

Bioinformatic Study on the *Arabidopsis* UV-B Photoreceptor UVR8

G02B

Reporter: Jiajun WANG

Members: Huihui Wu Jiajun WANG

Qing Liang Diqu Ren

January 10, 2014

Outline

1. Analysing the basic properties and structure of UVR8

- Background and questions
- Analysis procedure
- Experimental evidences
- Conclusions

2. Analysing how UVR8 positively regulates HY5

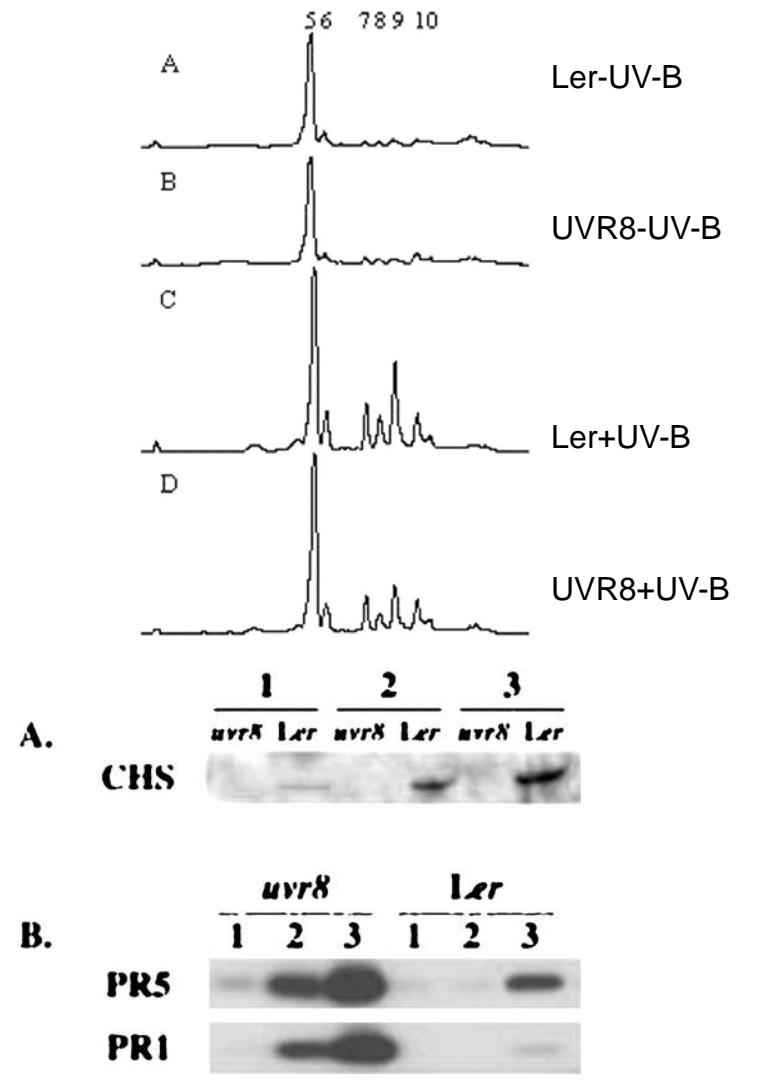
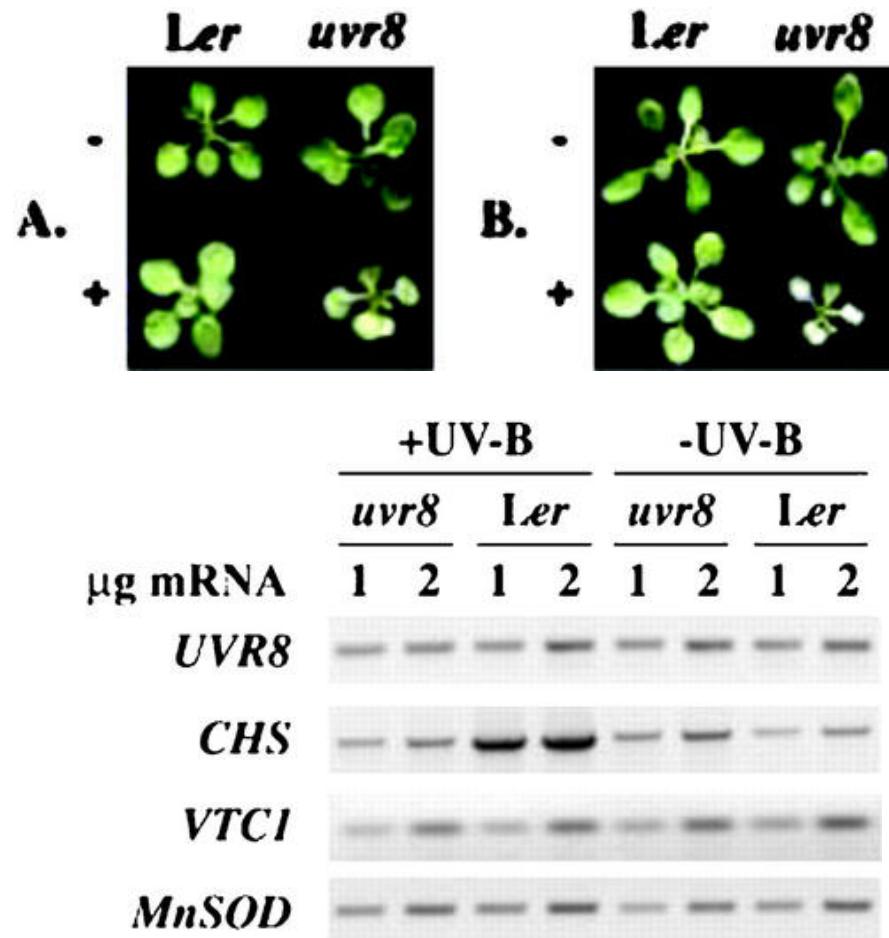
by COP1

- Background and question
- Analysis procedure
- Hypothesis

Part I : Analysing the basic properties and structure of UVR8

Background

1. Discovery of UVR8



Kliebenstein *et al.*, 2002

Background

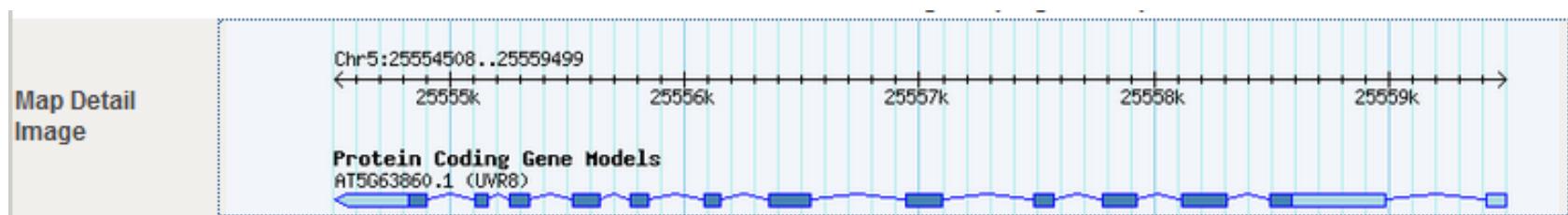
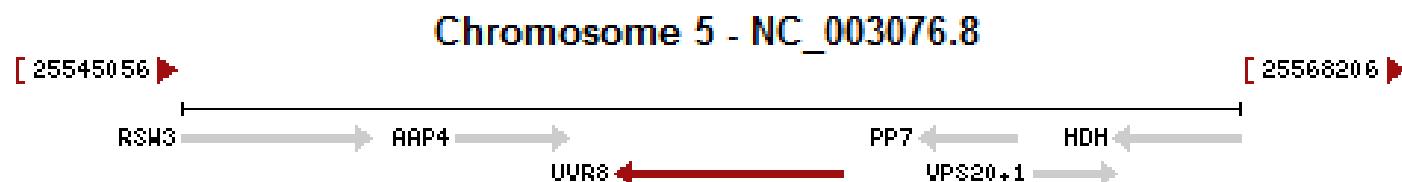
2. *UVR8* and *UVR8*

Gene ID: 836506

TAIR:AT5G63860

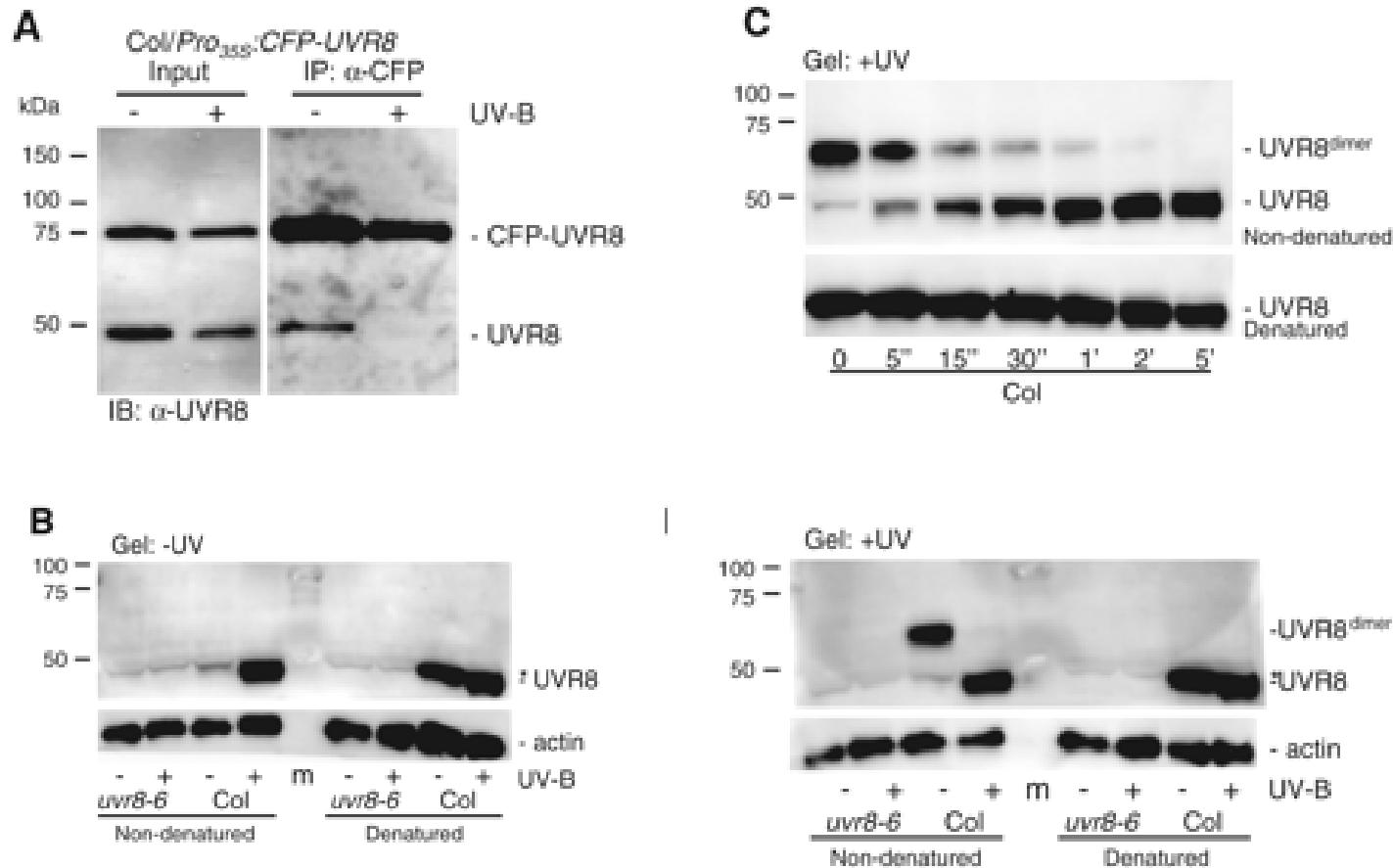
UniProt: Q9FN03

Sequence length: 440AA



Background

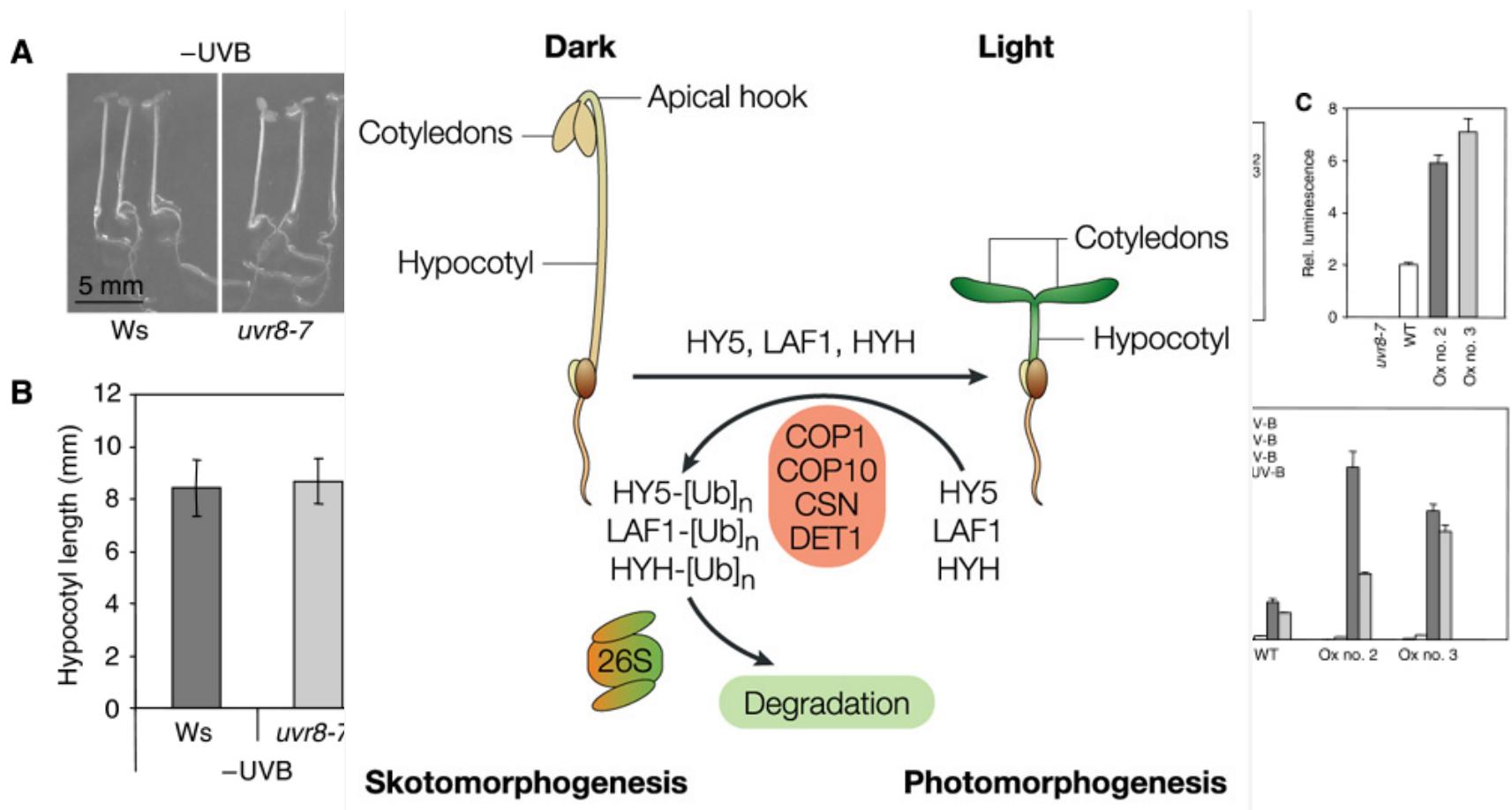
3. UVR8 responses UV-B



Rizzini et al., 2011

Background

4. UVR8 signal transduction

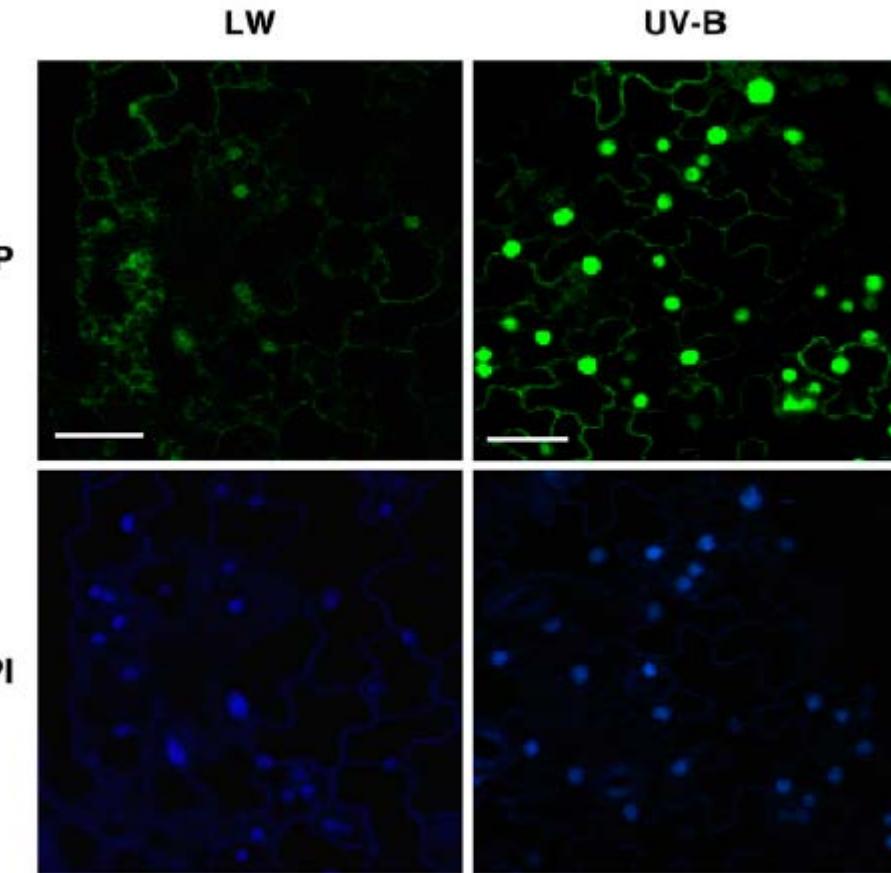


Fulleray et al., 2003

Background

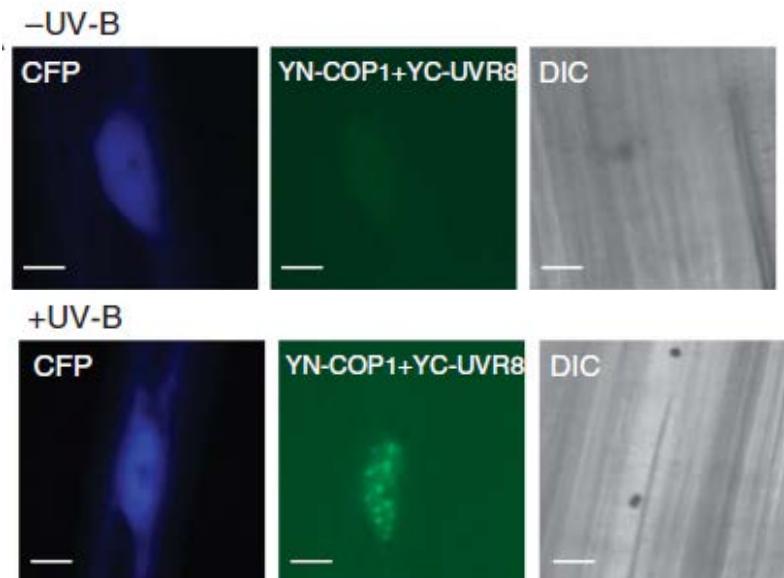
5. UVR8 subcellular localization

A



DAPI

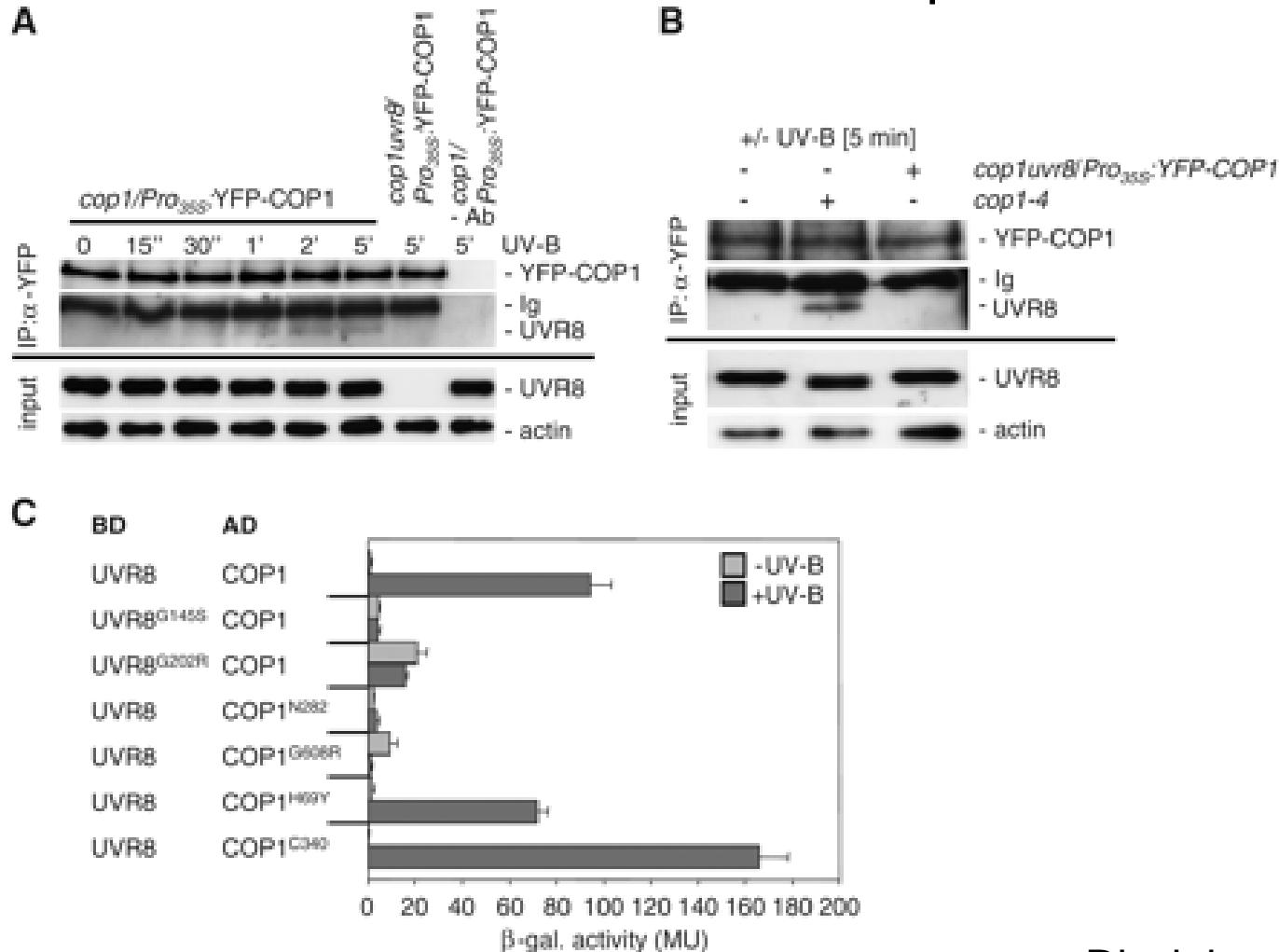
Kaiserli et al., 2007



Favery et al., 2009

Background

6. UVR8 interacts with COP1 in a UV-B-dependent manner



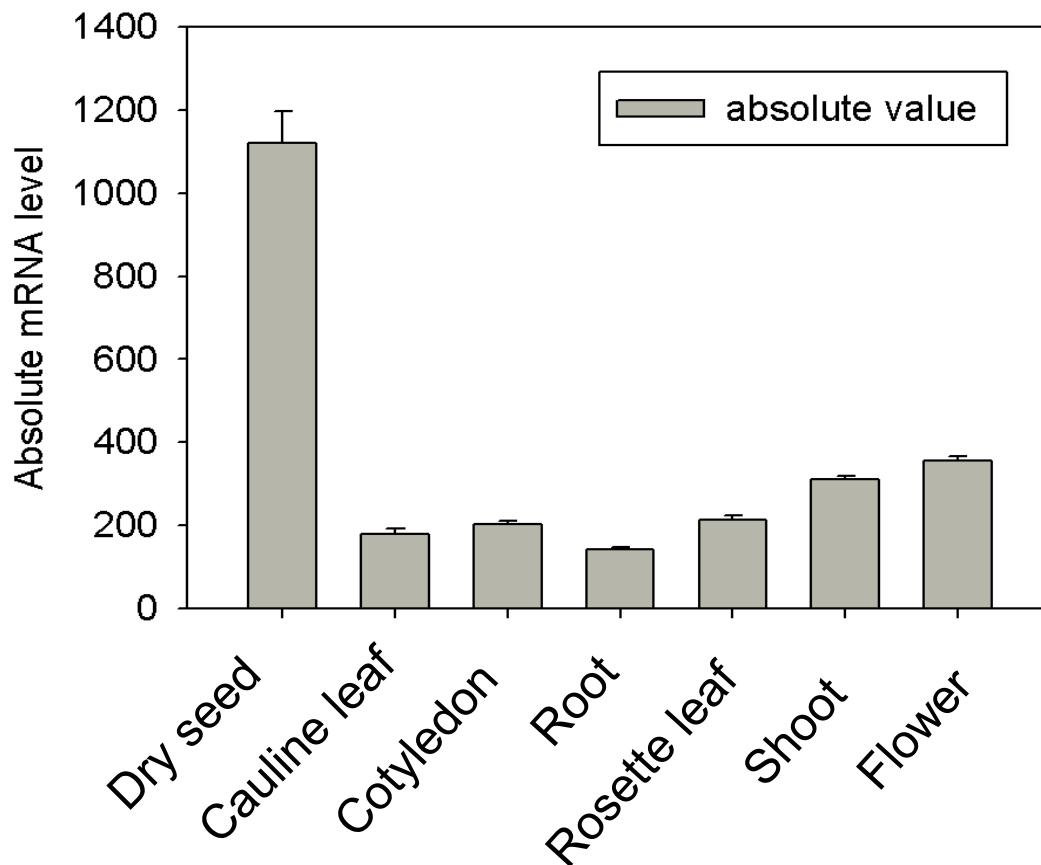
Questions

1. How does the UVR8 maintain homodimer?
2. What is the UVR8's chromophores?

Analysis procedure

e-FP Browser

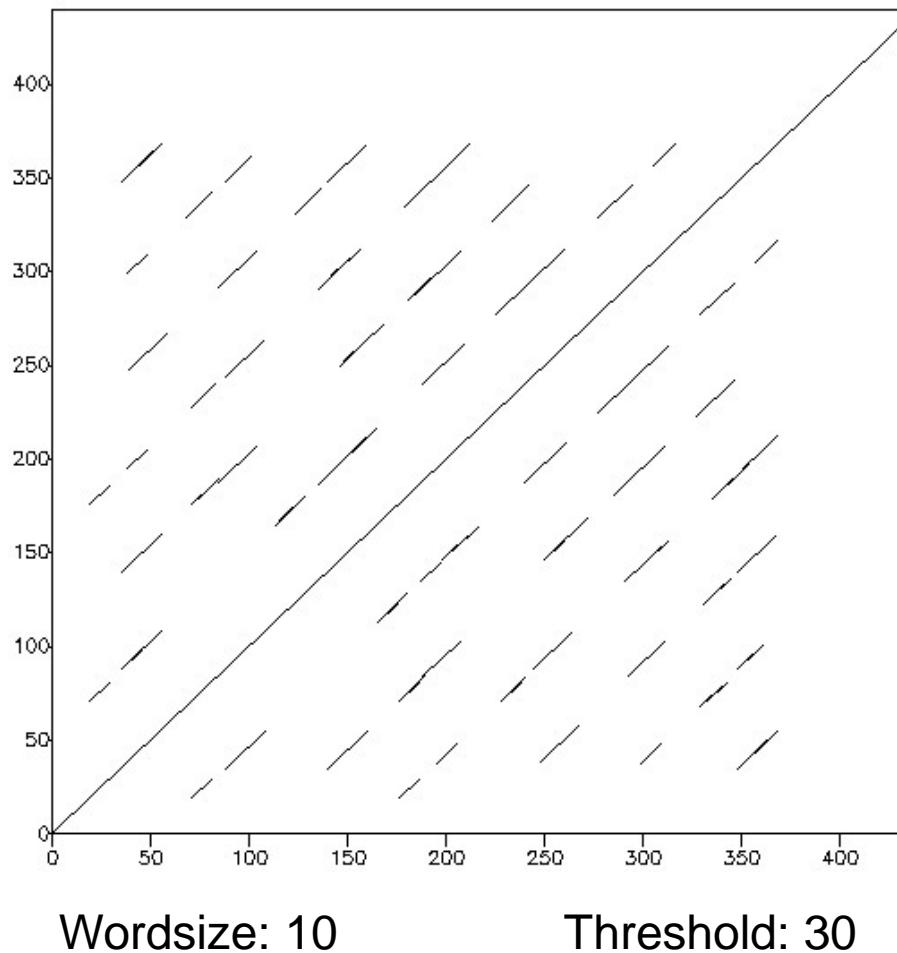
UVR8 tissue-specific expression



Analysis procedure

Dotmatcher

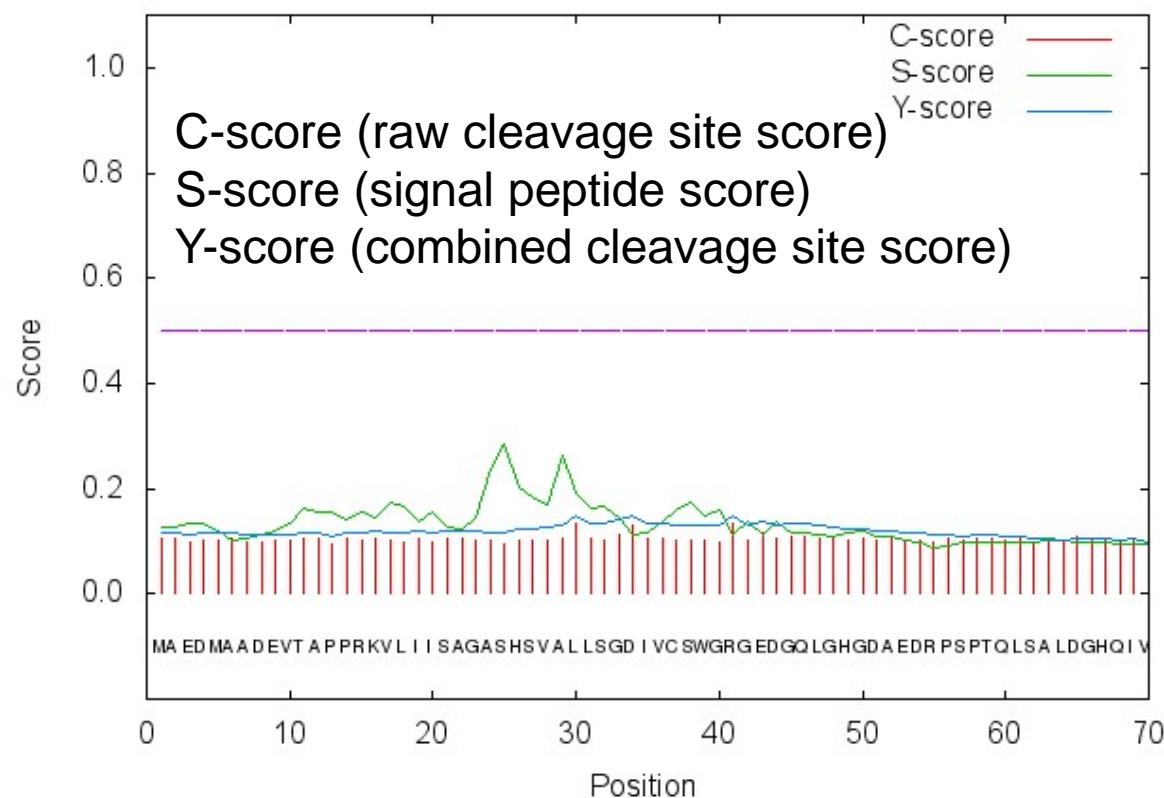
Repeated sequence of UVR8



Analysis procedure

SignalP

Signal peptide prediction



Analysis procedure

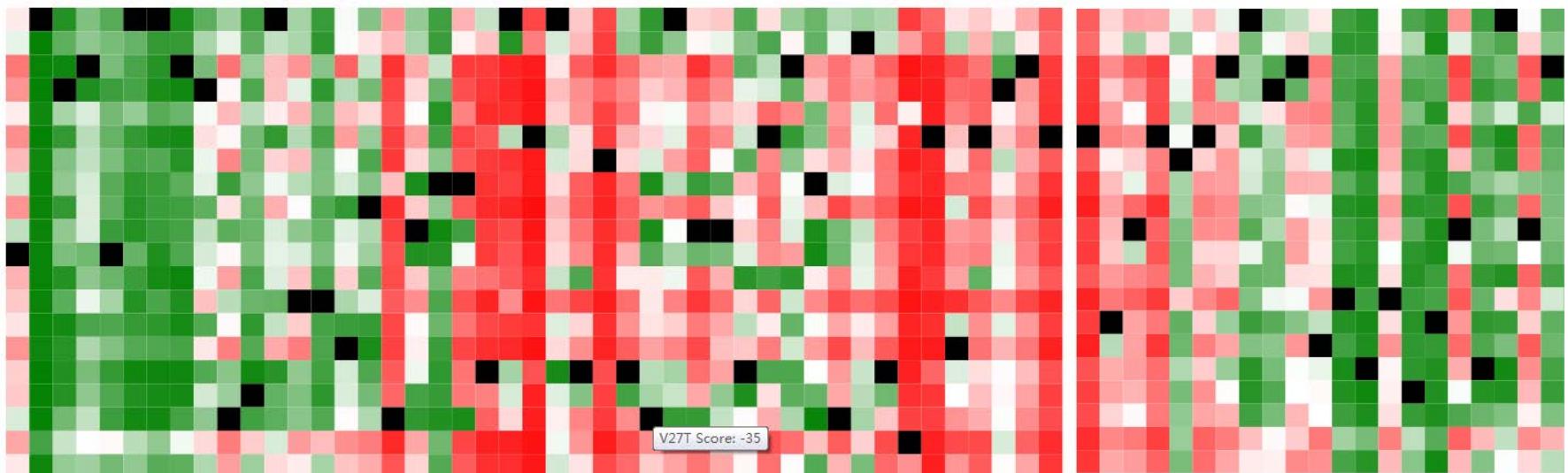
PredictNLS

Predict nuclear localization signal(NLS)

Prediction of Nuclear Localization Signals (NLS)

This protein does not contain a nuclear localization signal

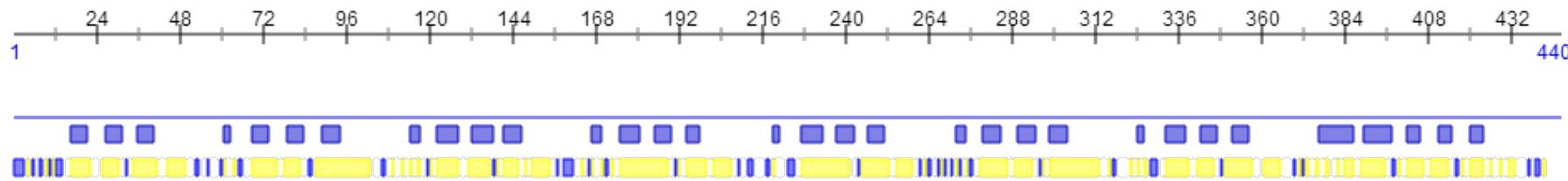
Effect of Point Mutations Prediction



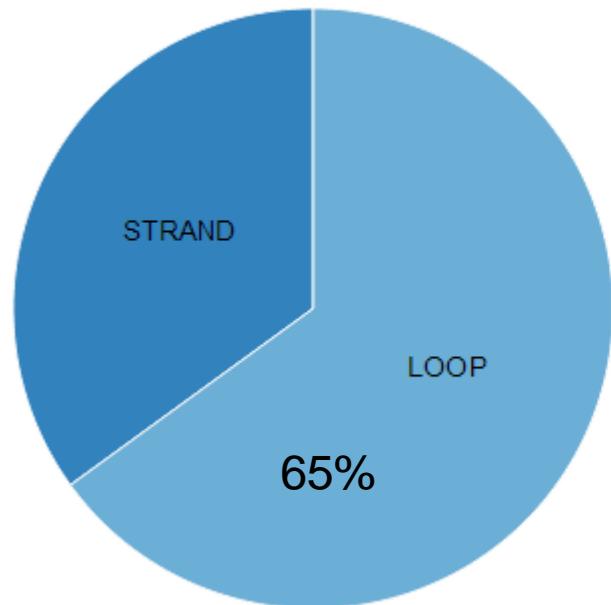
Analysis procedure

PredictNLS

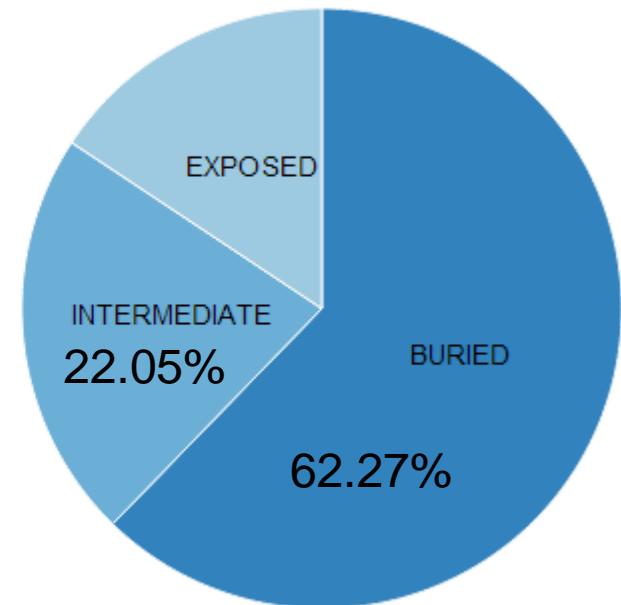
Secondary Structure and Solvent Accessibility Prediction



Secondary Structure Composition



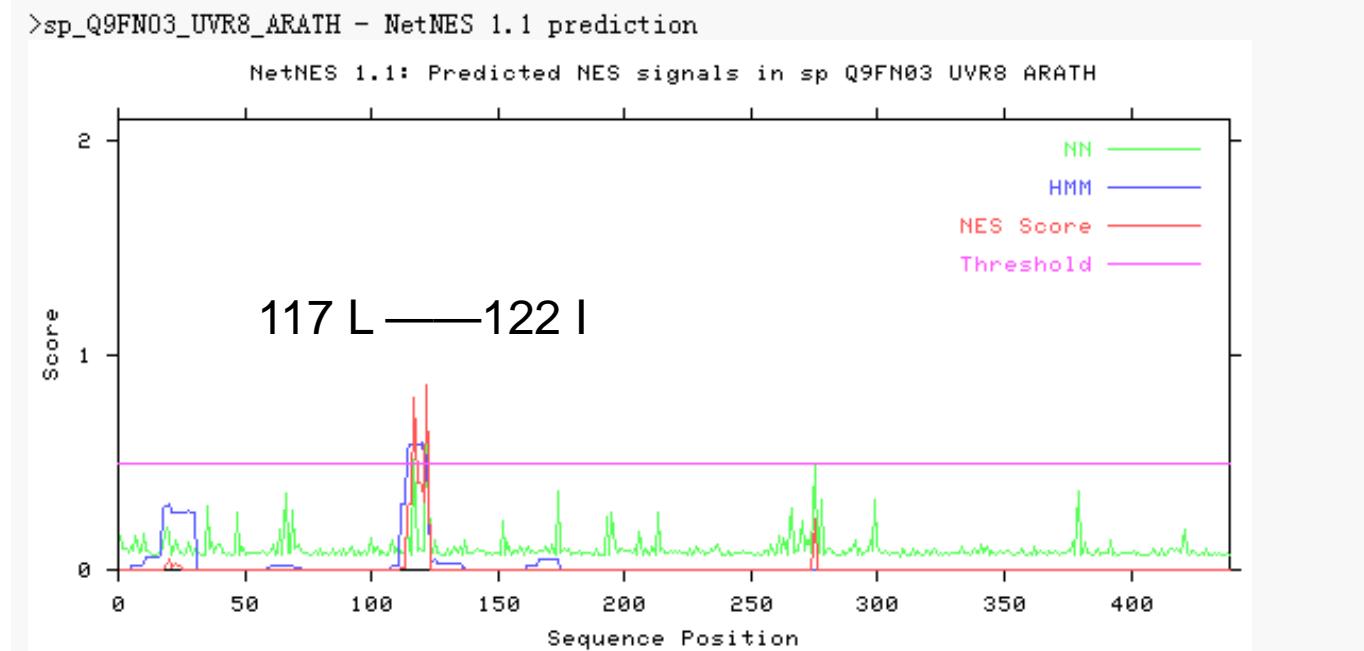
Solvent Accessibility



Analysis procedure

NETNES

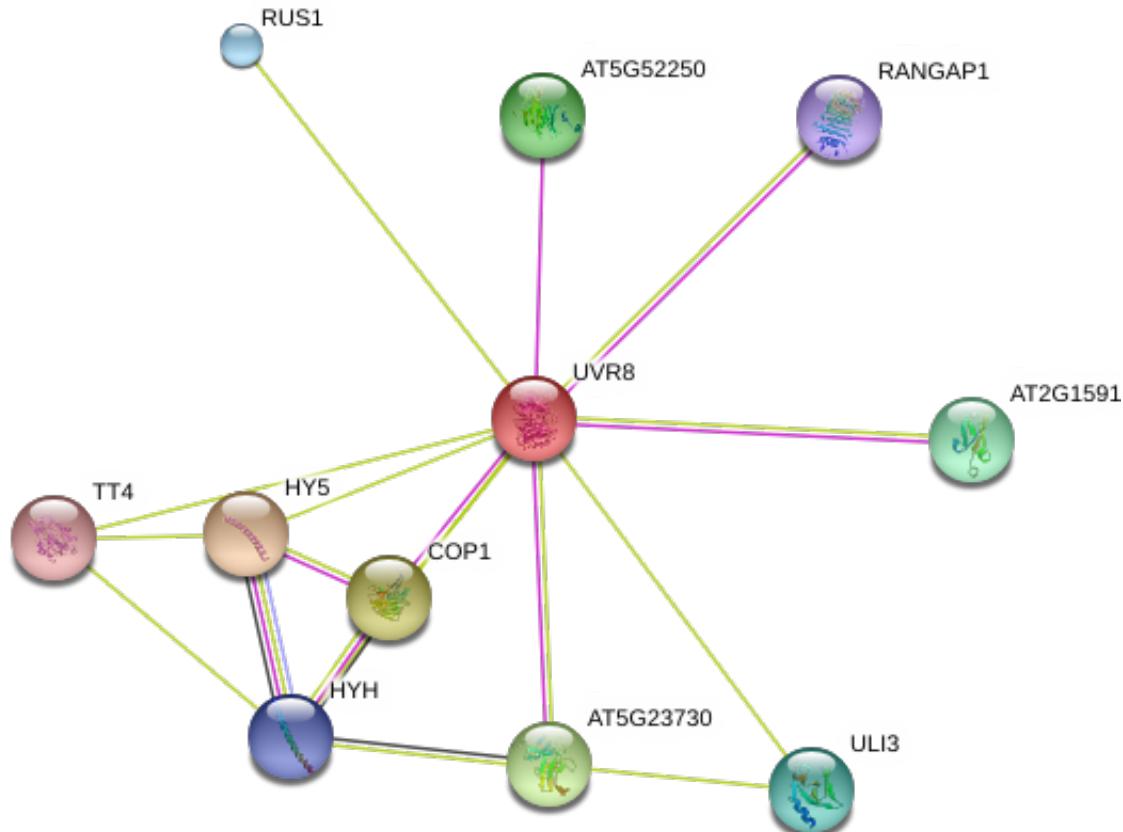
Predict nuclear export signal(NES)



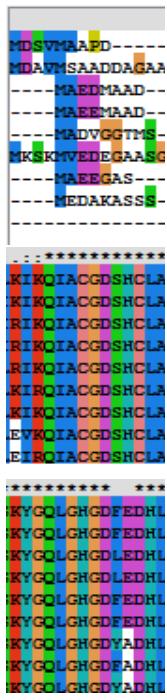
Analysis procedure

STRING 9.1

Interaction proteins



Multiple



```

1 -----M--AEDMAAD--EVTAPPKVLIIISAGASHSVALLSGDIVCSWGRGEDGQLGHGDAEDRISPTQLSALDGHQ--I 69
1 -----M--AEEMAAD--EVTAPPRTVLIISAGASHSVALLSGDIVCSWGRGEDGQLGHGDAEDRISPTQLSALDGHQ--I 69
1 -----M--ADVGGM--SEVSAPVRVLLISAGASHSVALLLGNCVCSWGRGEDGQLGHGDAEDRISPTYLSLALNGHE--V 70
1 mkskmv--EDEGAASgSEVSGPIRQVLLISAGASHSVALLSGNIVCWSWGRGEDGQLGHGDAEDRITPTQLSTLDGLD--I 76
1 -----M--AEEGA--SEVTAPVRRVLLISAGASHSVALLSGNVVCTWGRGEDGQLGHGDAEDRISPTILSTLEGLE--I 68
1 mds-vMaaPDS-----PPQTVVVLVSAGASHSVALLTGNVLCWSWGRGEDGQLGHGDAEDRIVPTVLSGFDA--pai 67
1 mda-vMsaADDAGAA-SGREDPPPPVAVLVSAGASHSVALLAGNVLCSWSWGRGEDGQLGHGDAEDRIVPTVLSGFDAApqi 78
1 -----M--EDAKASSsRGVR--RQVIALSAGASHSVALLSGDLVCSWSWGRGEDGQLGHGDAEDRIFPTIVSALDDCE--I 68
1 -----M--AGE-----RKVLLVSAGASHTVALLSGQVVCsWSWGRGEDGQLGHGDAEDRILPRIIGALNDCE--I 59

```

```

70SVSTCGADHTVAYSQSGMEVYSWGWGDFGRLGHGNSSDLFTPLPIKALHGIRIKQIAGDSHCLAVTMEGVQSWGRNQN 149
70SVSTCGADHTVAYSQSGMEVYSWGWGDFGRLGHGNSSDLFTPLPIKALHGIRIKQIAGDSHCLAVTMEGVQSWGRNQN 149
71SVSTCGADHTTAYSESFTQVYWGWGDFGRLGHGNSSDLFTPQPKIKALHGLRIKQIAGDSHCLAVTMDGEVQSWGRNQN 150
77ISITCGADHTTSYESRMEVYSWGWGDFGRLGHGNSSDLFTPQPKIKALHSLIKIRQIAGDSHCLAVTMDGEVQSWGRNQN 156
69ISVICGADHTTAYSESHTDVYWGWGDFGRLGHGNSSDLFTPQPPIRQLHGLKIKQIAGDSHCLAVTMEGVQSWGRNQN 148
68TSVICGADHTTAYSEDELQVYWGWGDFGRLGHGNSSDVFTPQPVKALQGLKIKQIAGDSHCLAVTMAGEVQSWGRNQN 147
79TSVICGADHTTAYSEDEQQVYWGWGDFGRLGHGNSSDVFTPQPVKALQGKIKIKQIAGDSHCLAVTMAGEVQSWGRNQN 158
69SSVSTCGADHTTALSNSTKTVYWGWGDFGRLGHGNSSDLFIPQPKIKALQGLEVKQIAGDSHCLAITTDGEVLGWWGRNQN 148
60SLVACGADHTTAYSATRLTIYWGWGDFGRLGHGNSSDLFVPHPKALQGLEIRQIAGDSHCLAVTADGEVYWGRNQN 139

```

```

150GQLGLGDTEDSLVPQKIQAFEGIRIKMVAAGAEHTAAVTEDGDLYGWGWGGRYGNLGLGDRDRNDLVPERVTSTGGEKMSMV 229
150GQLGLGDTEDSLVPQKIQAFEGIRIKMVAAGAEHTAAVTEDGDLYGWGWGGRYGNLGLGDRDRNDLVPERVTSAGGEKMSMV 229
151GQLGLGTTEDSLVPQKIQAFQGVSKVMVAAGAEHTAAVTEDGELYWGWGGRYGNLGLGDRDRNDLVPEKVSLVNGDKMMV 230
157GQLGLGTTEDSLVPQKIQAFQGVSKVMVAAGAEHTAAVTEDGELYWGWGGRYGNLGLGDRDRNDLVPEKVSLVNGDKMMV 236
149GQLGLGTTEDSLVPQKIQAFQGVPIKMVAAAGAEHTAAVTEDGELYWGWGGRYGNLGLGDRDRNDLVPEKVSLPHGEKMMMV 228
148GQLGLGTTEDSLLPQKIQAFQEVCVCKMIAAGAEHTAAVTEDGELYWGWGGRYGNLGLGDRDRNDLVPEKVSVEGEKMMVL 227
159GQLGLGTTEDSLLPQKIQFSFEGVCKVMIAAGAEHTAAVTEDGELYWGWGGRYGNLGLGDRDRNDLVPEKVSVEGEKMMVL 238
149GQLGLGTTEDVLTPHKLTAFEGVAVKMLAAGAEHTTAVTENGKLYWGWGGRYGNLGLGDRDRNDLVPDQVAAVAGELMMKV 228
140GQLGLGTTDDSLVPHKLLAFEGVSVKMLAAGAEHTAAVTDSGKLYWGWGGRYGNLGLGDRDRNDLPAEVPAVGDEKMMV 219

```

```

230ACQWRTITISVSYSGALTYYWSKYQQLGHGDLEDHLIPHKLEALSNSNSFISQISGGWRHTMALTSDGKLYWGWGWNKFQGVG 309
230ACQWRTITISVSYSGALTYYWSKYQQLGHGDLEDHLIPHKLEALSNSNSFISQISGGWRHTMALTSDGKLYWGWGWNKFQGVG 309
231ACQWRTITISVSSSGGLTYYWSKYQQLGHGDLEDHLIPHKLEALQENLISEISGGWRHTMALTSDGKLYWGWGWNKFQGVG 310
237ACQWRTITISVSSSGGLTYYWSKYQQLGHGDLEDHLIPHKLEALQENLISEISGGWRHTMALTSDGKLYWGWGWNKFQGVG 316
229ACQWRTITISVSSSGGLTYYWSKYQQLGHGDDYADHLIPHKLEALQGSFISQISGGWRHTMATSDGKLYWGWGWNKFQGVG 308
228ACQWRTITITVSDSGNLLTYYWSKYQQLGHGDDYADHLIPHKVEALKDSTISQISGGWRHTMALTSEGKLYWGWGWNKFQGVG 307
239ACQWRTITICVVAESGNMTYYWSKYQQLGHGDDYADHLIPHKVEALKDSSSKQIISGGWRHTMALTSEGKLYWGWGWNKFQGVG 318
229ACQWRTITICVVAESGNMTYYWSKYQQLGHGDDYADHLIPHKVEALKDSSSKQIISGGWRHTMALTSEGKLYWGWGWNKFQGVG 308
220ACQWRTITIAVSQSGKLYYWSKYQQLGHGDYADHLLPHQVAALAEFNITQVSGGWRHTVALTEAGKLYWGWGWNKFQGVG 299

```

```

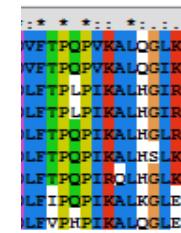
310VGNNLDQCSPVQVRFP--DDQ--KVVQVSCGWRHTLAVTERNNVFANGRTGNGQLGIGESVDRNFPKIIIEALSDGASQHQ 386
310VGNNLDQCSPVQVRFP--DDQ--KVVQVSCGWRHTLAVTERNNVFANGRTGNGQLGIGESVDRNFPKIIIEALSDGASQHQ 386
311VGDNVDHCSPVQVRFP--HEQ--KVVHISCGWRHTLAVTERQNFVSNGRTGNGQLGIGESIDRNPKMIEVLSADGSSGQQ 387
317VGDNIDHCSPVQVRFP--HEQahSLVQISCGWRHTLAITERQNFVSNGRTGNGQLGIGESMDRNPKMIEVLSADGSSGHHQ 395
309VGDNVDRSSPQVQLKP--LEQ--KVILSCGWRHTLAVTERQNFVSNGRTGNGQLGIGESMDRNPKMIEVLSADGSSGHHQ 385
308VGNNDDHSSPGQVSFP--EDQ--KISQVACGWRHTLALSEKKNFVSNGRTGTSQQLGIGESIVDRNTPVLIALSDGSACKK 384
319VGDDDDHCPVQVKFP--EDQ--KVAQVACGWRHTLATEKKNFVSNGRTGTSQQLGIGESIVDRNTPVLIALSDGSACKK 395
309CGNTEDQSSPQLVKG1QDQ--VVTVVQCGWRHTLAMTDRGNVYSNGRTGTSQQLGIGESIVDRNTPVLIALSDGSACKK 385
300SGDVDDHTSPQLISIPgQHQ--RVVQVSCGWRHTVAVTDKNNVYSNGRTGTSQQLGIGESIVDRNTPVLIALSDGSACKK 377

```

```

387IESSNIDPSSG--KSWSPAERYAVVPDETGLTDG--S-----SKGN-----GGDISPVQTDVKRV 438
387IESSNIDPSSG--KSWSPAERYAVVPDETGLTDG--S-----NKGN-----GGDISPVQTDVKRV 438
388IETSKVDPSTG--RWSPSERYAVVPDETGSTQTgVs-----VKGN-----GNDASPVEDVKRI 441
396IEASTVDPSLG--KSWSPSDYAVVPDESAQTDG--aVS-----VGGN-----GNDASPVEDVKRI 446
386IESSMVDSSSG--KTWVLPSERYAVVPDESAQTDG-----SGK-----GSDVSPEGVDVKRI 435
385LESSTAAFAAA--KVWVSPSERYAVVPDENVRKAGgGT-----ARGN-----GADANPVEDVKRM 438
396LEPTAVPFA--KVWVSPSERYAVVPDEKVPNSGeGT-----ARGN-----GADANPVEDVKRM 449
386IESSGSSNCT-sLSNWISPSERYAVVPDE-----TLgplarrlhqqtlnFEGEeleVptaDddsSGDANPVDTDTKRM 457
378LEKSPEPAVTGsgvANWISPSERYAVVPDE-----AV-----LVGQ-----MGDASPVDTDIKRI 427

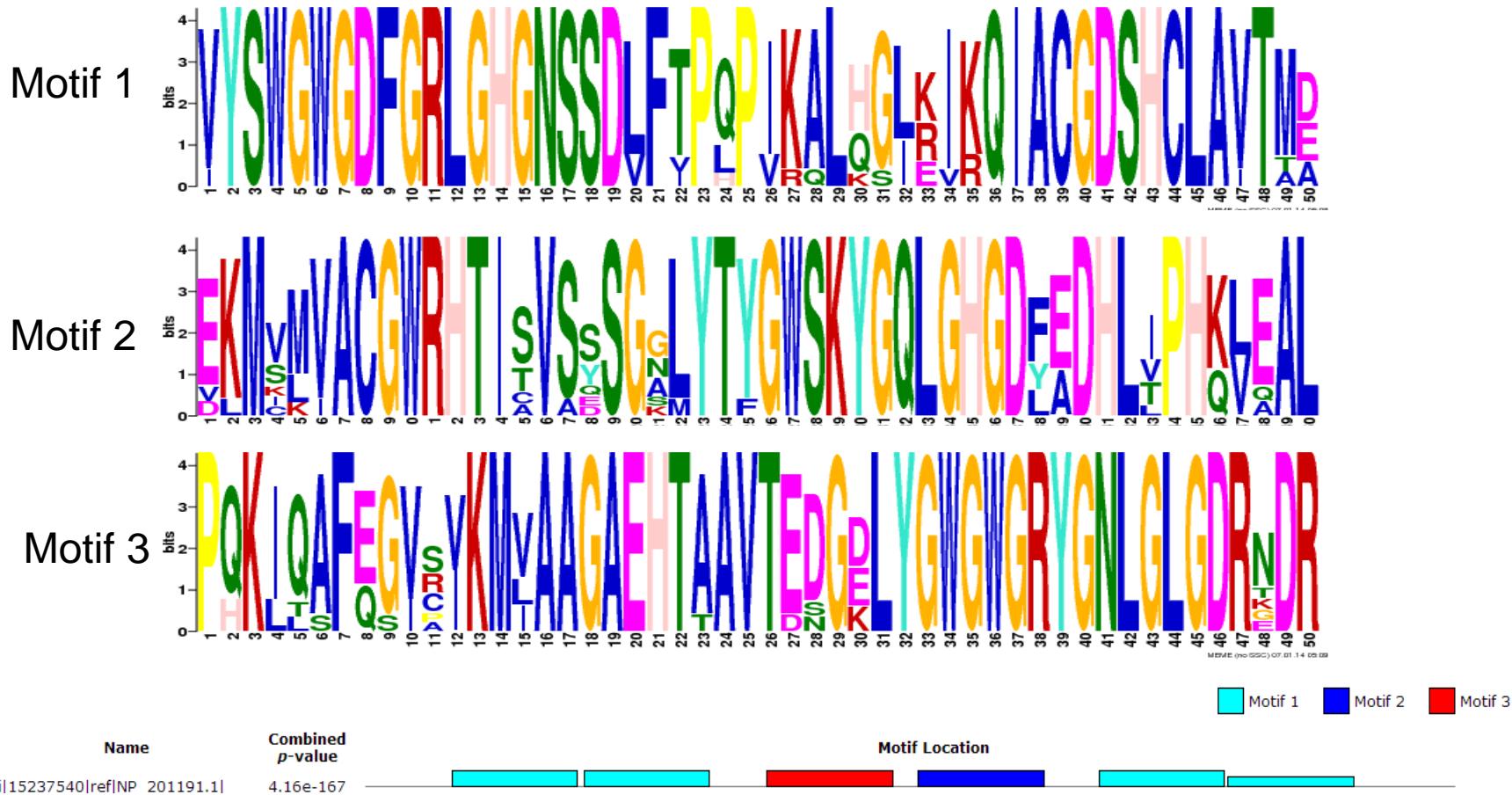
```



Analysis procedure

MEME 4.9.1

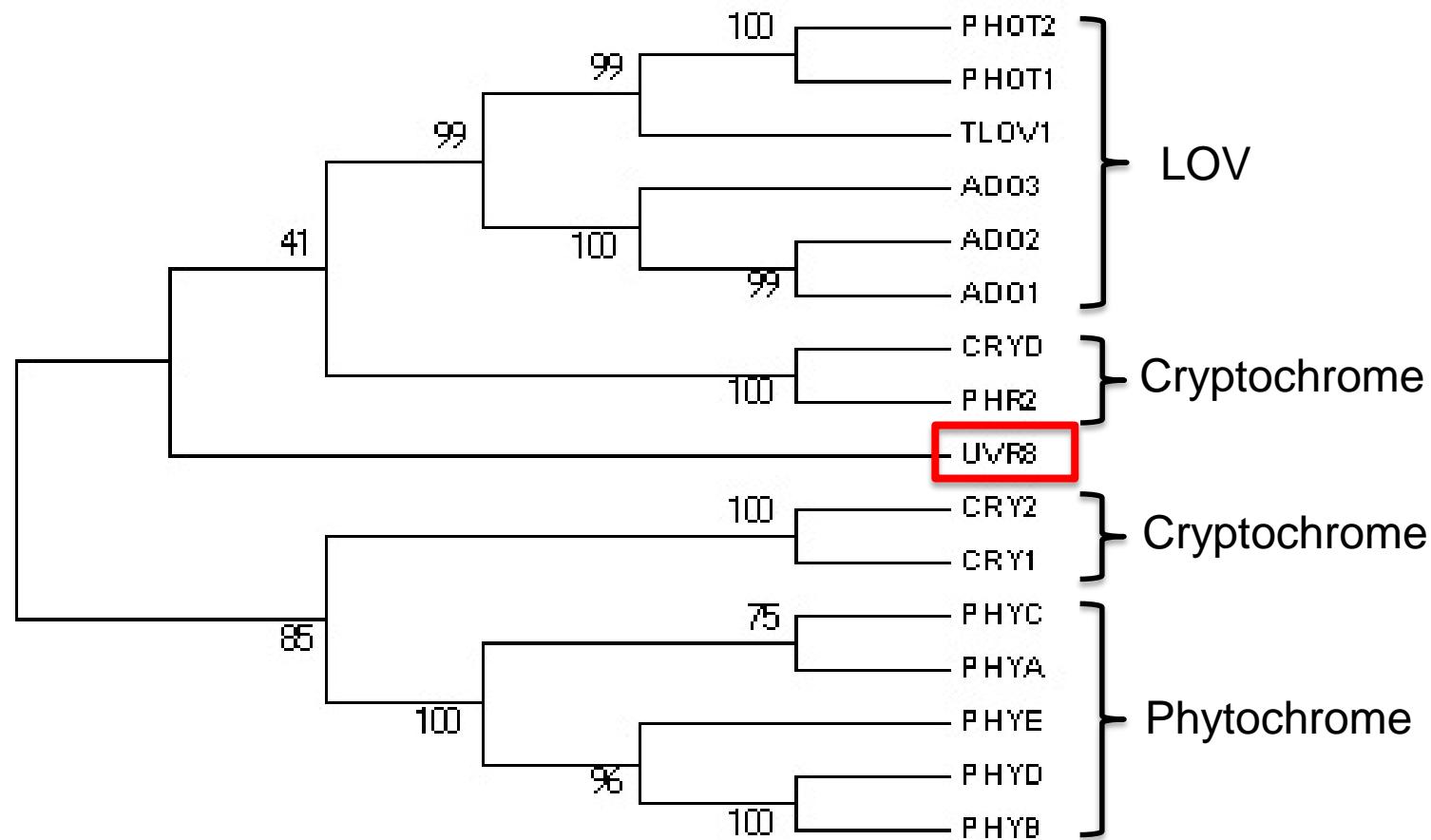
Motif finding



Analysis procedure

Neighbor-Joining Phylogenetic Tree (MEGA 5.2)

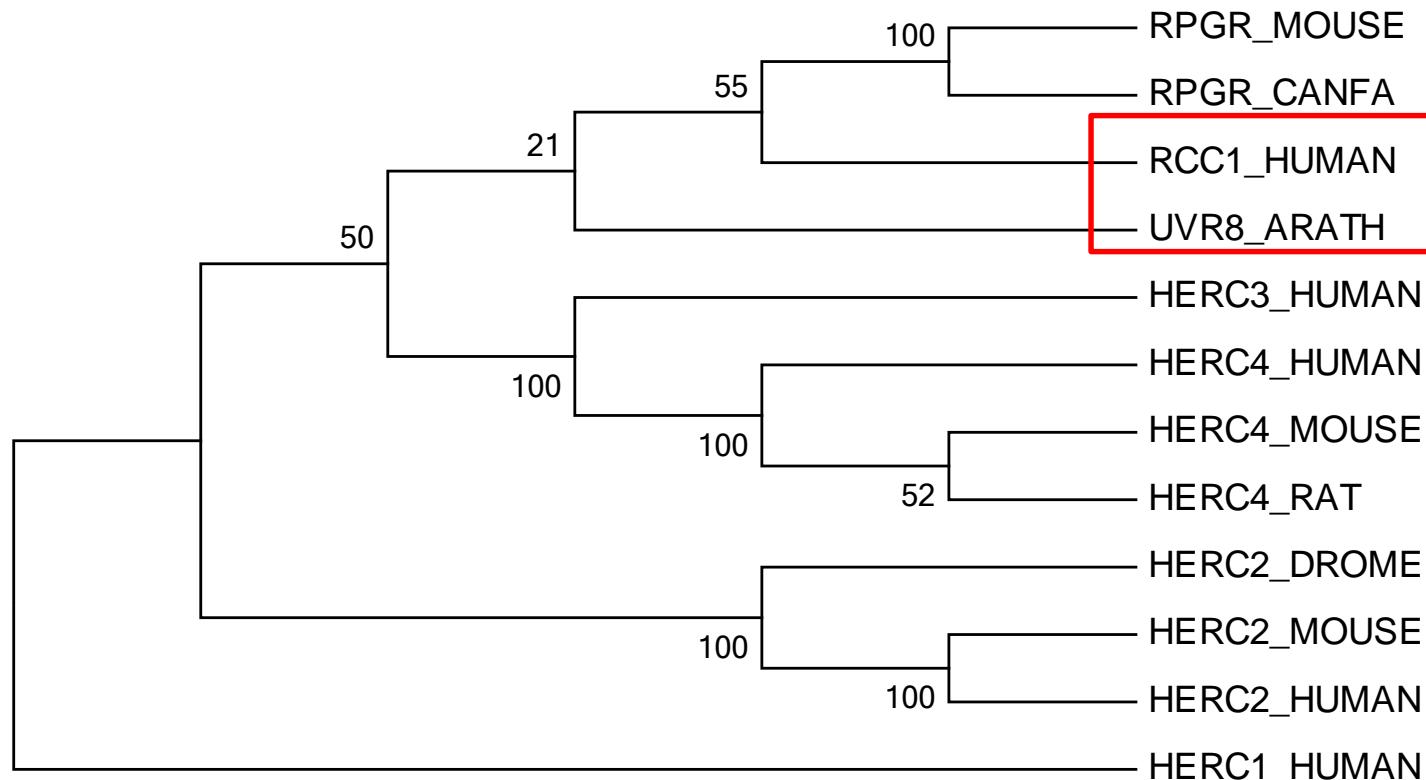
Photoreceptors in *Arabidopsis thaliana*



Analysis procedure

Neighbor-Joining Phylogenetic Tree (MEGA 5.2)

Homologous protein of UVR8



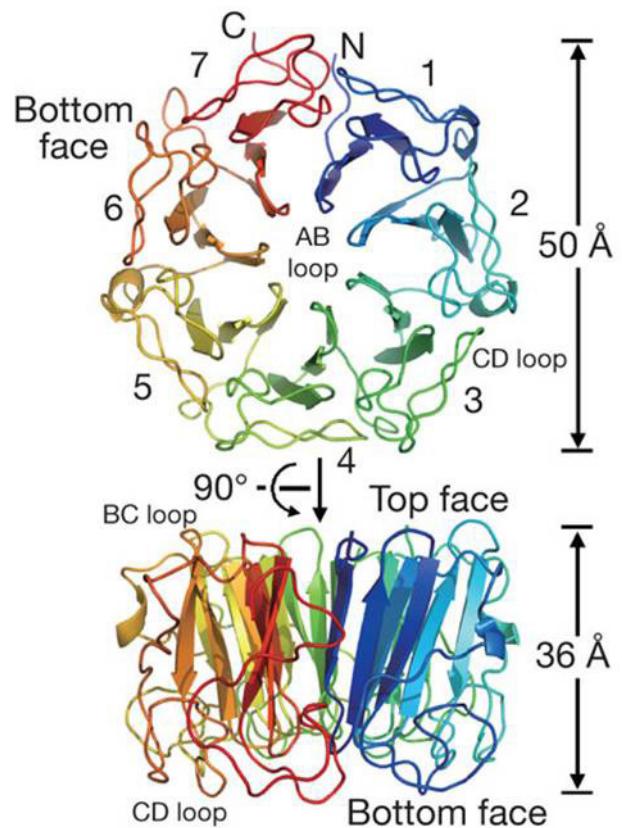
Analysis procedure

SWISS-MODEL

Homology model of *Arabidopsis thaliana* UVR8



VS

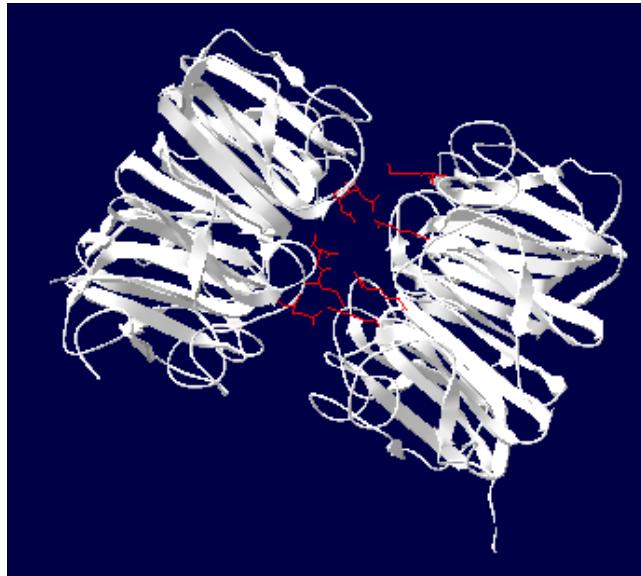


Wu et al., 2012

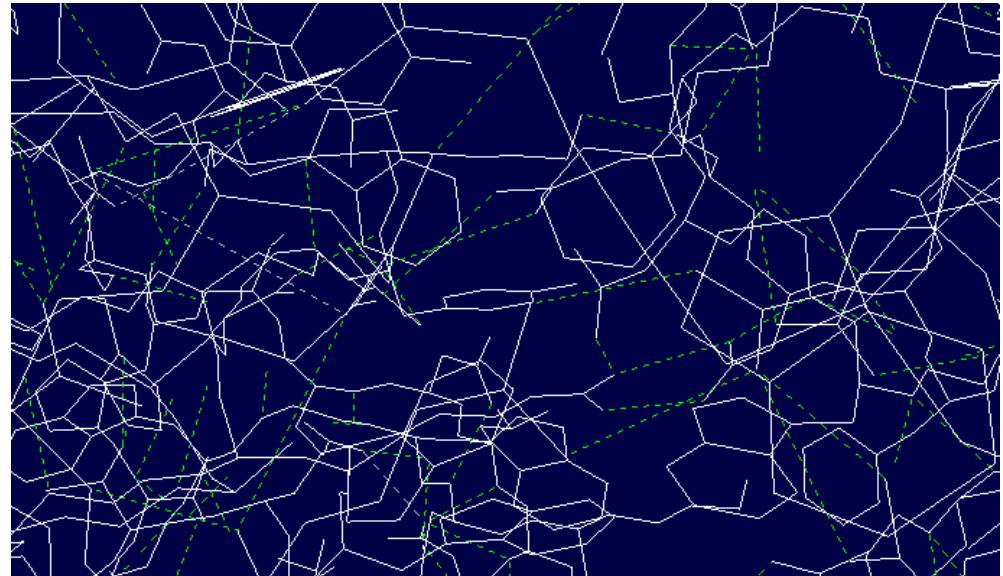
Analysis procedure

Swiss-PdbViewer 4.1.0

Structural basis of UVR8 homodimer formation



Charged attraction

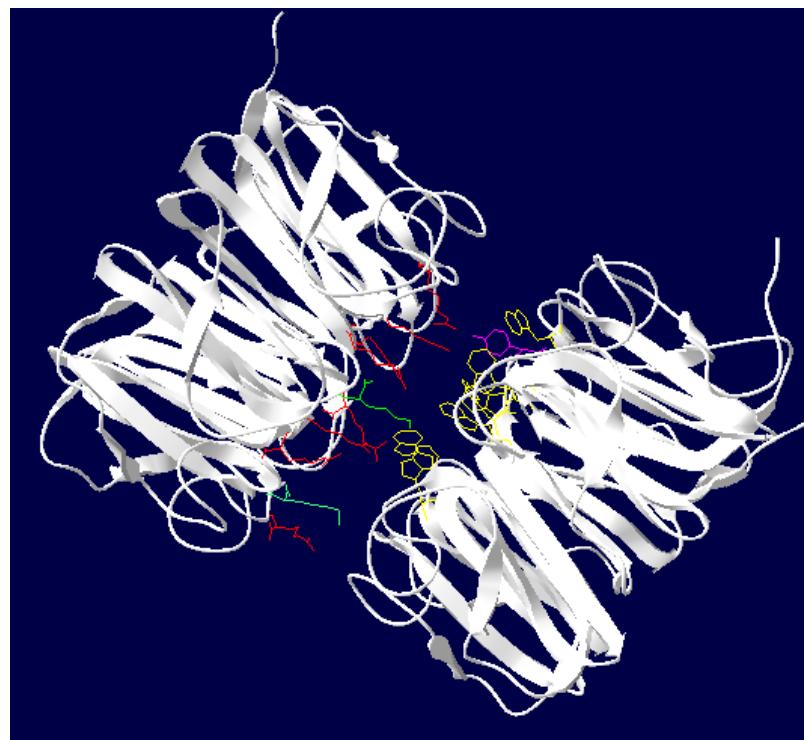


Hydrogen bond

Analysis procedure

Swiss-PdbViewer 4.1.0

Structural basis of UVR8 homodimer formation

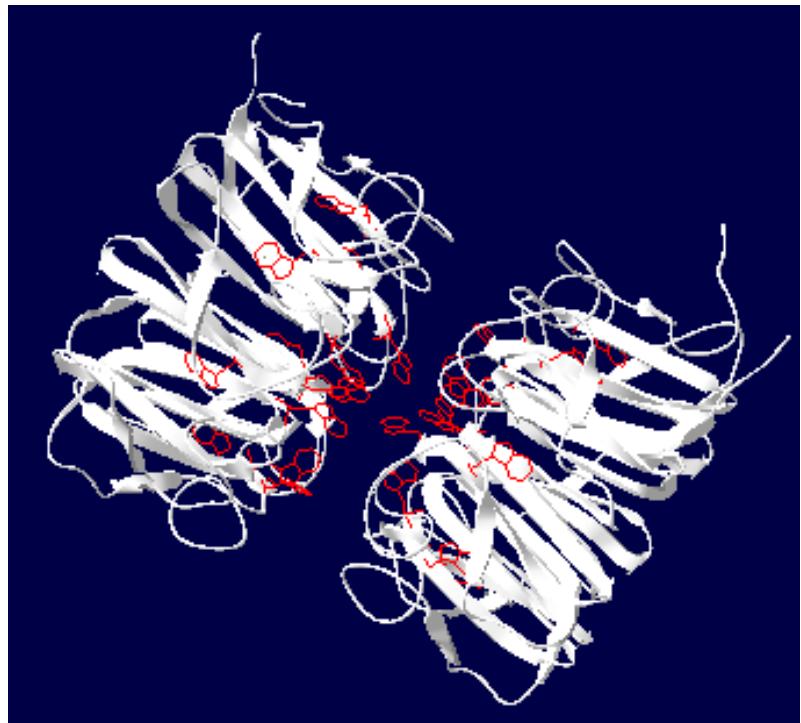


Cation–π interactions

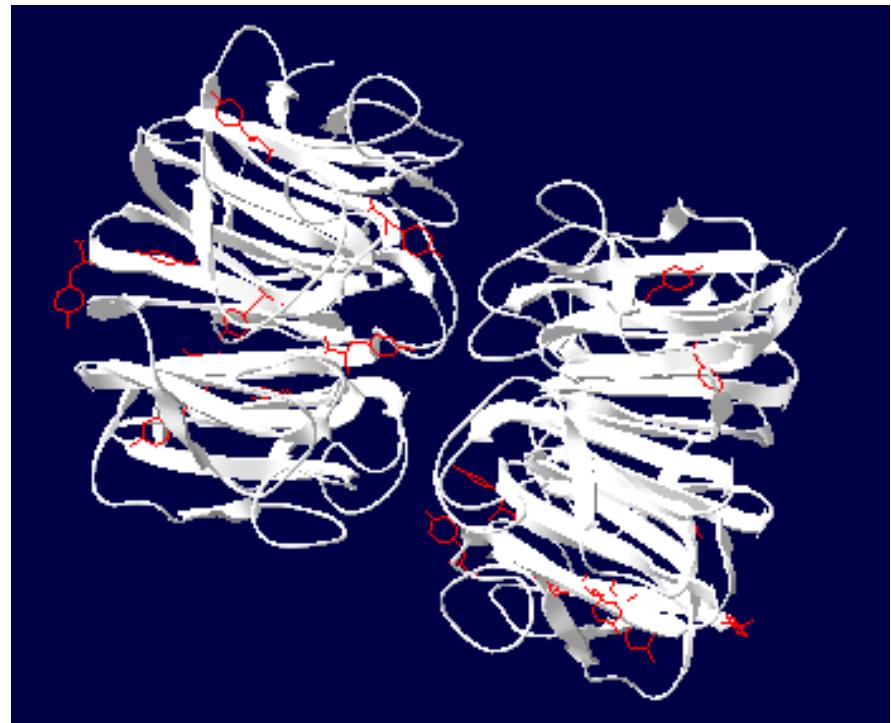
Analysis procedure

Swiss-PdbViewer 4.1.0

Identification of which amino acids as the UV-B chromophore

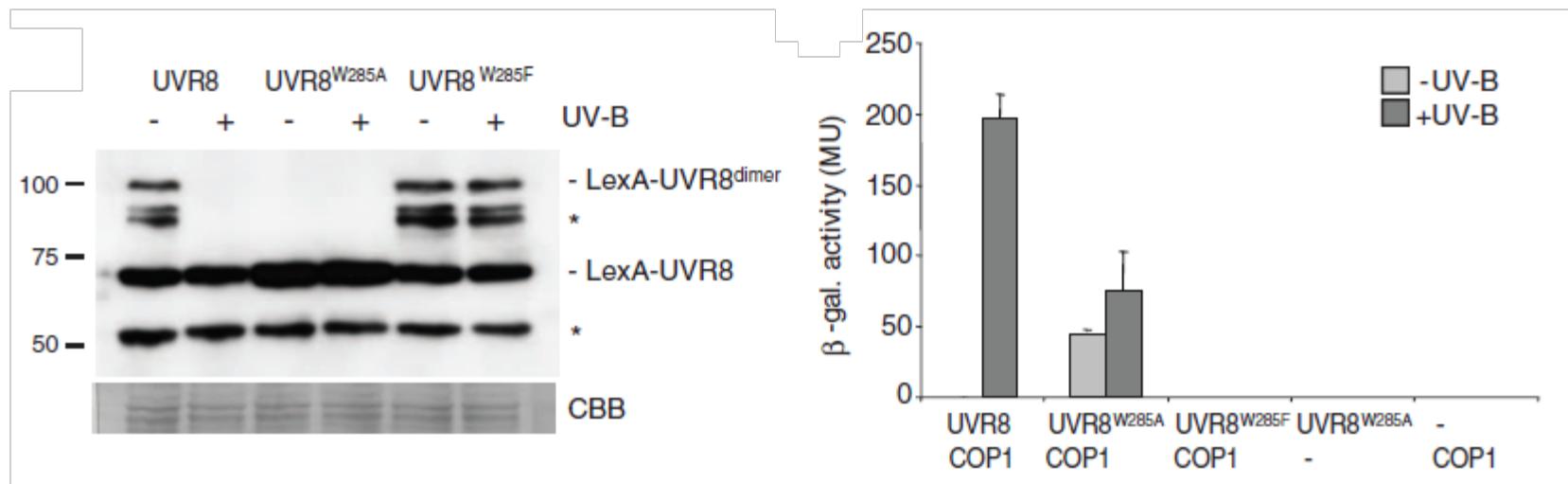


Try(W)



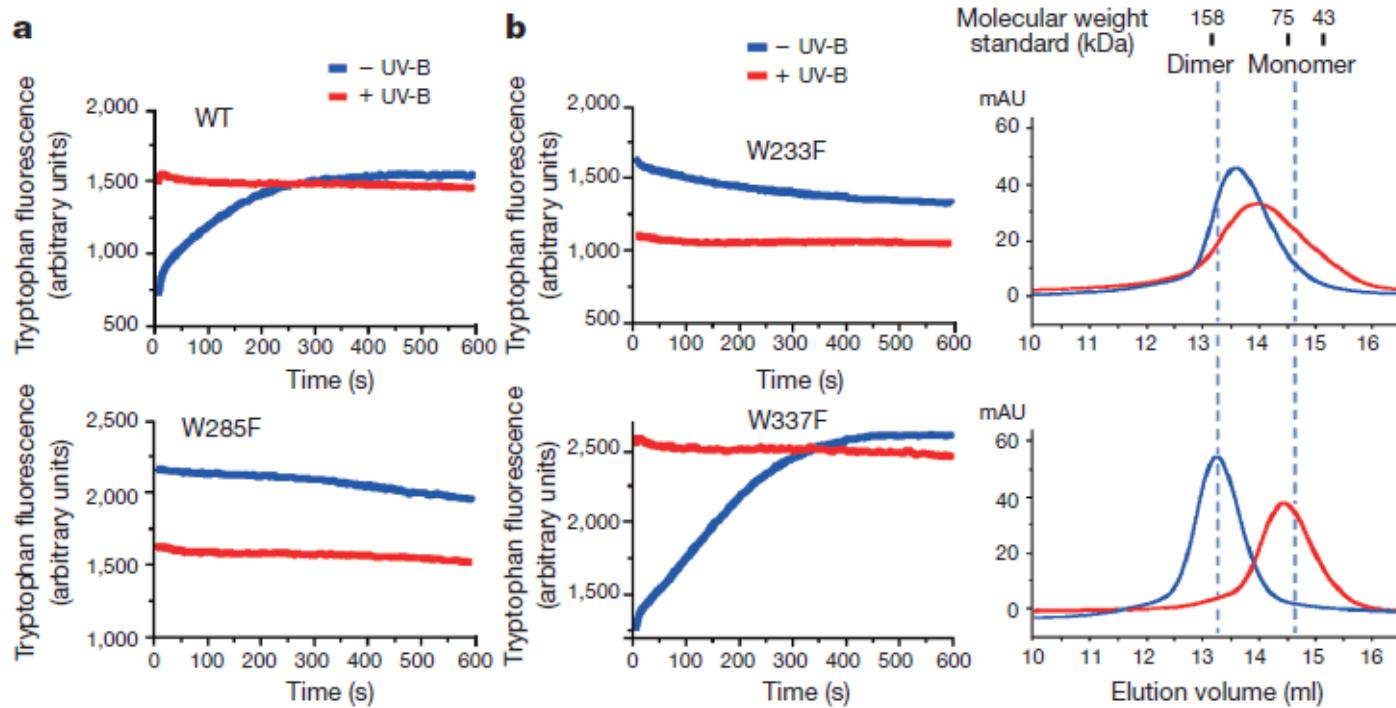
Tyr(Y)

Experimental evidences



Rizzini *et al.*, 2011

Experimental evidences



Wu *et al.*, 2012

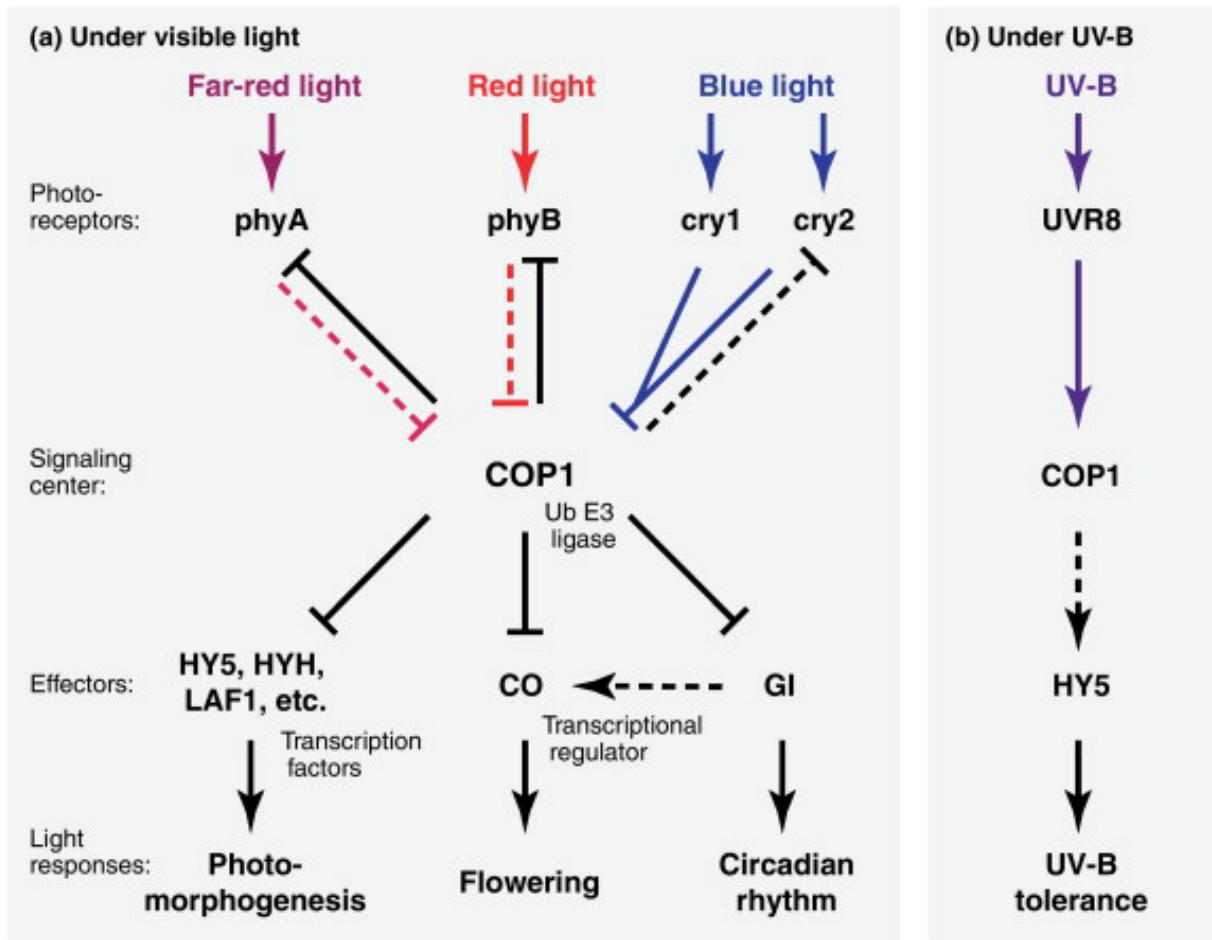
Conclusions

1. The acidic and basic surface patches from one UVR8 core domain associate with the complementarily charged surface patches of another core domain to form a symmetric homodimer. Intermolecular hydrogen bonds and cation–π interactions can stabilize protein structure.
2. These analyses unambiguously identify Trp 285 and Trp 233 collectively as the chromophore for ultraviolet-B perception.

Part II: Analysing how UVR8 positively regulates HY5 by COP1

Background

Signal paths of COP1



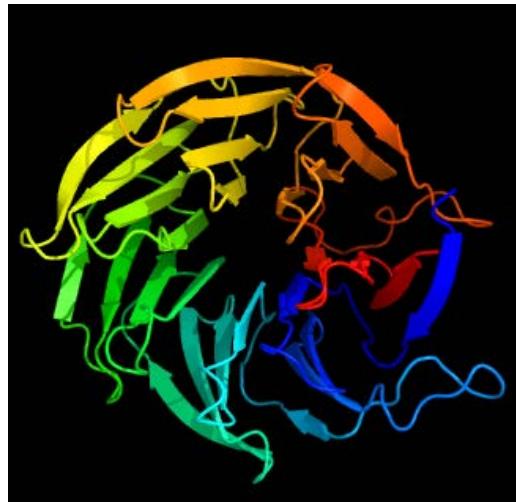
Question

How does the UVR8 positively regulate HY5 by COP1?

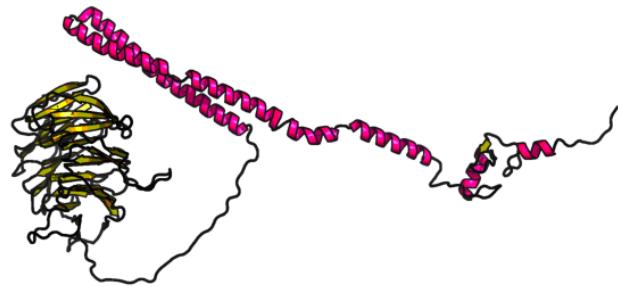
Analysis procedure

Protein modeling prediction

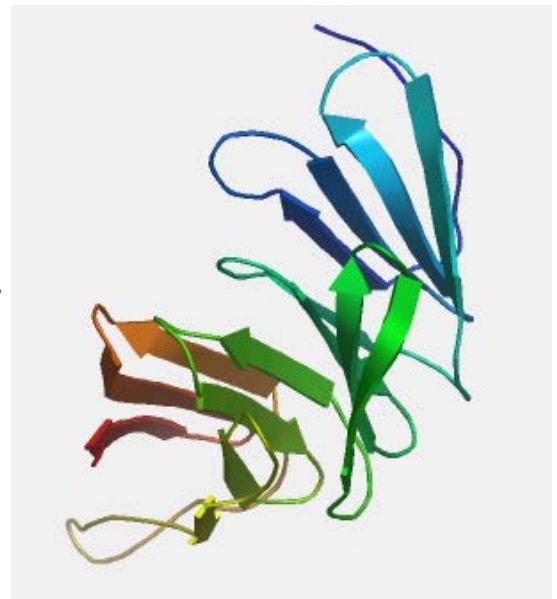
COP1 structure prediction



Phyre2



RaptorX

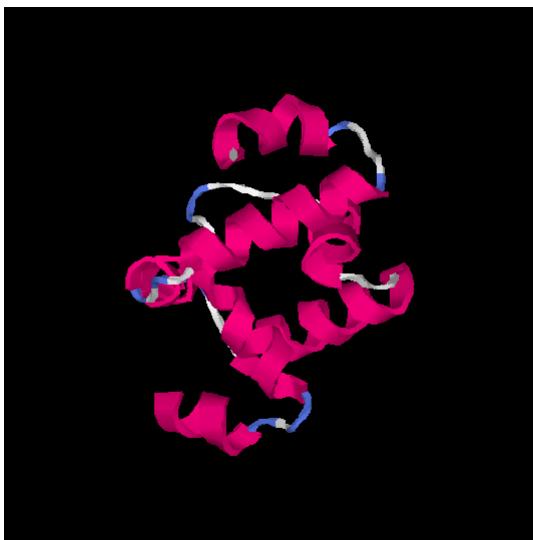


SWISS-MODEL

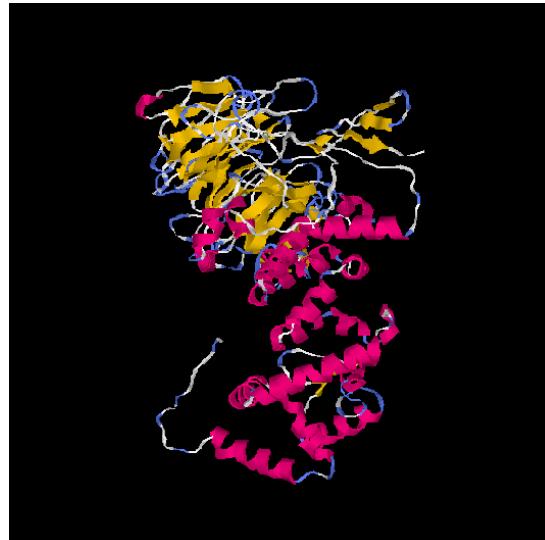
Analysis procedure

I-TASSER

COP1 structure prediction



Model 1



Model 2

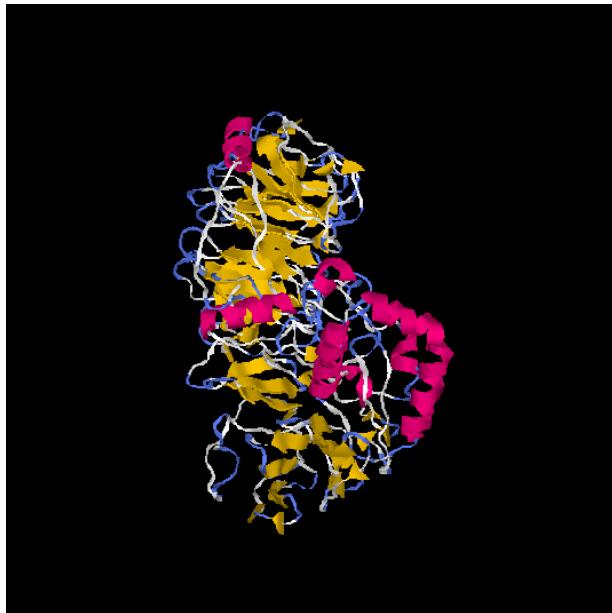


Model 3

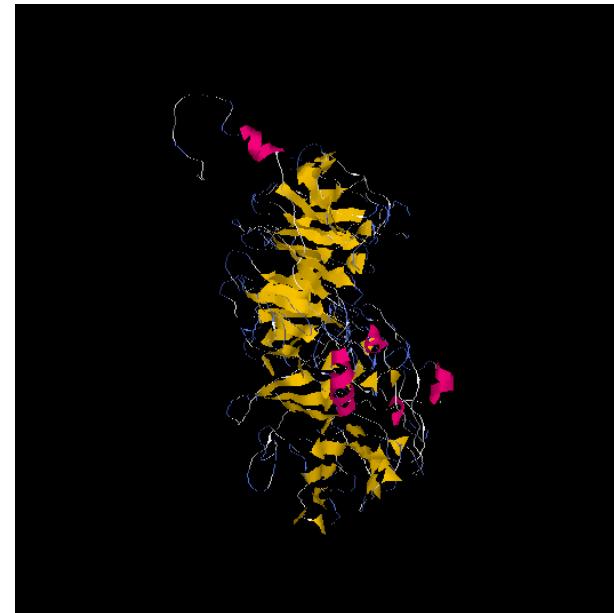
Analysis procedure

I-TASSER

COP1 structure prediction



Model 4

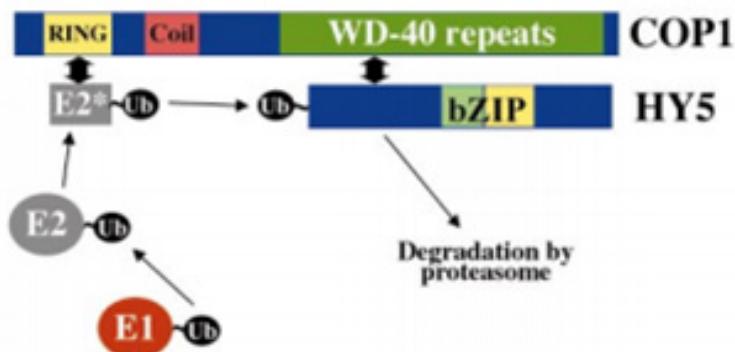
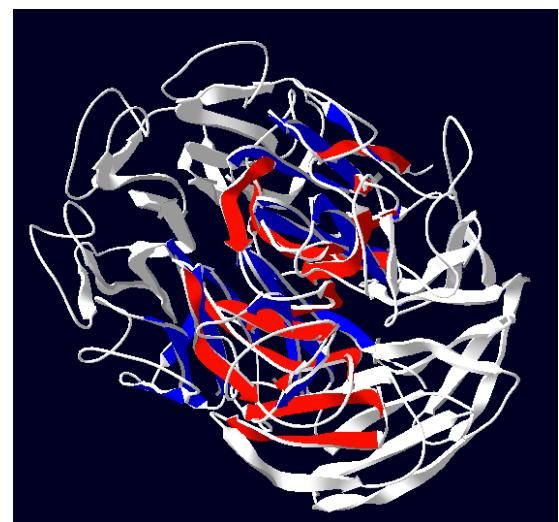
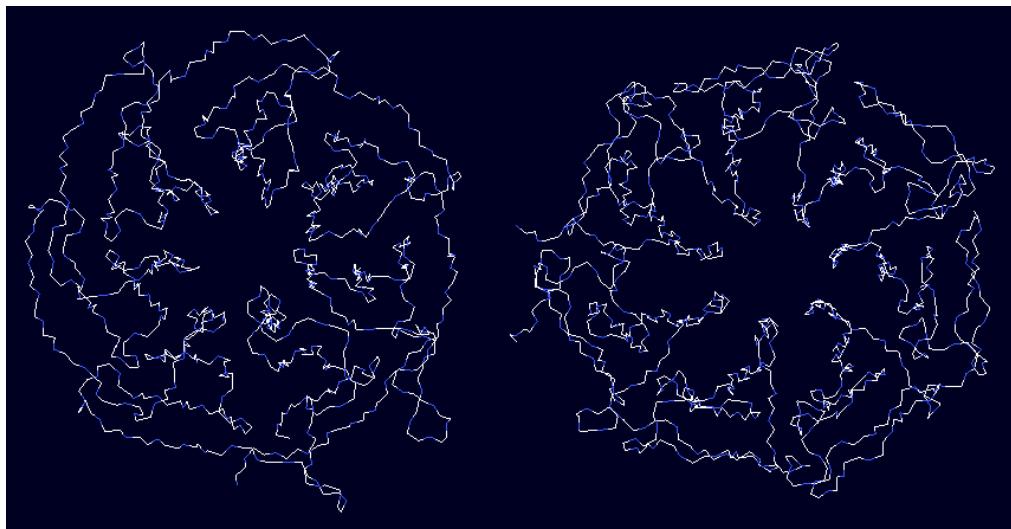


Model 5

Analysis procedure

Fit

COP1 and UVR8 can partially fit



Osterlund *et al.*, 2000

Hypothesis

UVR8 interacts with COP1 basing on the similar WD40 domain at the C-terminal that inhibits COP1 degrading HY5 by blocking COP1 WD40 domain binding HY5.

Acknowledgements

- Prof. Jingchu Luo
- Prof. Liumin Fan
- Dr. Haodong Chen
- All the members in the class
- Prof. Xing Wang Deng
- Prof. Hongwei Guo
- Dr. Guangming He
- All the members in Deng Lab, Fan Lab, Guo Lab

Thank you!