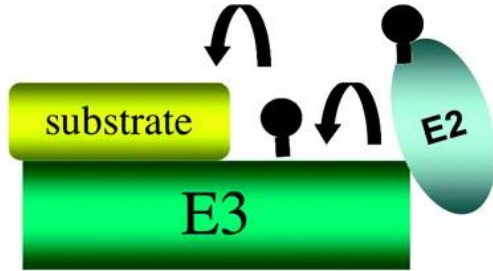
---Recognition of E3&Substrate



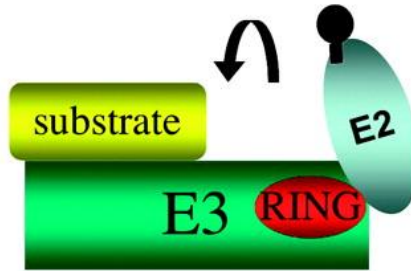
Universal E1, E2 and Proteasome with
Substrate **Unique** E3

Diverse UPS in Plant

Single Subunit:



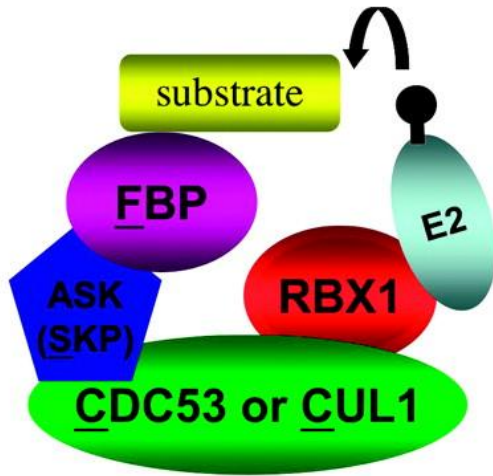
HECT



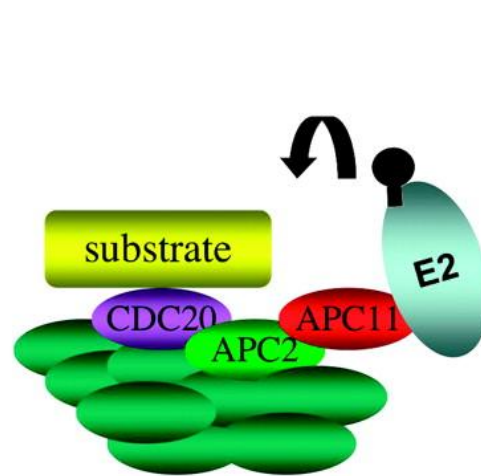
RING/U-box

● = Ubiquitin

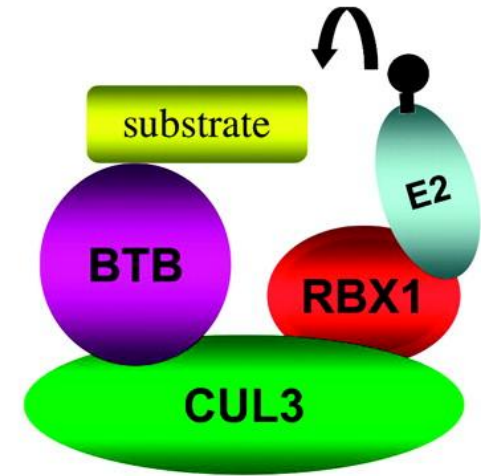
Multi Subunit:



SCF

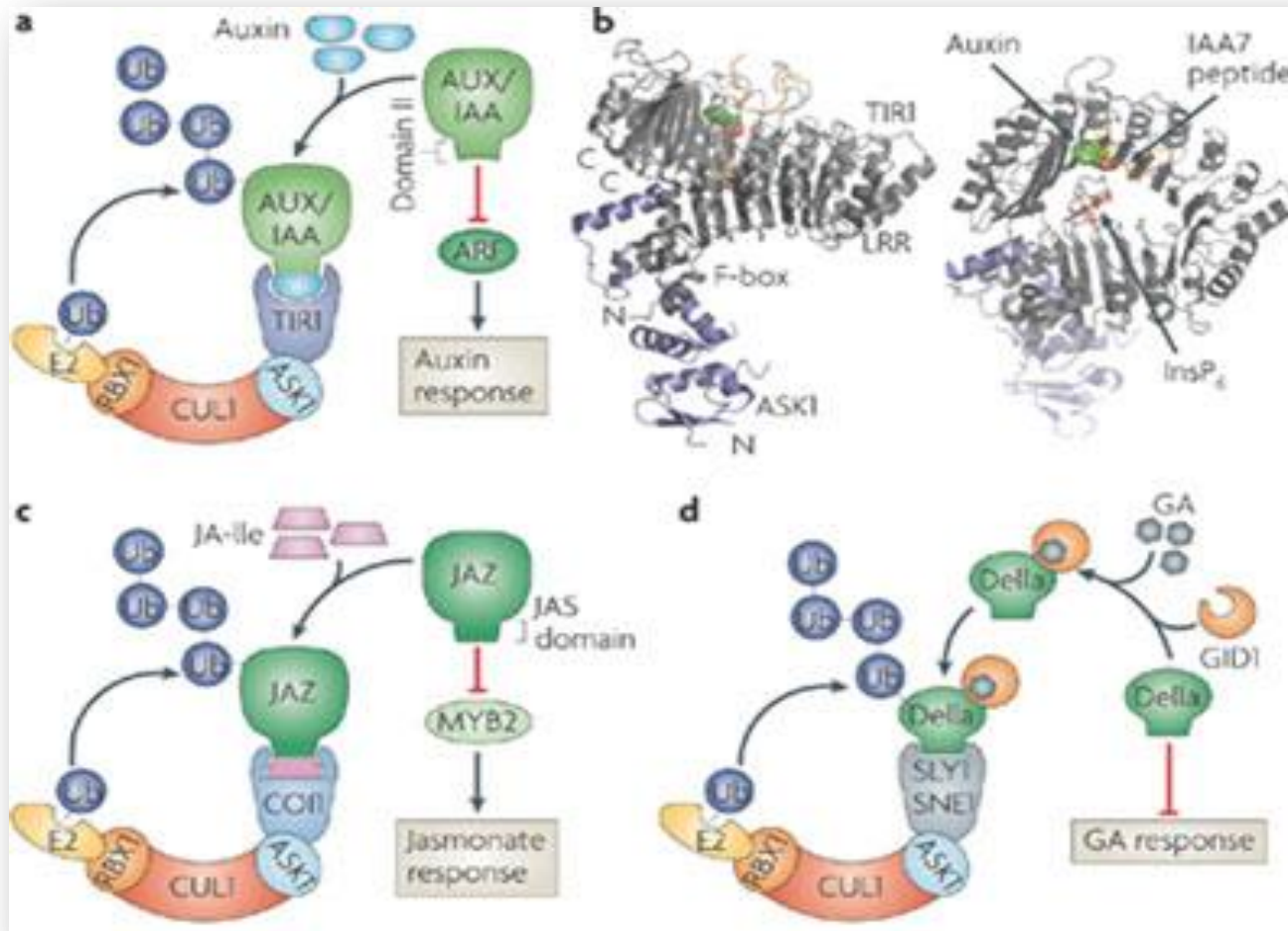


APC



CUL3-BTB

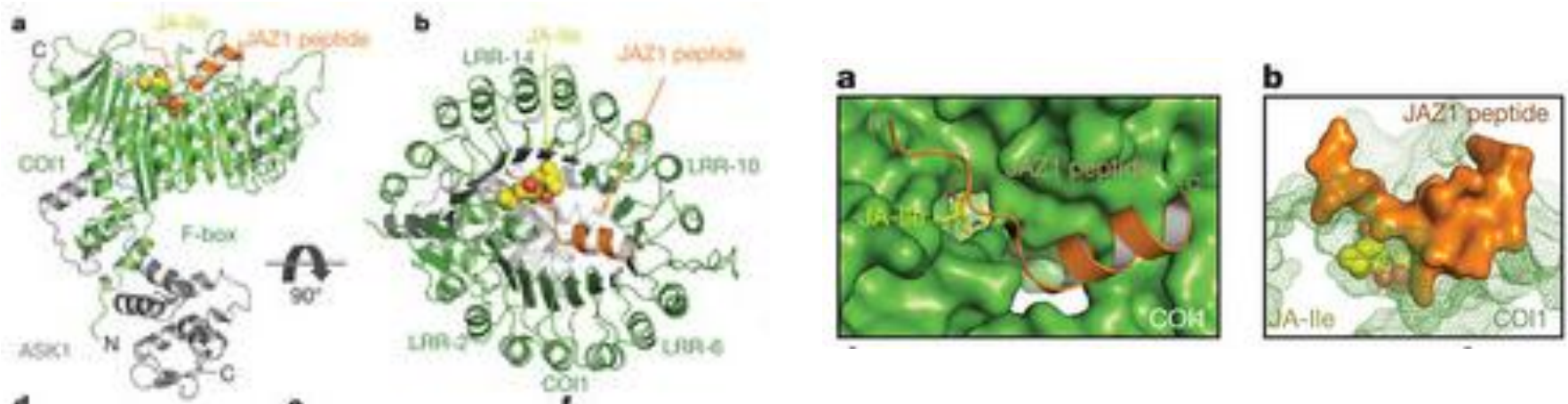
Diverse “SCF type” UPS in Phytohormone Signaling



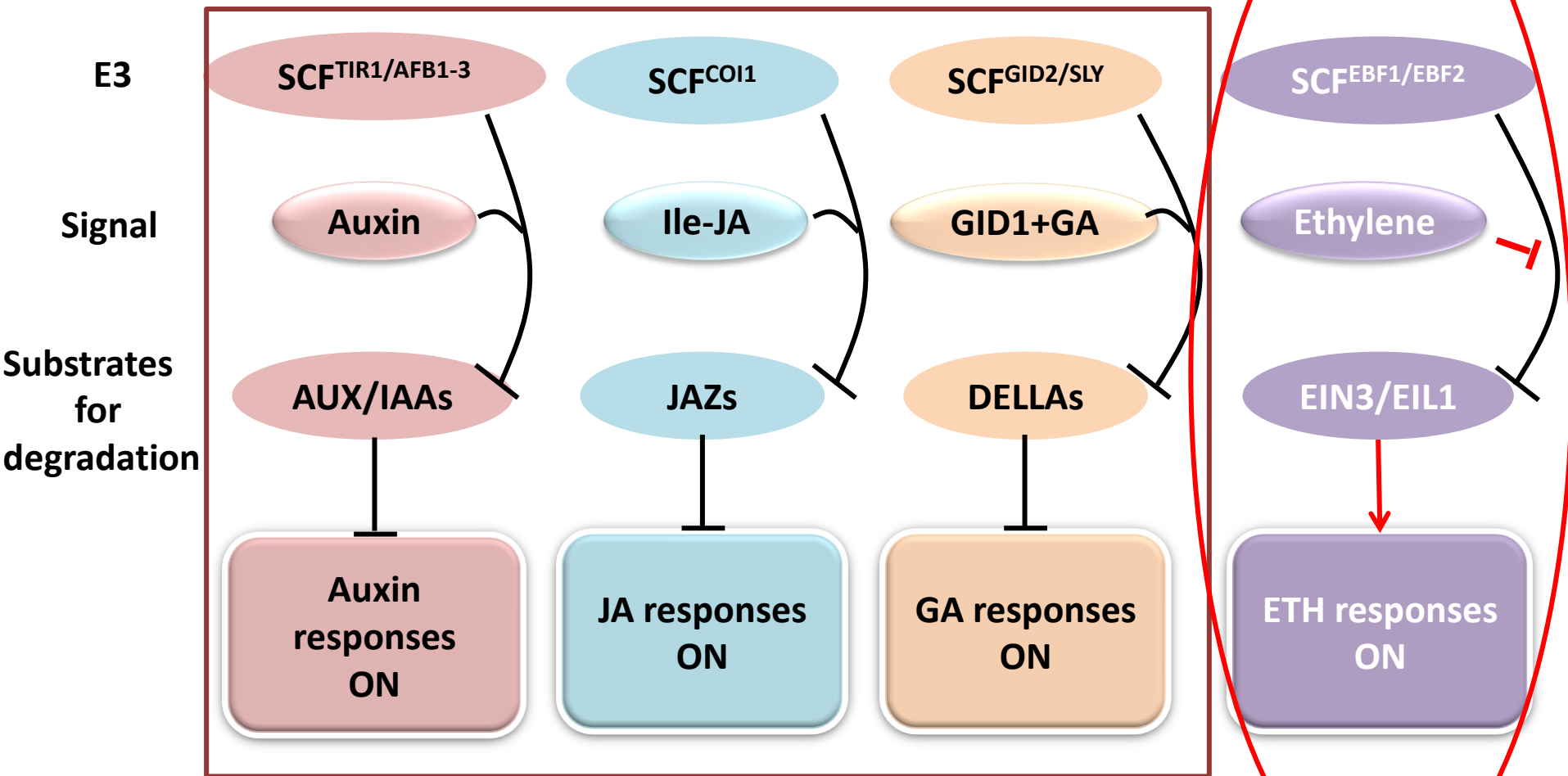
Auxin as “molecular glue” Regulates TIR1(F-box, Receptor)–AUX/IAA(TF) Interactions



COI1(F-box) Interacts with JAZs and Perceives Jasmonate as co-receptor



F-box Proteins in Plant Hormone Response



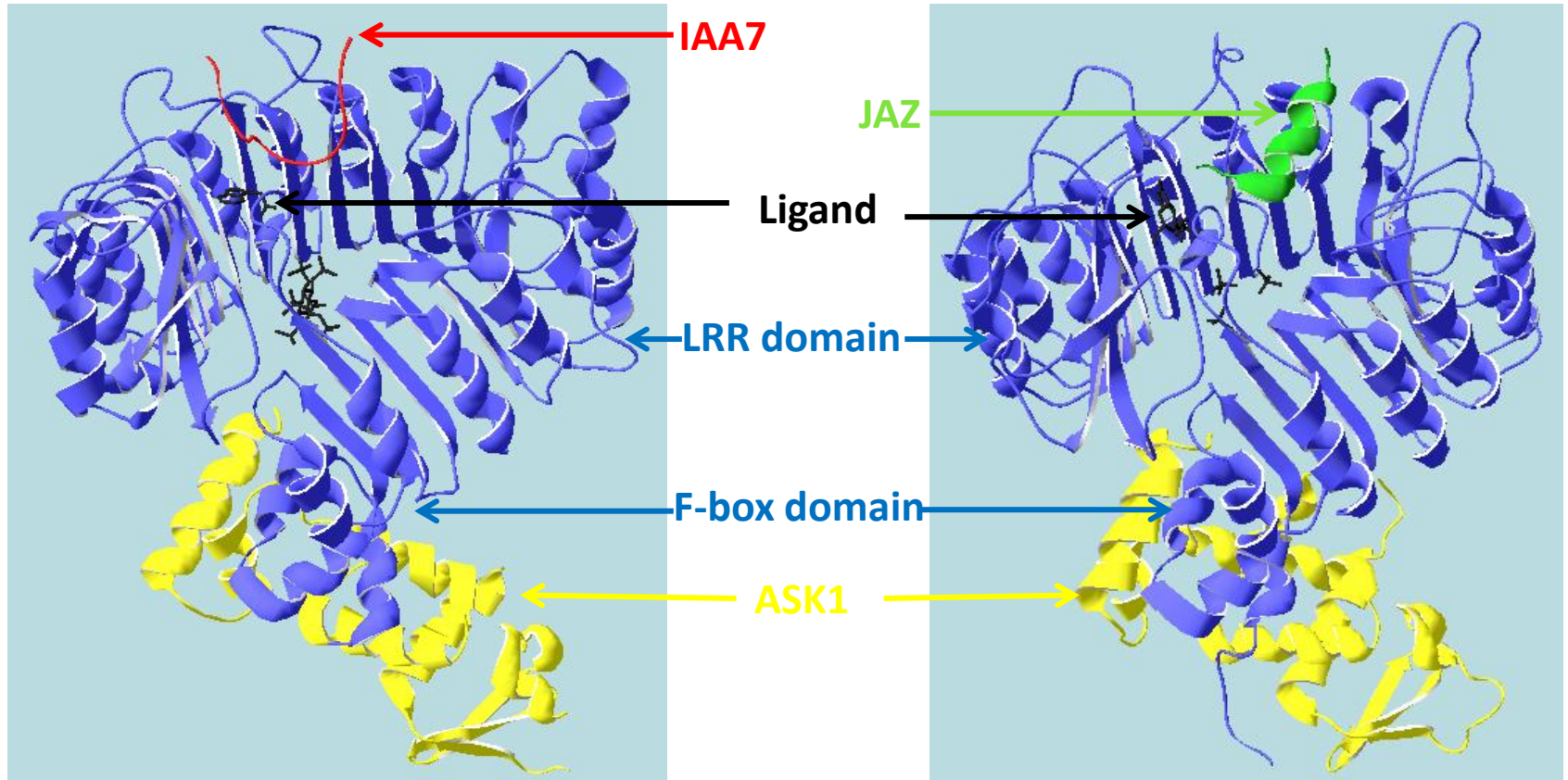
General secondary structure of F-box protein in *Arabidopsis*



Which region contributes to the specificity?

- >Response to different hormones**
- >Recognize different substrates**

3-D structure of SCF^{TIR1} & SCF^{COI1} in *Arabidopsis*



TIR1 & ASK1

COI1 & ASK1

What happened to EBF1&EBF2?

Nature (2007) 446:640-645

Nature (2010) 468:400-405

Major Goals



- Take TIR1&COI1 as templates to predict 3-D structure of EBF1
- Predict the region in EBF1 that is responsible for EIN3 recognition and binding specifically
- Predict the secondary structure of EIN3 and the possible 3-D conformation
- Speculate the possible interaction mechanism between EIN3 and EBF1

Approaches



- **Sequence Alignment:**
ClustalW, Muscle
- **Secondary structure prediction:**
PSIPRED V3.0, MEME, SMART,
- **Protein Modeling:**
Swiss-Model, 3D-JIGSAW, Swiss-PDB viewer

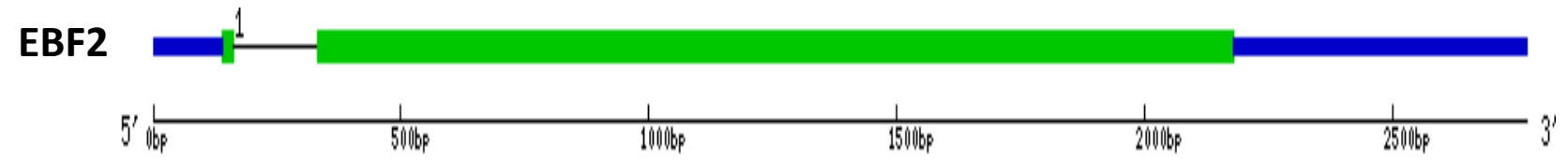
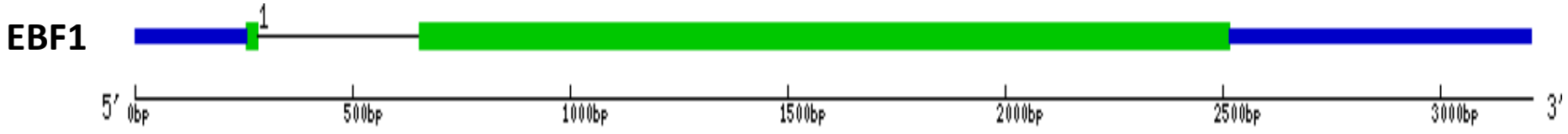
Results



- Structural information of EBF1
- Structural information of EIN3

EBF1 structural information

- 1、Sequence information of EBF1/EBF2

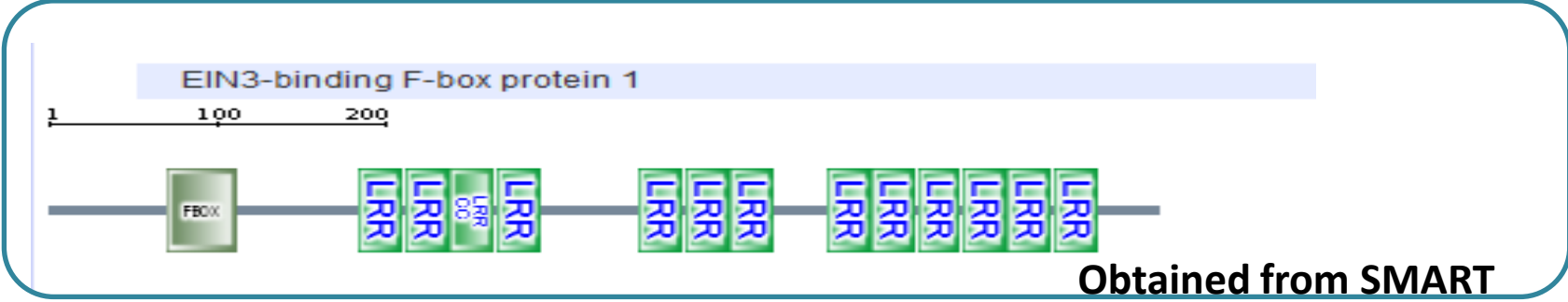
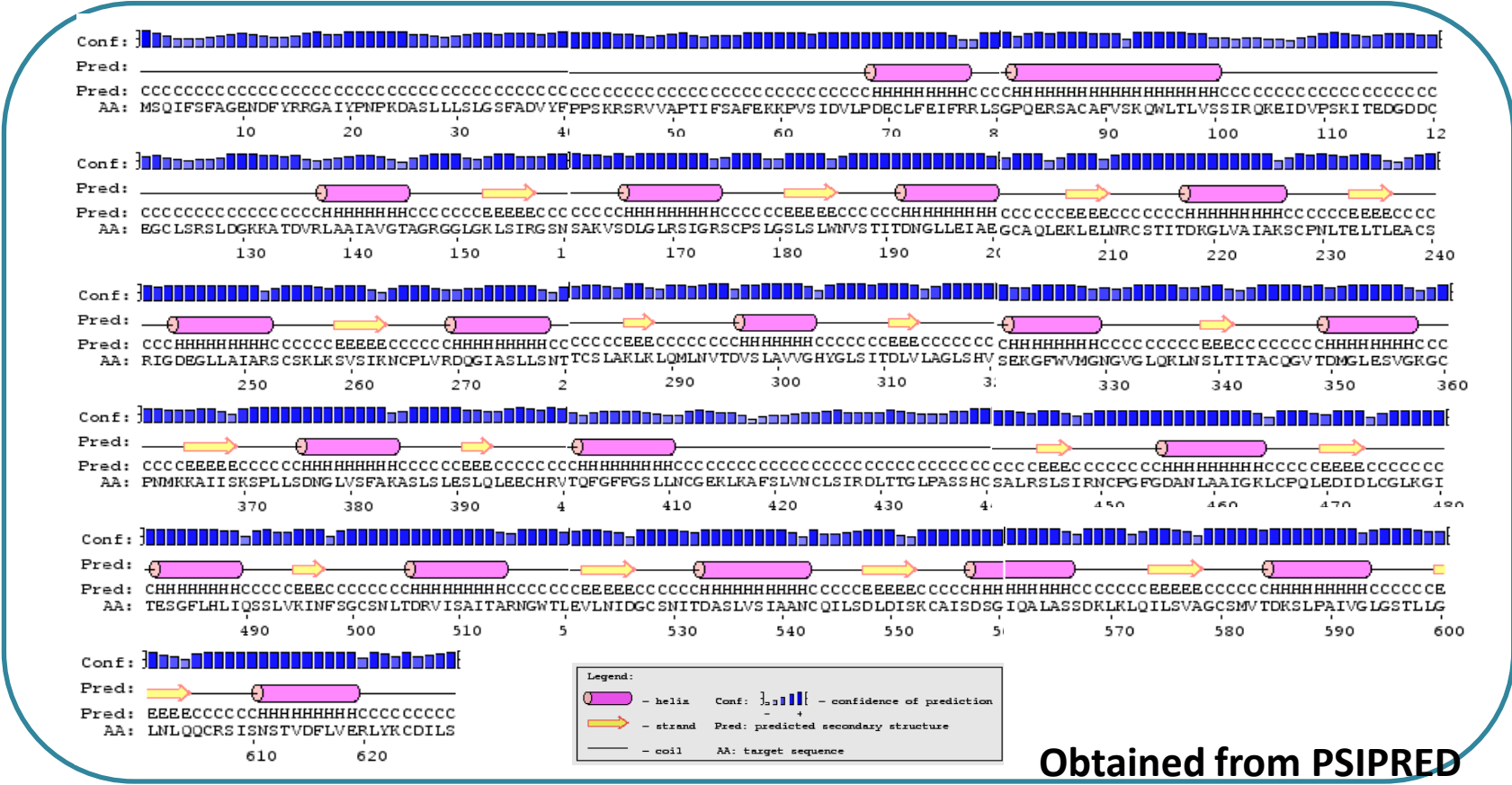


Legend:

- exon
- marked region
- intron
- UTR
- 0 1 2: intron phase

EBF1 structural information

- 2、 Prediction of the Secondary structure(EBF1)



EBF1 structural information

- 3、 Multiple sequence alignment between EBF1/EBF2 and TIR1、 COI1

```
EBF1_ARATH 1 MSQIFSGAGENDFYRRGAIYPNPKDASLLLSLGSFADVYPPSKRSRVVAPITISAFKPKVPSIDVLPDECLFEIFRRLSGQERSACAFVSKQWLELVSSIRCKEIDVPSKITEDGDDCGGLSRSLDGKMATDVRLLAA
EBF2_ARATH 1 MSGIFRSGGDEDCLLGGSMYLSP.....GSCPGVYYPARKRLRVAATSFYSGFEEKQTSIDVLPDECLFEIFRRLPSCQERSACACVSKHWNLLSSTSRSEVNESS..VQDVVEGEGFLSRSLDGKMATDRLAA
TIR1_ARATH 1 .....MOKRRLALS...F.....PPEVLEHVESFQLDKDRNSVSLVCKSWYETERWCRKVFICNCAVWSPAT....VIRRFKRVRSVLEK...
COI1_ARATH 1 .....MDEPDIKRCKLSCVAT.....VDDVLEQMTYITDEKDRDSASLVCRWFKLDSETRHEVTMALQYTAIPDR....LSRRFENLRSKLLK...

EBF1_ARATH 141 IAVGTAERGGGLGRLSIRGNSAKVSDLGLRSIGRSCPSLGSISLWNVSTLTDNGLLEIAEGCAQLEKLSLNRCSTITDKGLVAIAKSCPNLTELTLFAC.....SRIGDEGLL
EBF2_ARATH 130 IAVGTSSRGGGLGRLQIRGSGEESKVTDVGLGCAVHGCPSLRIVSLWNPAVSDLGLSEIARS..CPMTEKLDLSROPGHITDSGLVAIAENCVNLSDLTIDSC.....SGVNGEGLR
TIR1_ARATH 75 .....GKPHFADFNLVDPDGCGGVYYPW..EAMSSSYTWLEERLKRMLVVTDCLLELIAKS..FKMFKWLVLSGEGFTDGLAAIAAATCRNLKELDLRESDDVDVSGHWLSHPFDYTSLVSLNISCLA..SEVVSFALE
COI1_ARATH 82 .....GKPRAMFNLIIPENWGGYVTPW..VTELSNNLRQLKSVHFRRM..IVSDLDLRLAKARADLLELKLKDCSGETTDGLLSVWTHCRKIKKTLMESSFSKDKCWHLHQAQNTSLEVLNFMFTEFAKISPKDLE

EBF1_ARATH 249 AIARSCSKLKSVSIT.....KNOPLVRDQGIASLLSNT.....TOSLAKLKLQLNVDVSLAVVGHYGLSITDVLVLAGLSHWSEKGFWMGNGVGLQKLNLSLITACQGVTDMGLESVGGKGP
EBF2_ARATH 239 AIARRCNLRSISIT.....RSCPRHGDQGVAFLLAQA.....GSIYTKWKLQNLVSGLSIAVWGHYGAAVTDLVHLGLQGVNERGFWMGNAGLKKLRSLSVMSCRCMTDVGLEAVGNGGP
TIR1_ARATH 205 RLVTROCNLKSILKINRAVLEKLLATLQRAPOLEELCTCGYTAEW..RPDVYSGLSVALSCRELECLSGIWDVAVPAVYVCSRLITLNLISYATVQSYDLVKLIQCCPKLORLWVLDY....TEDAGLEVLAATCK
COI1_ARATH 214 TARNCRSLVSVKVGDFEILE..LVGFFKAAANLEBFCCGSLNEDIGMPEKYMNVVFRPKLORLGLSYWG...FNEMLIFPFAAQIRKLDLYALLETEDHCTLIQKCNLEVLLETNRV....IGDRGLEVLAAQYCK

EBF1_ARATH 362 NKKWAIISKSP.....LLSDNGLVSAKASLSLESLELECHRVTQFGFGSLIN...CGEKLAFSLVNCLSIRD...LITGLEA.SSHCSALRSLSIR.NCGFGDANLAAIKLCPQLEDIDLGLKIGITES
EBF2_ARATH 352 DLKHEVSNKCL.....LVSGKGLVALAKSALSLESLELECHRINQFGLMGLMN...CGSKLAFSLANCLGSDENSESSLHS..PSGSSLRSLIR.CCPGFGDASLAFI GKFKHQIQDVELCGINGVIDA
TIR1_ARATH 339 DLRELRMF..PSEPFVMEPNVALTEQGLVSVSMGQPKLESV..LYFCROMTAAALITARNRPNMTRFRLCIIEPKADYITLEPLDITCFGAVEHCKDLRRLSL...SGLLTDKVFVEYIGTYAKKMEMI..SVFAQDSDL
COI1_ARATH 343 QLKELRIEFGADPQGMDEEGLVSRGLIALAQCCQLEBYMAVY.VSDITNESLESIGTYLKNICDFRVLVLDREE..RITDLPDNGVRSLLIGCKKLRRFAYLRQCGITDGLGLSYIGQYSPNWRW..LIGYVGSDE

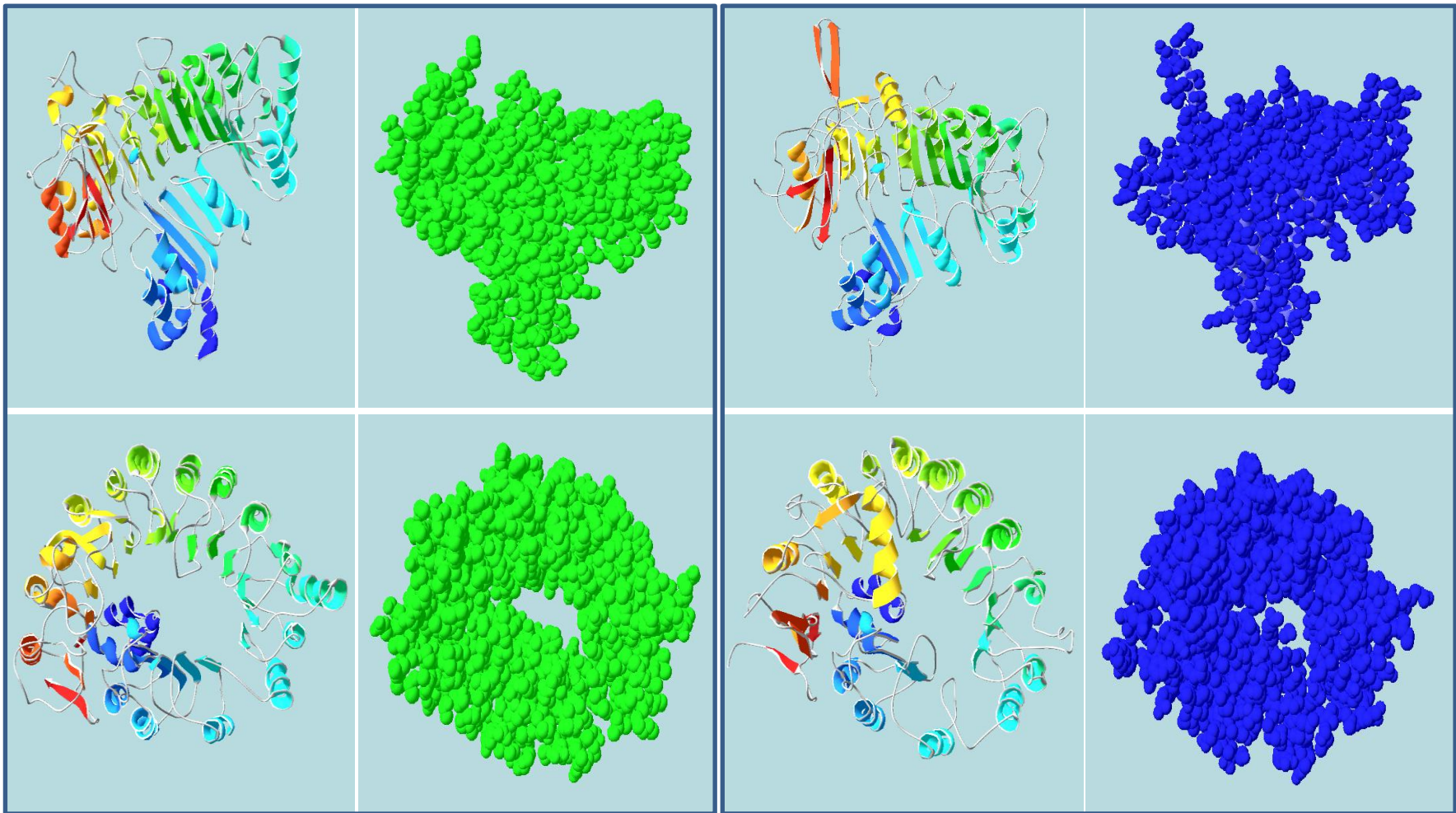
EBF1_ARATH 484 GFLHLIQSS...LVKINFGCCSNLTDRTVSAIARNGMTLEVINIDGCSNITDASLVSTANCOILSDLDISKCAISDSGIQALASS..DKLKLQILSVAGCS.....MVIDKSLPA..IVGLGSLTLLGLNIQCRSISN.
EBF2_ARATH 475 GVRLLQSNVGLVKWNLSECNVSDNTVSAISVCHRTLESINIDGCRNITNASLVAIAKNCYSVNDLISNTLVSDHGTRALASSPNHLNLQVLSIGCS.....SITDKSNAC..IQKLGRTLLGLNIQCRSIS.
TIR1_ARATH 472 GMHHVL.....SGDS.....IRKLEIRDCP.FGDKALLANASKLETMRSLMSSCSWSFGACKLLQKMPKLNVEVIDERGAP.....DSRPFSCPVERVFIYRTVACPRFDMPEGVNM
COI1_ARATH 479 GLMEFS.....RGCPN.....LCKLEWRGCC.FSERALAAAVYKLSLRYLWQGYRASMTGQDLWQMARFYWNEITLPSRRVPEVNOQGETREMEHPA..HGLAYYSLAGQRTDCP.....

EBF1_ARATH 612 ...STVDFLVRRLYKCDILS
EBF2_ARATH 607 ...STVDTLLNLRWCDILY
TIR1_ARATH 577 DQDSTVRRSRQIITNGH..
COI1_ARATH 583 ...ITVRYLKEPI.....
```

Obtained from Muscle

EBF1 structural information

- 4、 Prediction of 3-D structure(EBF1)



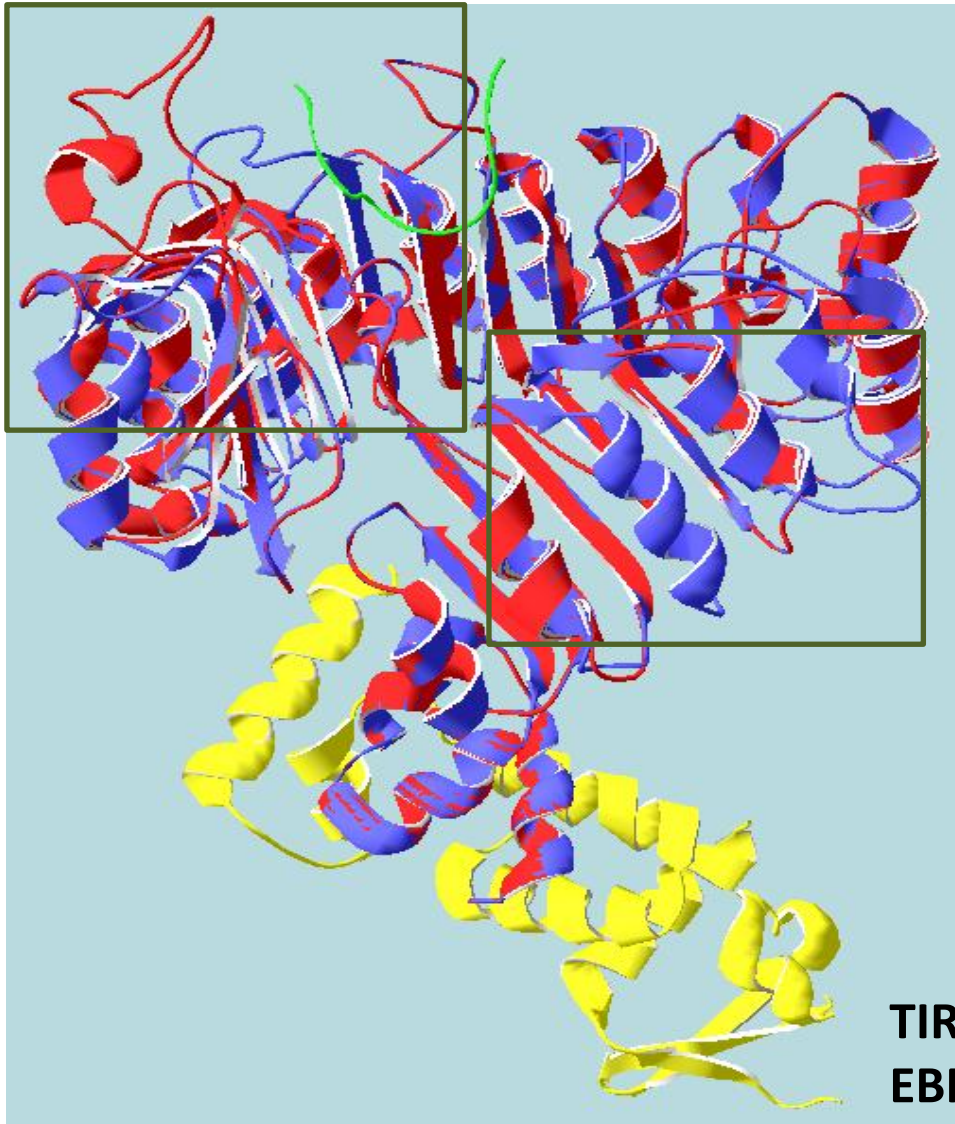
TIR1 as Template

COI1 as Template

Predicted from Swiss-Model and viewed by Swiss-PDB viewer

EBF1 structural information

- 5、 Analysis of the predicted 3-D structure

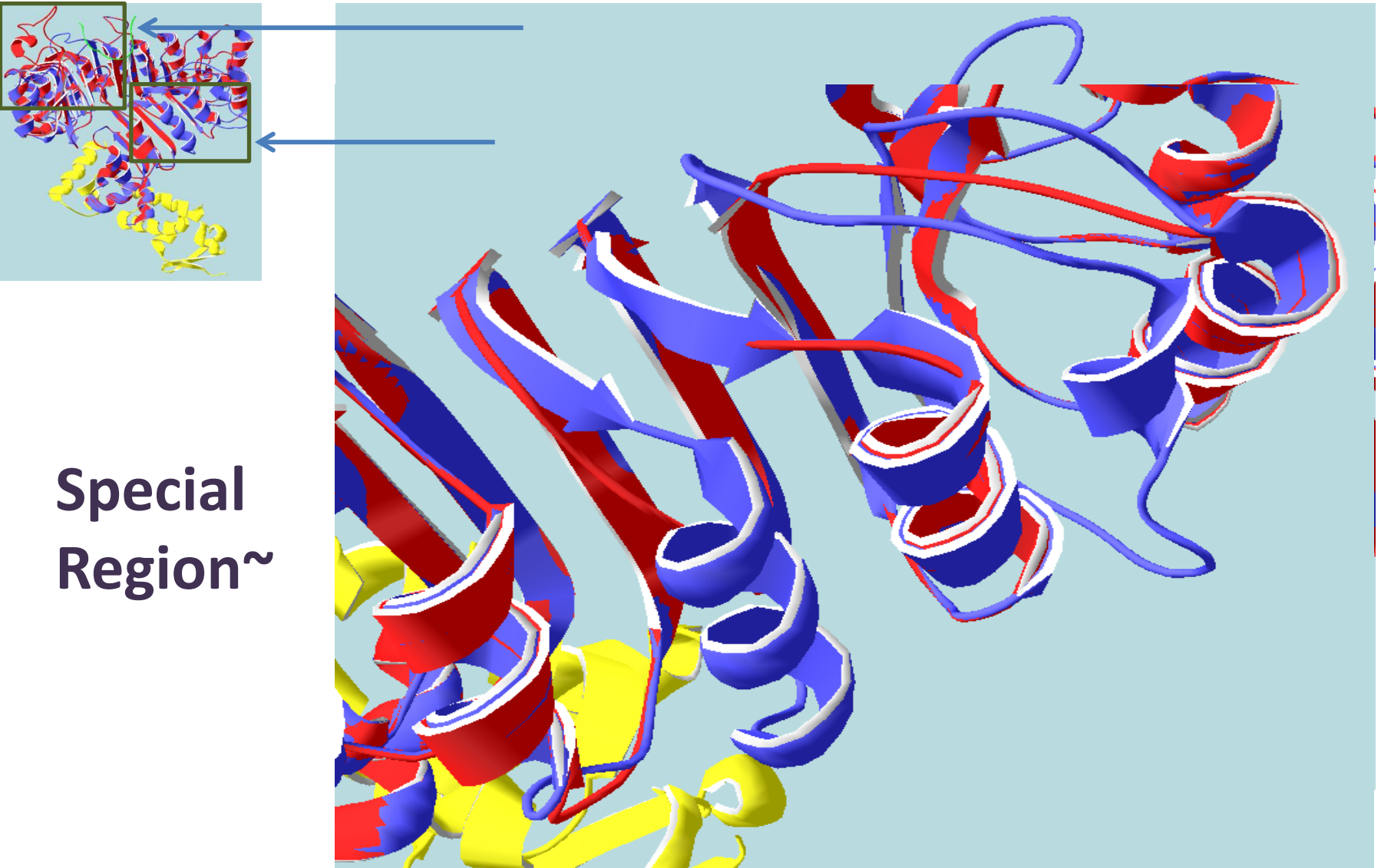


Share high similarity

TIR1 as Template;
EBF1(RED) fit with TIR1(BLUE)

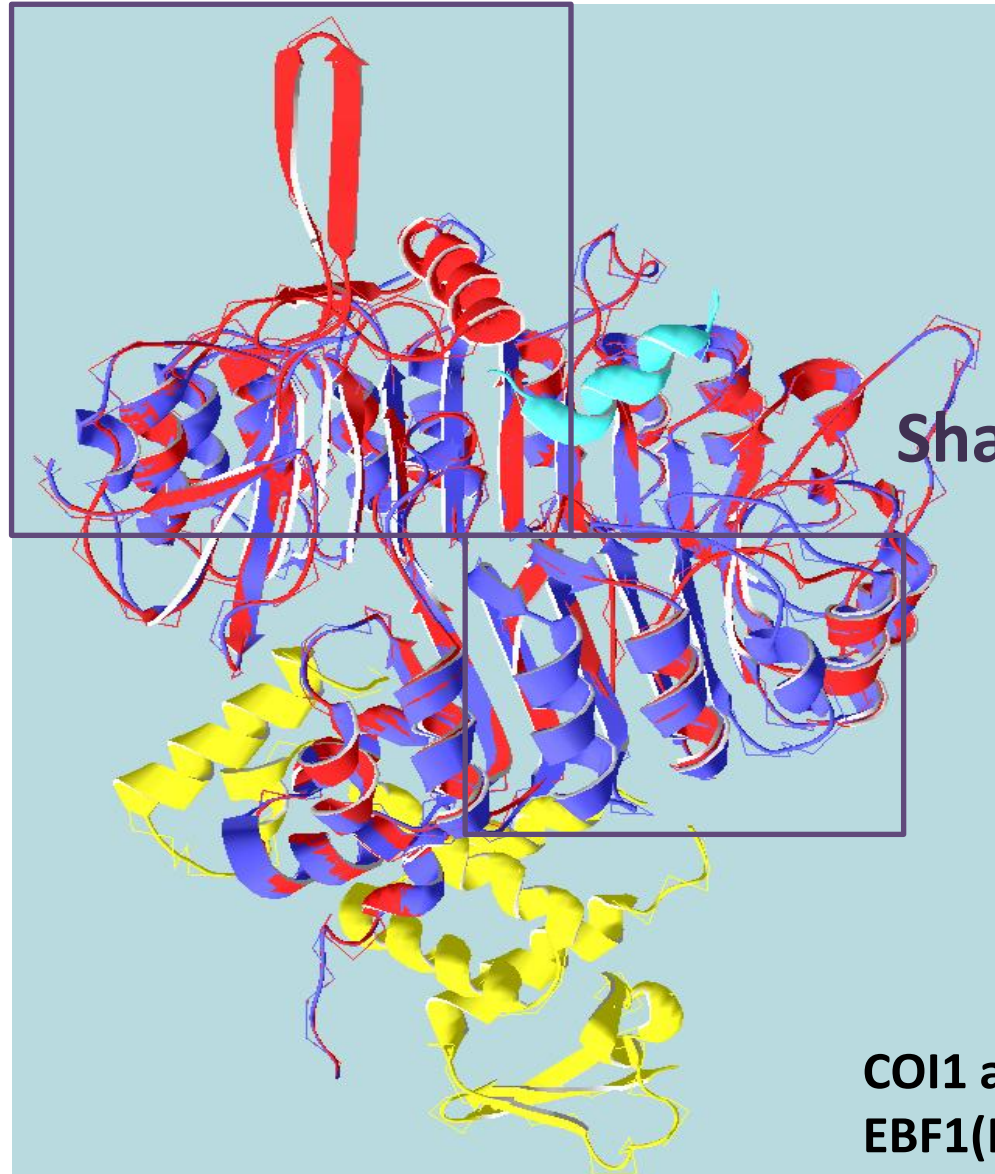
EBF1 structural information

- 5、 Analysis of the predicted 3-D structure



EBF1 structural information

- 5、 Analysis of the predicted 3-D structure

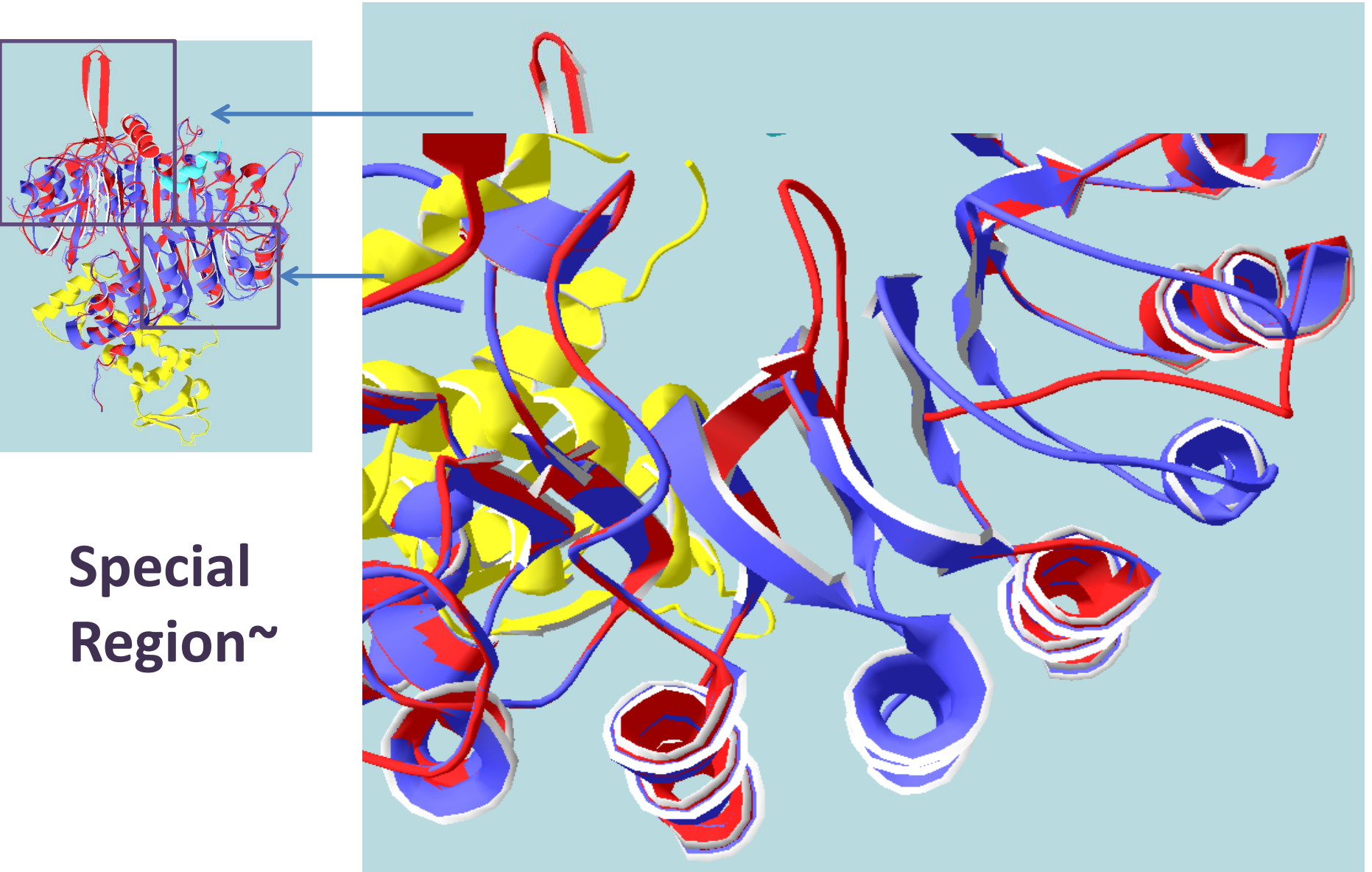


Share high similarity

COI1 as Template;
EBF1(RED) fit with COI1(BLUE)

EBF1 structural information

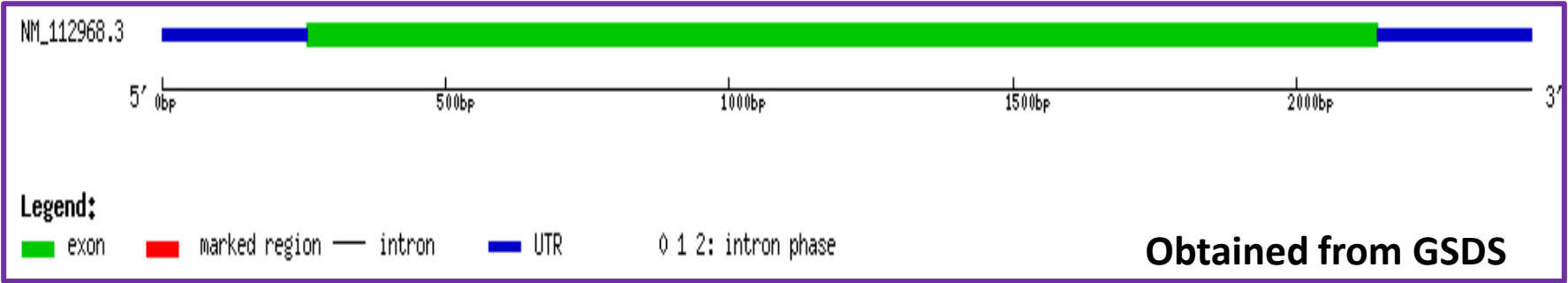
- 5、 Analysis of the predicted 3-D structure



EIN3 structural information

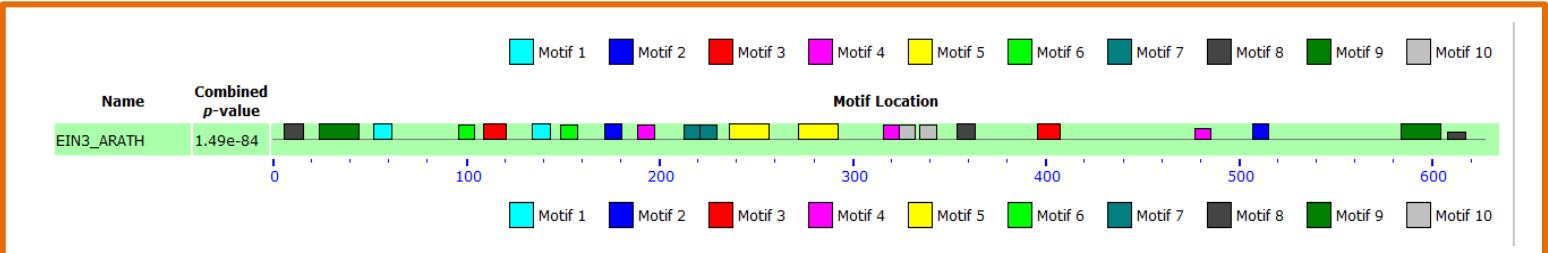
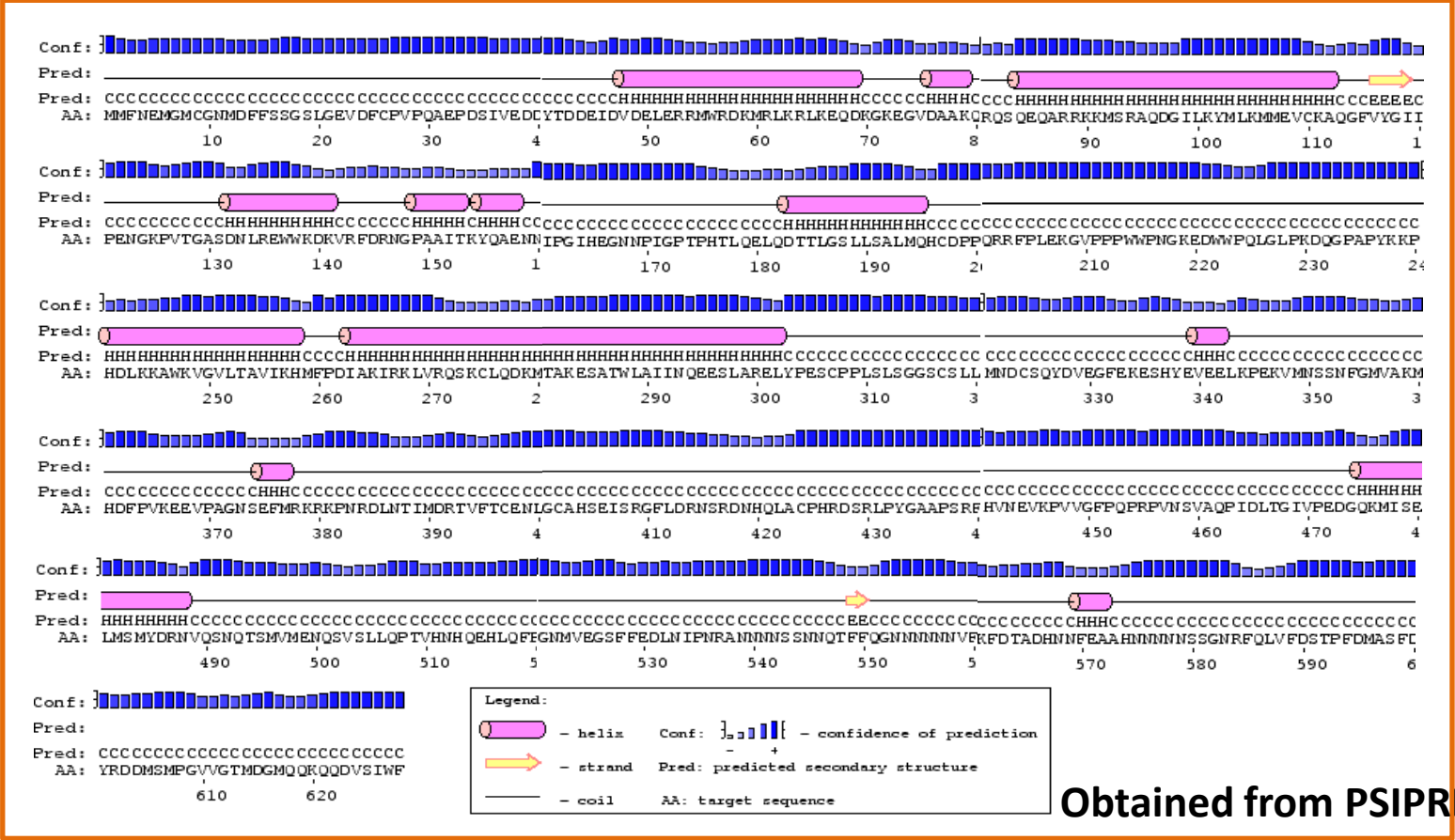
EIN3 structural information

- 1、Sequence information of EIN3



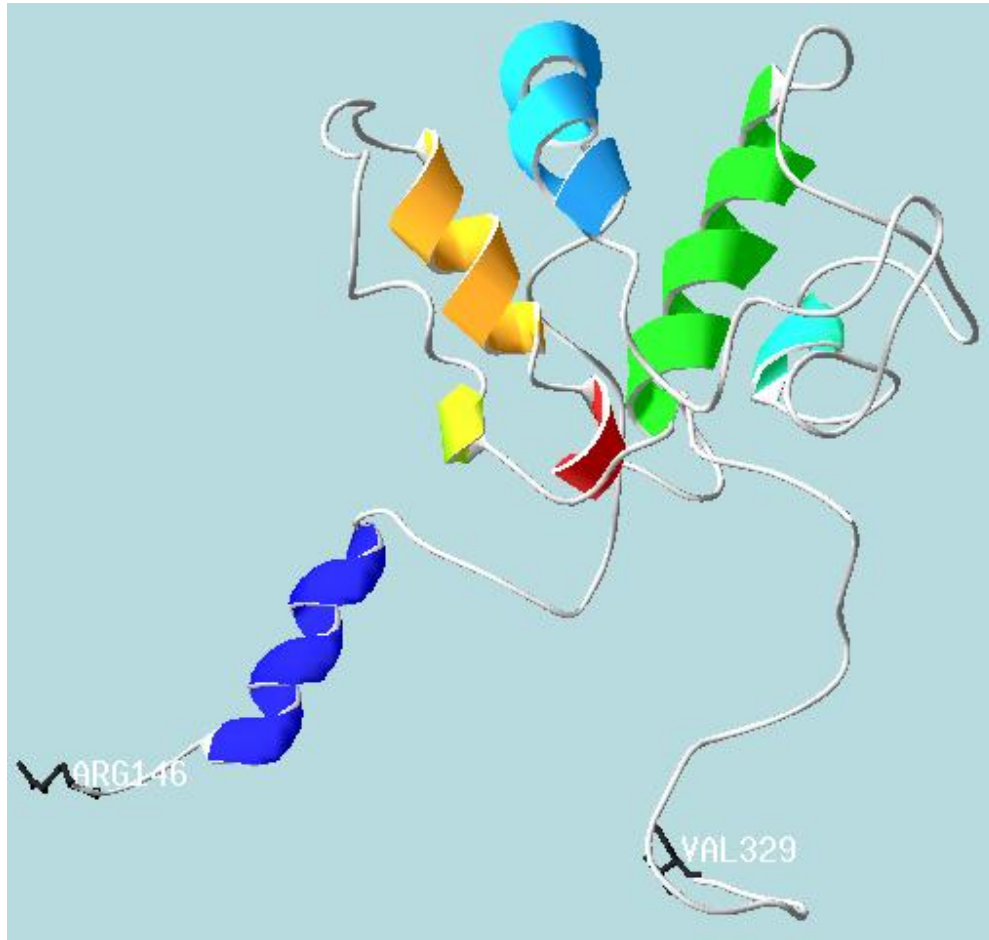
EIN3 structural information

- 2、Secondary structure of EIN3(putative motif)



EIN3 structural information

- 3、 Predicted 3-D structure(146aa-329aa)



EIL3 as template(1WIJ),
predicted by 3D-JIGSAW,
Viewed by Swiss-PDB viewer

De-novo prediction has failed~~~

i.e. conformation of full-length protein is still totally unclear~~~

Discussion



Hint from the bioinformatic analysis:

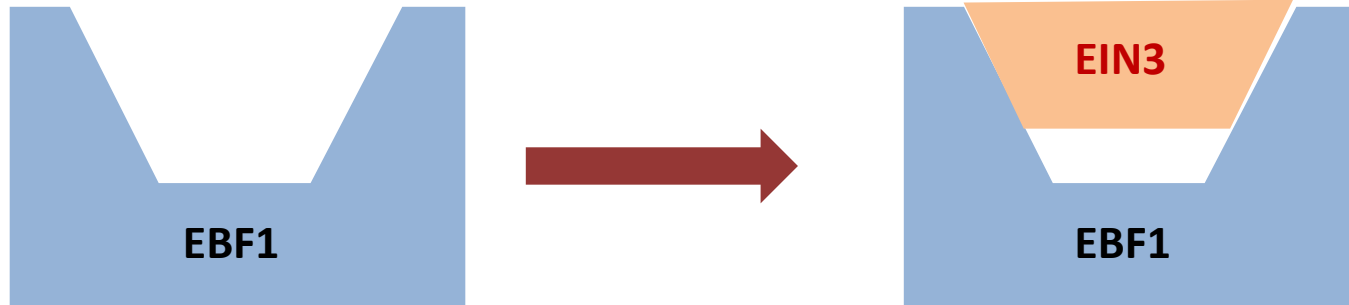
- 1、 The putative region in EBF1 that is responsible for EIN3 recognition and binding

The special loop between different LRR domain!

The unique sidechain residues that contributes to the pocket size and the hydrophobic atmosphere!

Point-mutation that can disrupt EIN3&EBF1 interaction!

- 2、 The putative interaction mechanism between EIN3 and EBF1



Questions Remain: from predict to prove!

- Accuracy of the prediction?
- How to confirm the possible model?



**More experimental evidences
are desperately needed!!!**

Acknowledgement

- Prof. Luo & Prof. Guo
- Chongshu Jane, Cuifang Zhang, Tong Wei
- Wenyang Li, Yanpeng Xi , all Guolab members

Thanks for your attention~
Merry Christmas😊

