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CHINESE ACADEMY OF AGRICULTURAL SCIENCES

水稻APO1-APO2模块的互作机制分析

Analysis of the Interaction Mechanism of the
APO1-APO2 Module in Rice

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明德格物 博学笃行

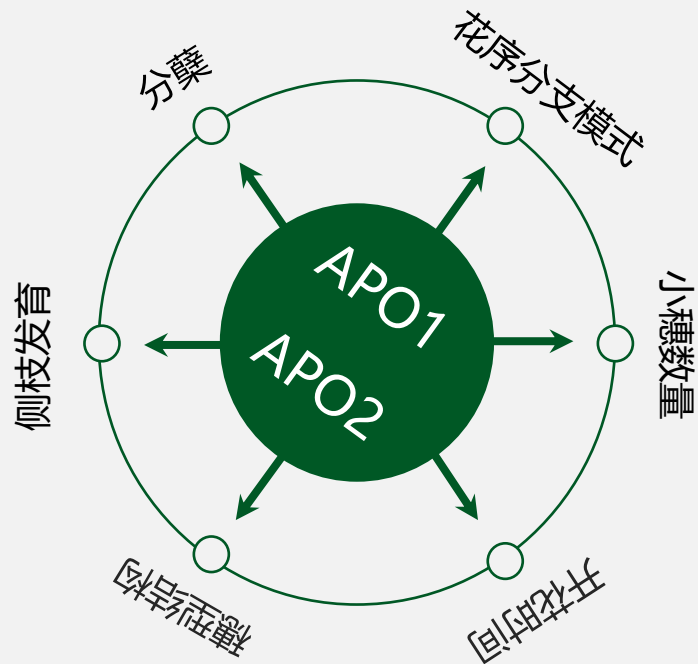


Part 1

研究背景

APO1和APO2基因

APO1和APO2基因是调控水稻 (*Oryza sativa*) 生殖发育的重要遗传因子，特别是在调控分蘖、穗型结构、开花时间以及产量相关性状方面具有核心功能。APO1和APO2基因的突变会导致显著的形态学改变，从而影响最终的籽粒产量。因此，厘清APO1和APO2基因互作的机制能够深化对水稻花序发育分子机制的理解，也为高产育种提供了重要的候选基因资源。



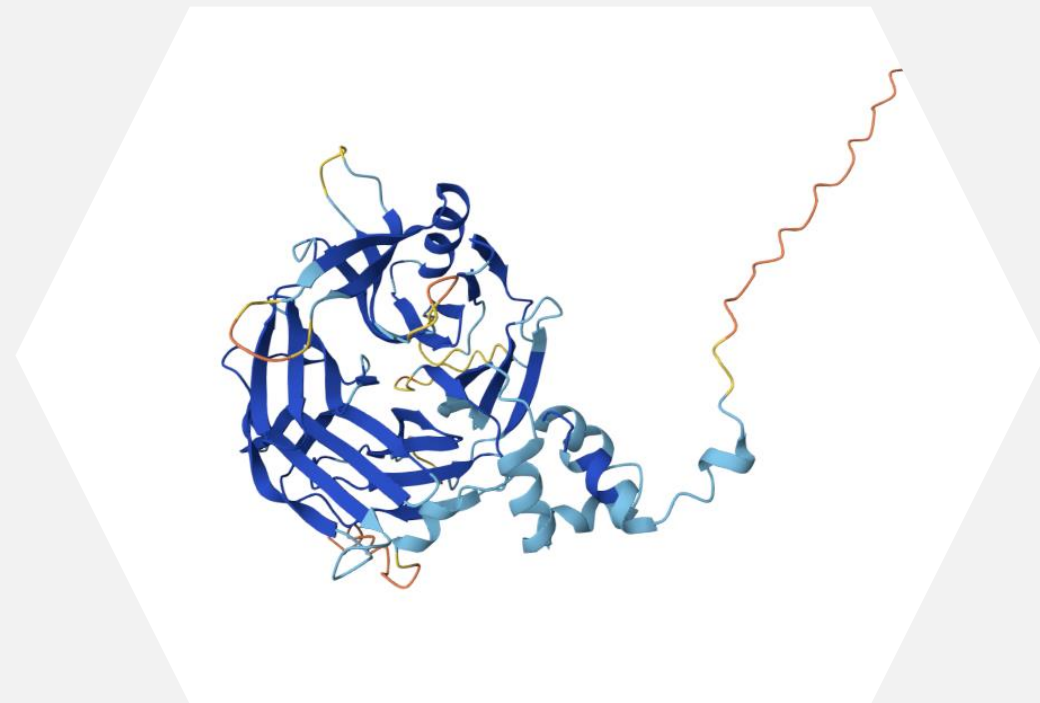
研究背景



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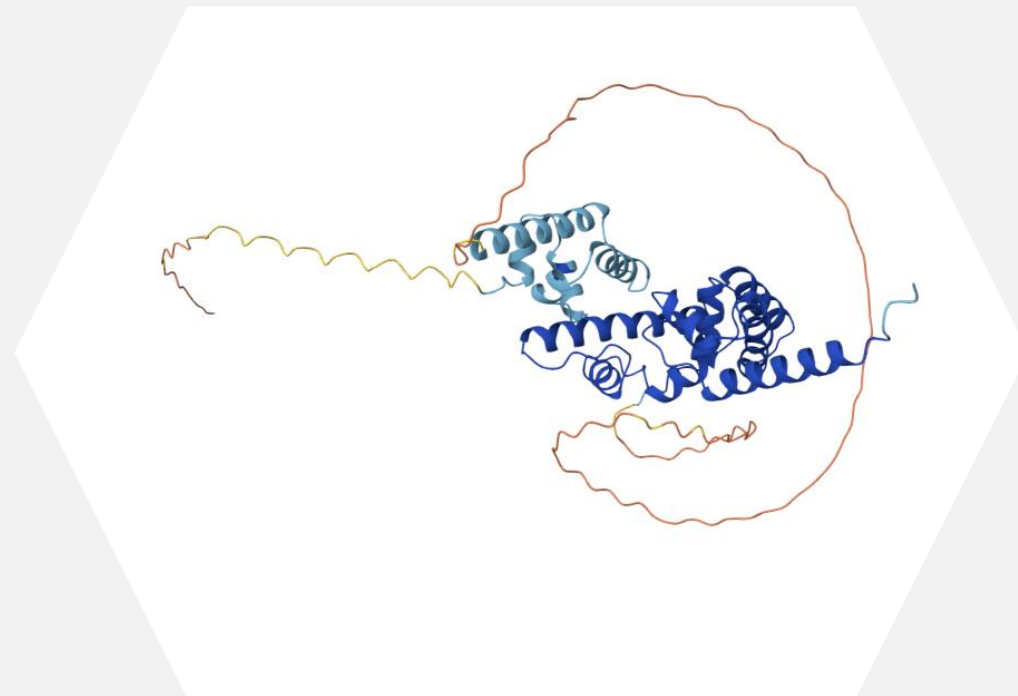
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APO1 (ABERRANT PANICLE ORGANIZATION 1)是一个F-box蛋白编码基因，主要通过控制分生组织中细胞增殖来调节水稻穗部结构。F-box蛋白是一类在真核生物中广泛存在的关键调控蛋白，是E3泛素连接酶复合物的核心识别亚基，主要是在蛋白质降解、信号转导、细胞周期调控及发育过程中发挥重要作用。在野生型水稻中，主轴分生组织通常会形成10-12个初级枝梗，而在*apo1*突变体中，该分生组织提前转变为小穗分生组织，导致初级枝梗数量显著减少，进而降低每穗粒数和总产量。



APO1

APO2 (ABERRANT PANICLE ORGANIZATION 2)也称为RFL基因，在调控水稻从营养生长向生殖生长转变的过程中发挥重要作用。该基因的主要功能包括抑制花序分生组织向花分生组织的过早转化，并参与多个阶段的穗形成过程。研究表明，*apo2*突变体会表现出小型化穗部结构，初级枝梗数量减少，且由于小穗分生组织提前形成而导致穗粒数下降。此外，APO2还影响节间伸长速率和开花时间，*apo2*突变体通常表现为晚花表型。



APO1



Part 2

互作位点分析

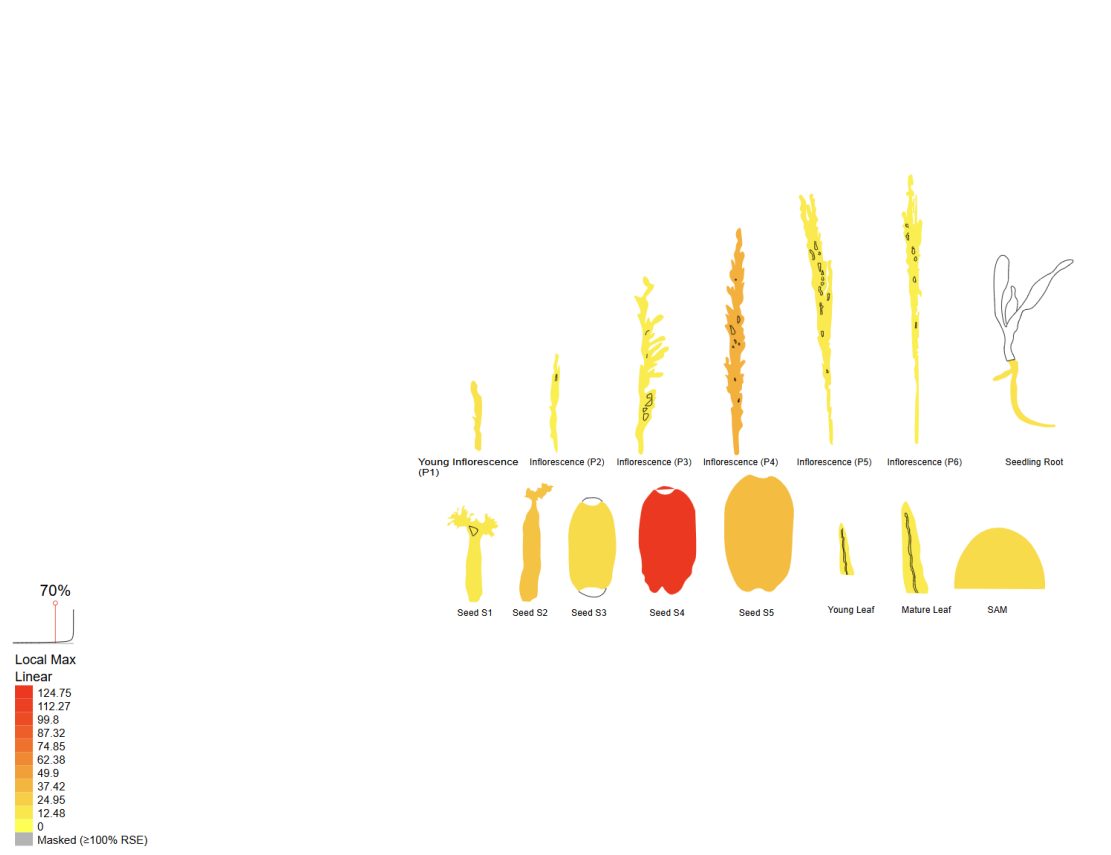
APO1与APO2表达部位预测



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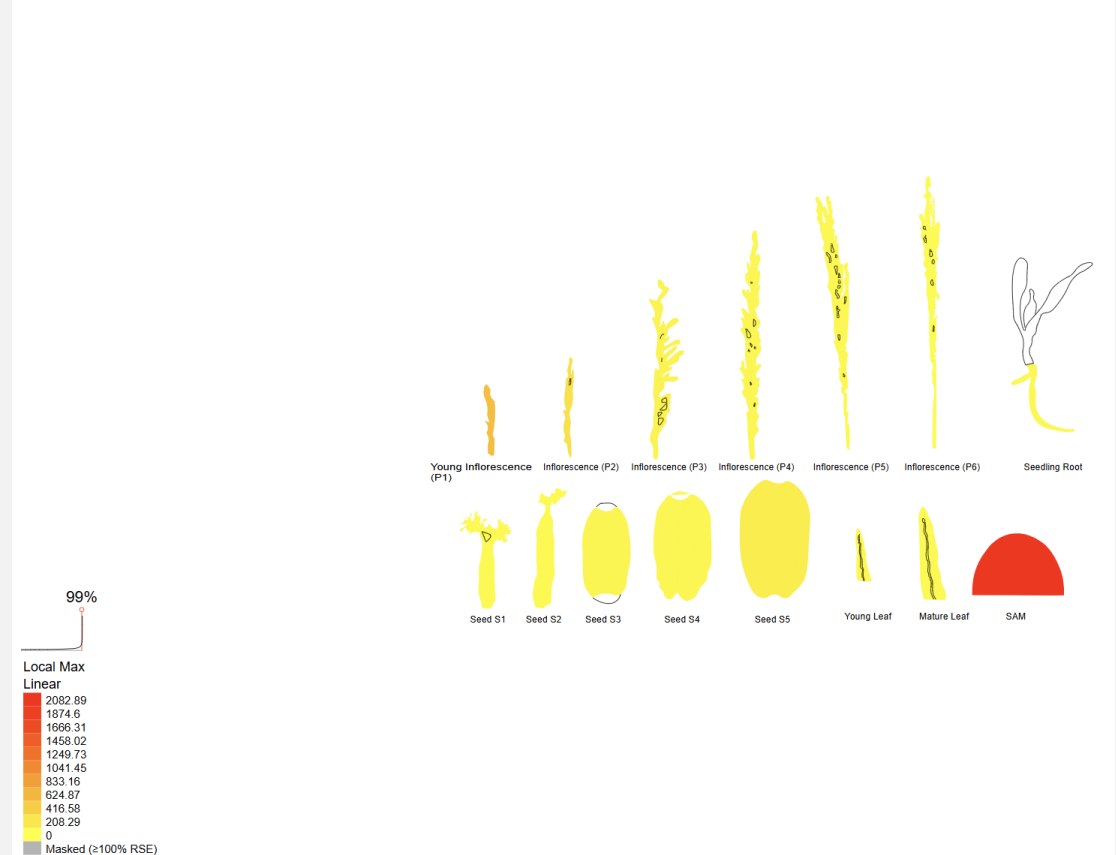
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Plant eFP: LOC_Os06g45460



APO1

Plant eFP: LOC_Os04g51000



APO2

APO1理化性质的预测分析



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Number of amino acids: 429
Theoretical pI: 9.95
Molecular weight: 45022.11

Amino acid composition:

Ala (A)	66	15.4%
Arg (R)	33	7.7%
Asn (N)	4	0.9%
Asp (D)	17	4.0%
Cys (C)	5	1.2%
Gln (Q)	4	0.9%
Glu (E)	10	2.3%
Gly (G)	36	8.4%
His (H)	12	2.8%
Ile (I)	7	1.6%
Leu (L)	52	12.1%
Lys (K)	4	0.9%
Met (M)	8	1.9%
Phe (F)	22	5.1%
Pro (P)	48	11.2%
Ser (S)	39	9.1%
Thr (T)	15	3.5%
Trp (W)	8	1.9%
Tyr (Y)	5	1.2%
Val (V)	34	7.9%
Pro (O)	0	0.0%
Sec (U)	0	0.0%
(B)	0	0.0%
(Z)	0	0.0%
(X)	0	0.0%

Total number of negatively charged residues (Asp + Glu): 27
Total number of positively charged residues (Arg + Lys): 37

Atomic composition:

Carbon	C	2045
Hydrogen	H	3190
Nitrogen	N	572
Oxygen	O	551
Sulfur	S	13

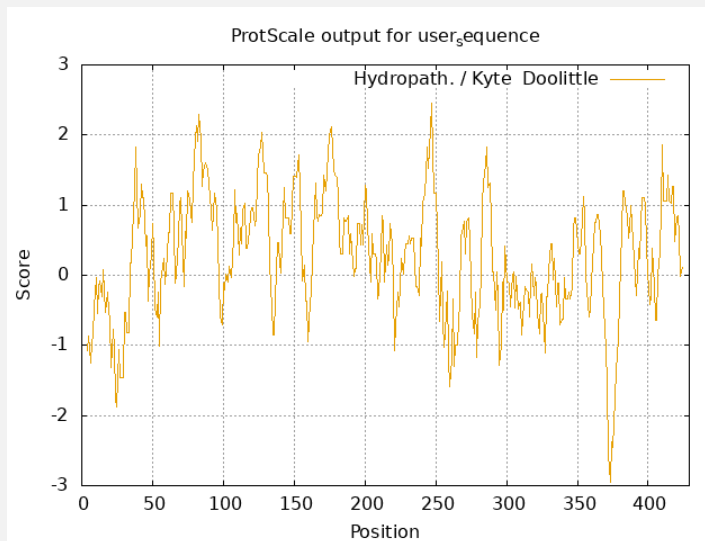
Formula: $C_{2045}H_{3190}N_{572}O_{551}S_{13}$
Total number of atoms: 6371

Instability index:

The instability index (II) is computed to be 64.28
This classifies the protein as unstable.

Aliphatic index: 92.00

Grand average of hydropathicity (GRAVY): 0.253



应用 ExPASy 系统中的 ProtParam 软件
(<https://web.expasy.org/protparam/>)
分析基本理化性质;

应用在线软件 ProtScale
(<https://web.expasy.org/protscale/>)
分析亲疏水性

APO2理化性质的预测分析



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CES

Number of amino acids: 389
Theoretical pI: 8.59
Molecular weight: 42530.27

Amino acid composition:

Ala (A)	48	12.3%
Arg (R)	33	8.5%
Asn (N)	7	1.8%
Asp (D)	25	6.4%
Cys (C)	7	1.8%
Gln (Q)	12	3.1%
Glu (E)	23	5.9%
Gly (G)	37	9.5%
His (H)	16	4.1%
Ile (I)	5	1.3%
Leu (L)	35	9.0%
Lys (K)	19	4.9%
Met (M)	13	3.3%
Phe (F)	12	3.1%
Pro (P)	29	7.5%
Ser (S)	22	5.7%
Thr (T)	11	2.8%
Trp (W)	4	1.0%
Tyr (Y)	10	2.6%
Val (V)	21	5.4%
Pyl (O)	0	0.0%
Sec (U)	0	0.0%
(B)	0	0.0%
(Z)	0	0.0%
(X)	0	0.0%

Total number of negatively charged residues (Asp + Glu): 48
Total number of positively charged residues (Arg + Lys): 52

Atomic composition:

Carbon	C	1857
Hydrogen	H	2922
Nitrogen	N	562
Oxygen	O	548
Sulfur	S	20

Formula: $C_{1857}H_{2922}N_{562}O_{548}S_{20}$

Total number of atoms: 5909

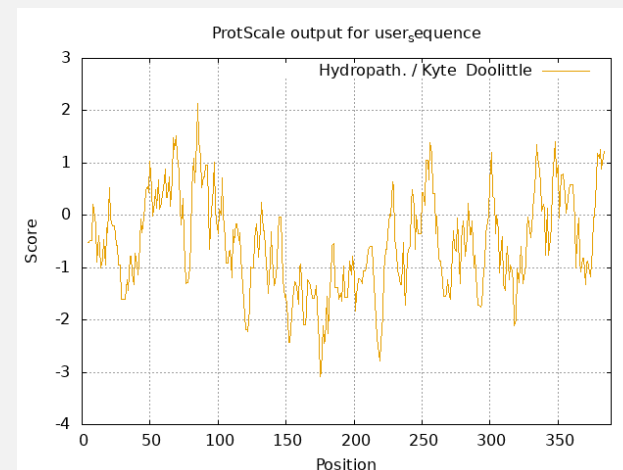
Instability index:

The instability index (II) is computed to be 54.26

This classifies the protein as unstable.

Aliphatic index: 68.10

Grand average of hydropathicity (GRAVY): -0.528

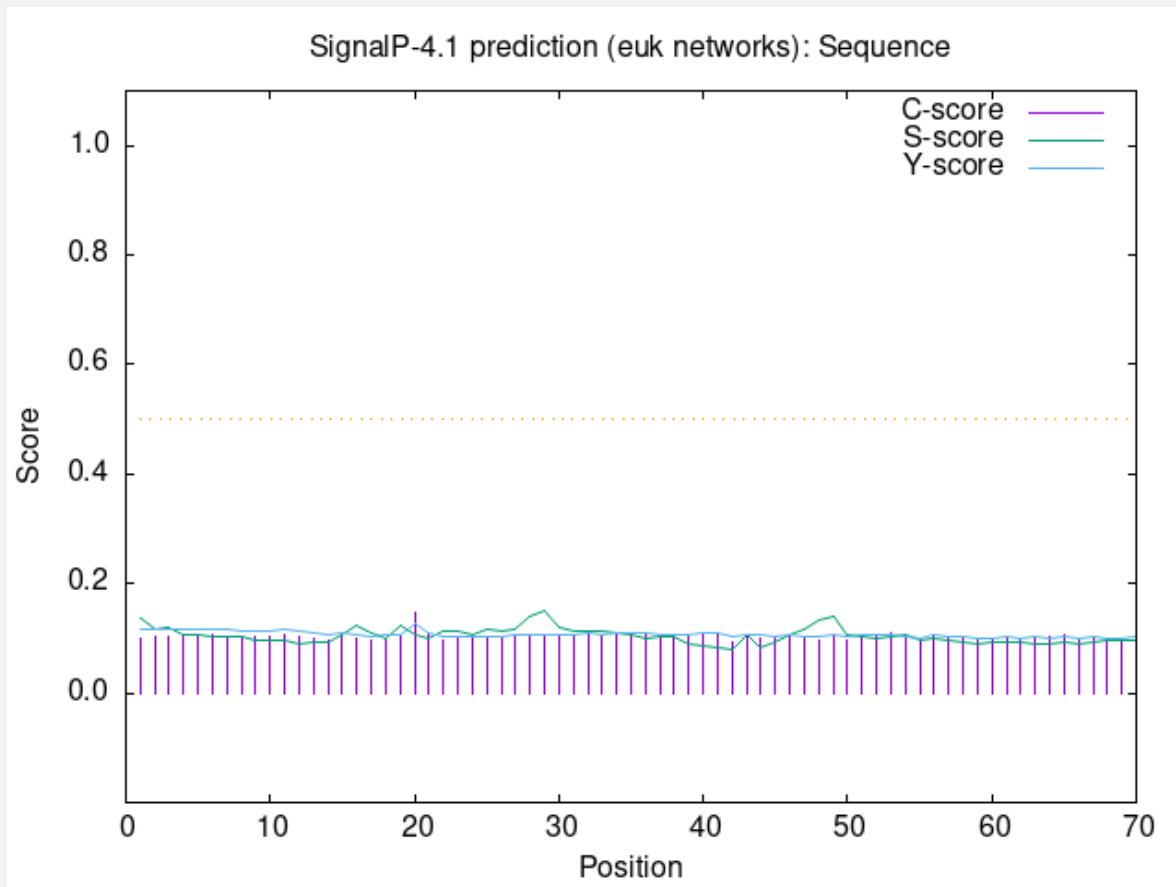


APO1与APO2的信号肽预测分析

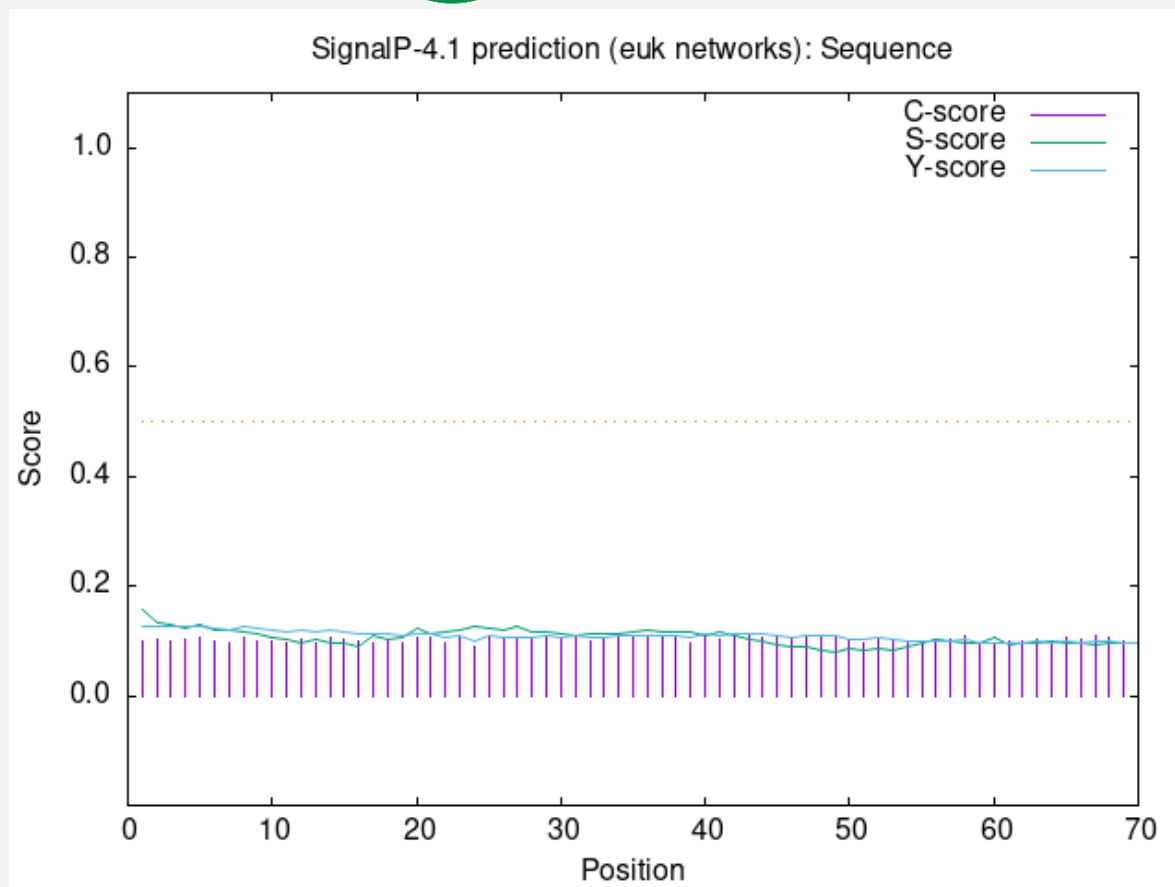


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APO1



APO2

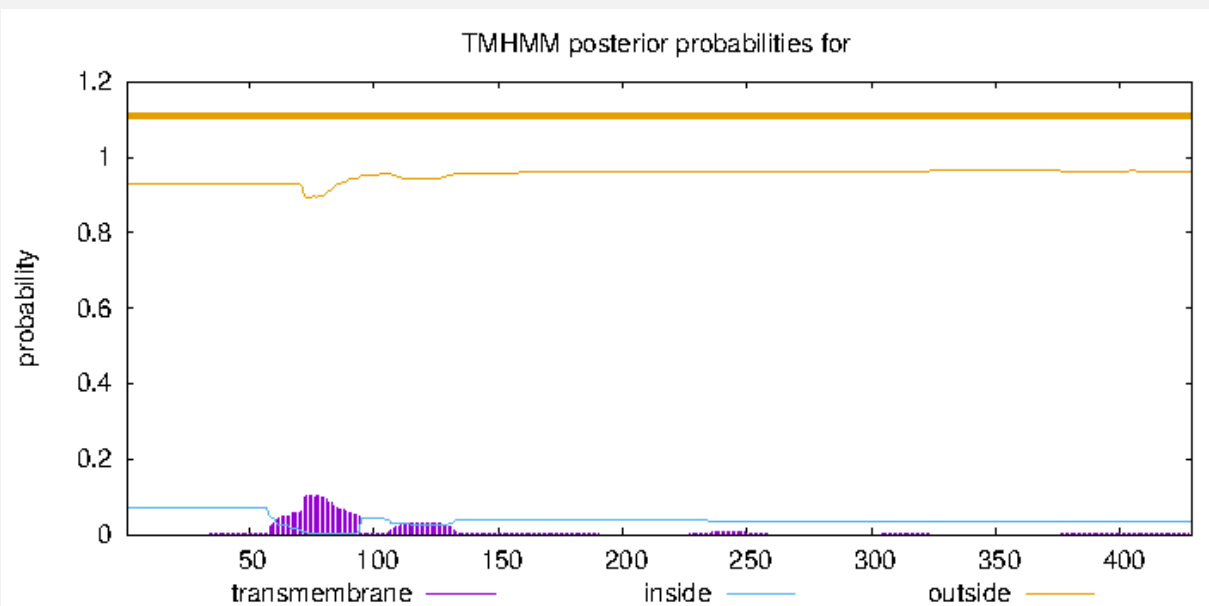
应用<https://services.healthtech.dtu.dk/services/SignalP-4.1/>预测信号肽结构

APO1与APO2跨膜结构的预测分析

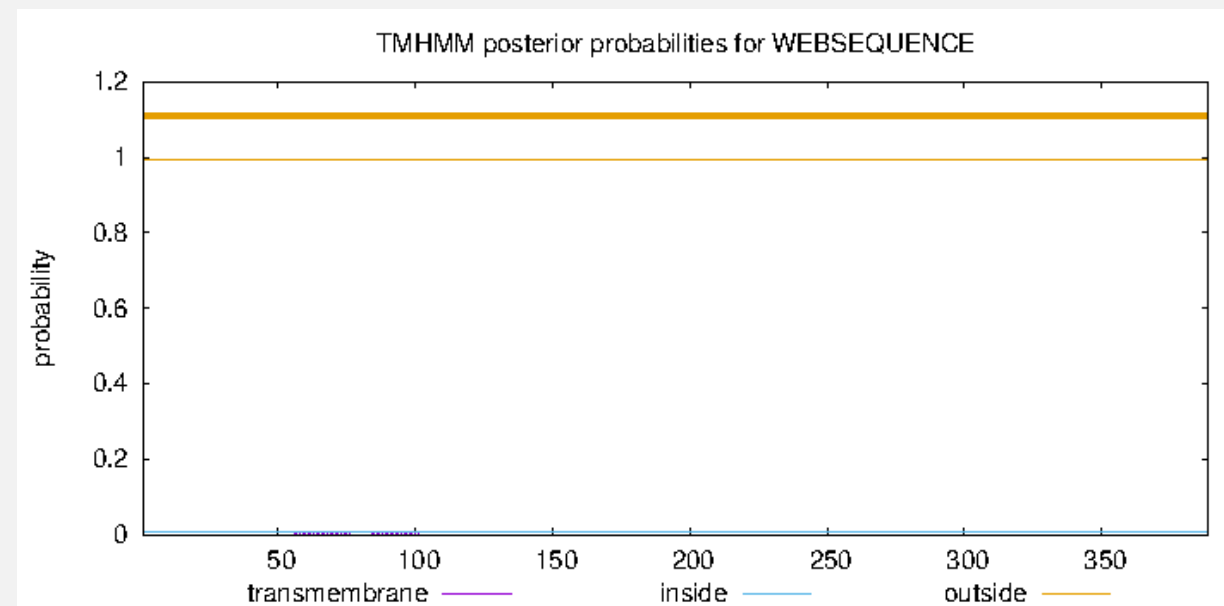


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APO1



APO2

应用<https://services.healthtech.dtu.dk/services/TMHMM-2.0/>预测跨膜结构

APO1亚细胞定位的预测分析



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Results of the *k*-NN Prediction

k = 9/23

- 43.5 %: mitochondrial
- 21.7 %: cytoplasmic
- 17.4 %: endoplasmic reticulum
- 13.0 %: Golgi
- 4.3 %: vacuolar

>> prediction for QUERY is mit (k=23)

Plant-mPLoc: Predicting subcellular localization of plant proteins including those with multiple sites

[Read Me](#) | [Data](#) | [Citation](#)

Your input sequence (429aa) is:

```
>APO1
MMNPRRLPPLPSSTSSASAADDMDPRVWRRLPQPLVDRILACLPTPSFLRLRAACRRFYH
LLFSSPFLHSHLLSPHLPFAFVVPAAAGHLLLDPTATASWSRLPLPPVAGGPAAFS
PAAASAGLLAFLSDASGHKTLNPIITRLLAALPISPTPRLSPTVGLAAGPTSHAVVA
GDDLVSFPAVKNISADTFVADAASVPPSGFWAPSSLLPRLSSLDPRAGMAFASGRFYCMS
SSPFAVLVFDVAENVWSKVQPPMRRFLRSPALVELGGREGGAARVALVSAVEKSRLSVPR
SVRLWTLRGGGGGGGGAWTEVARMPPEVHAQFAAAEGRGFCAAHGDYVVLAPRGPVA
QAPTSALVFDSSRRDEWRWAPPVVAHGGGAGAAGRVFAYPEPRLATPAIGLLDATAP
VALHGMHDG
```

----- Plant-mPLoc Computation Result -----

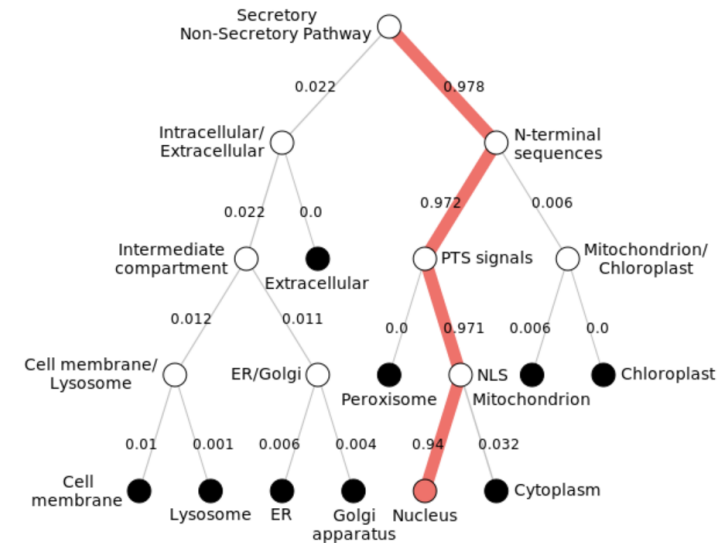
Query protein	Predicted location(s)
APO1	Nucleus.

Sequence

Prediction: Nucleus, Membrane

Localization	Nucleus	Cytoplasm	Cell membrane	Endoplasmic reticulum	Golgi apparatus	Mitochondrion	Peroxisome	Lysosome/Vacuole	Plastid	Extracellular
Likelihood	0.8685	0.0759	0.0166	0.0154	0.0085	0.0084	0.0028	0.0027	0.001	0.0003

Type	Soluble	Membrane
Likelihood	0.3132	0.6868



APO2亚细胞定位的预测分析



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Results of the *k*-NN Prediction

k = 9/23

- 52.2 %: cytoplasmic
- 26.1 %: nuclear
- 8.7 %: mitochondrial
- 4.3 %: endoplasmic reticulum
- 4.3 %: vesicles of secretory system
- 4.3 %: plasma membrane

>> prediction for QUERY is cyt (k=23)

Plant-mPLOC: Predicting subcellular localization of plant proteins including those with multiple sites

[Read Me](#) | [Data](#) | [Citation](#) |

Your input sequence (389aa) is:

```
>APO2
MDPNDAFSAAHPRWDLGPPAPAPVPPPPPPPPANVPRELEELVAGYVGRMSTVAR
ISELGFSTILLAMIERELDDMMAALAGLFRWDLILLGERFGLRAALRAERGLMSLGGRH
HGHQSGSTVDGASQEVLSDEHDMAGSGGMDDDNGRRMVTGKQAKKGSAAKGGKARRK
KYDDLRLDMQDEMDCCDEDDGGGSESTESSAGGGGERQREHPFVVTPEGEVARAKKNG
LDYLFHLYEQCRLLFLLQVQSMALKHGHKSPKVTNQVFRYAKKVGASYNPKMRHYVHC
YALHCLDEEASDALRRAYKARGENVGAWRQACYAPLVDISARHGFDIDAVFAAHPRLAIW
VYPTRLRLQLCHQARSSHAATAAALPPPLF
```

----- Plant-mPLOC Computation Result -----

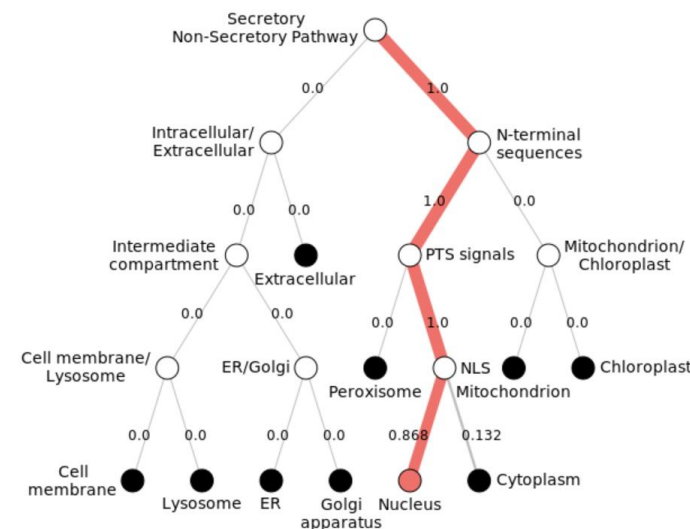
Query protein	Predicted location(s)
APO2	Nucleus.

Sequence

Prediction: Nucleus, Soluble

Localization	Nucleus	Cytoplasm	Cell membrane	Peroxisome	Mitochondrion	Endoplasmic reticulum	Golgi apparatus	Extracellular	Plastid	Lysosome/Vacuole
Likelihood	0.9088	0.0904	0.0005	0.0001	0	0	0	0	0	0

Type	Soluble	Membrane
Likelihood	0.8755	0.1245





Part 3

序列保守性与系统发育分析

序列比对

APO1序列比对

Tool: UniProt



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Species	Scientific name	Accession	AA Length
Rice	<i>Oryza sativa</i>	B8B183	429
Sorghum	<i>Sorghum bicolor</i>	A0A0A0UMU7	196
Yellow bluestem	<i>Bothriochloa ischaemum</i>	A0A0A0UNU7	194
Ravanna grass	<i>Triplidium ravennae</i>	A0A0A0UP06	193
Chinese silver grass	<i>Miscanthus sinensis</i>	A0A0A0UQ41	196

<input type="checkbox"/> sp B8B183 APO1_ORYSI	MMNPRRLPPLPSSTSSASAADDMDPRVWRRLPQPLVDRVLACLPTPSFLRLRAACRRFYHLLF	63
<input type="checkbox"/> tr A0A0A0UNU7 A0A0A0UNU7_BOTIS	-----	0
<input type="checkbox"/> tr A0A0A0UP06 A0A0A0UP06_TRIRV	-----	0
<input type="checkbox"/> tr A0A0A0UMU7 A0A0A0UMU7_SORBI	-----	0
<input type="checkbox"/> tr A0A0A0UQ41 A0A0A0UQ41_MISSI	-----	0
<input type="checkbox"/> sp B8B183 APO1_ORYSI	SSPFLHSHLLLSPHLPFFAFVVPAAAGHLLLDPTA--TASWSRLPLPLPPVAG-GPAAFSPAA	123
<input type="checkbox"/> tr A0A0A0UNU7 A0A0A0UNU7_BOTIS	-----HSHLLLSPHLPFFAFVVPSSGYLPLLDPTTRPEAPSWSRRLPLPLPA--GHQAASFSPAA	56
<input type="checkbox"/> tr A0A0A0UP06 A0A0A0UP06_TRIRV	-----HPHLLLSPHLPFFAFVPSAGYLLLLDPTTR-QAPSWSRRLPLPLPAP-G-AGQAFSPAA	55
<input type="checkbox"/> tr A0A0A0UMU7 A0A0A0UMU7_SORBI	-----HSHLLLSPHLPFFAFVPSAGYLLLLDPTTRPEAPSWSRRLPLPLPAPGAGHQAFSPAA	58
<input type="checkbox"/> tr A0A0A0UQ41 A0A0A0UQ41_MISSI	-----HSHLLLSPHLPFFAFVPSAGYLLLLDPTTRPQAPSWSRRLPLPLP-APGAGHQAFSPAA	57
<input type="checkbox"/> sp B8B183 APO1_ORYSI	ASAGLLAFLSDASGHKTLLLANPITRLLAALPLISPTPRLSPTVGLAAGPTSIIAVVAGDDLVS	186
<input type="checkbox"/> tr A0A0A0UNU7 A0A0A0UNU7_BOTIS	ASAGLLAFLSDASGHKTLLLVNPIITRLLAPLPLSPTARLSPTVGLAAGPTSFIAVIAGDDLVS	119
<input type="checkbox"/> tr A0A0A0UP06 A0A0A0UP06_TRIRV	ASAGLLAFLSDASGHKTLLLANPITRILAPLPLCPTARLSPTVGLAAGPTSFIAVIAGDDLVS	118
<input type="checkbox"/> tr A0A0A0UMU7 A0A0A0UMU7_SORBI	ASAGLLAFLSDASGHKTLLLANPITRLLAPLPLCPTARLSPTVGLAAGPTSFIAVIAGDDLVS	121
<input type="checkbox"/> tr A0A0A0UQ41 A0A0A0UQ41_MISSI	ASSGLLAFLSDASGHKTLLLANPITRLLAPLPLCPTARLSPTVGLAAGPTSFIAVIAGDDLVS	120
<input type="checkbox"/> sp B8B183 APO1_ORYSI	PFVAVKNISADTFVADAASVPPSGFWAPSSLLPRLSSLDPRAGMAFASGRFYCMSSSPFAVLVF	249
<input type="checkbox"/> tr A0A0A0UNU7 A0A0A0UNU7_BOTIS	PFVAVKNISADTFVADAASVPPSGFWAPSSILPRLSSLDPRAGMAFASGRFYCMSSSPFAVLVF	182
<input type="checkbox"/> tr A0A0A0UP06 A0A0A0UP06_TRIRV	PFVAVKNISADTFVADAASVPPSGFWAPSSILPRLSSLDPRAGMAFASGRFYCMSSSPFAVLVF	181
<input type="checkbox"/> tr A0A0A0UMU7 A0A0A0UMU7_SORBI	PFVAVKNISADTFVADAASVPPSGFWAPSSILPRLSSLDPRAGMAFASGRFYCMSSSPFAVLVF	184
<input type="checkbox"/> tr A0A0A0UQ41 A0A0A0UQ41_MISSI	PFVAVKNISADTFVADAASVPTSGFWAPSSILPRLSSLDPRAGMAFASGRFYCMSSSPFAVLVF	183
<input type="checkbox"/> sp B8B183 APO1_ORYSI	DVAENVWSKVQPPMRRFLRSPALVELGGGREGAARVALVSAVEKSRLSVPRSVRLWTLRGGGG	312
<input type="checkbox"/> tr A0A0A0UNU7 A0A0A0UNU7_BOTIS	DVATNVWSKVQP-----	194
<input type="checkbox"/> tr A0A0A0UP06 A0A0A0UP06_TRIRV	DVATNVWSKVQP-----	193
<input type="checkbox"/> tr A0A0A0UMU7 A0A0A0UMU7_SORBI	DVATNVWSKVQP-----	196
<input type="checkbox"/> tr A0A0A0UQ41 A0A0A0UQ41_MISSI	DVATNVWSKVQP-----	195

结果分析

INTERPRETATION OF RESULT

1

APO1

水稻、高粱、白羊草、蒲苇和芒草5种禾本科植物中的APO1蛋白序列是高度保守的。

2

APO2

在植物界不同物种中，APO2同源的概率是很大的，它们编码的氨基酸序列中有大量的保守结构域。

系统发育分析

APO1系统发育分析

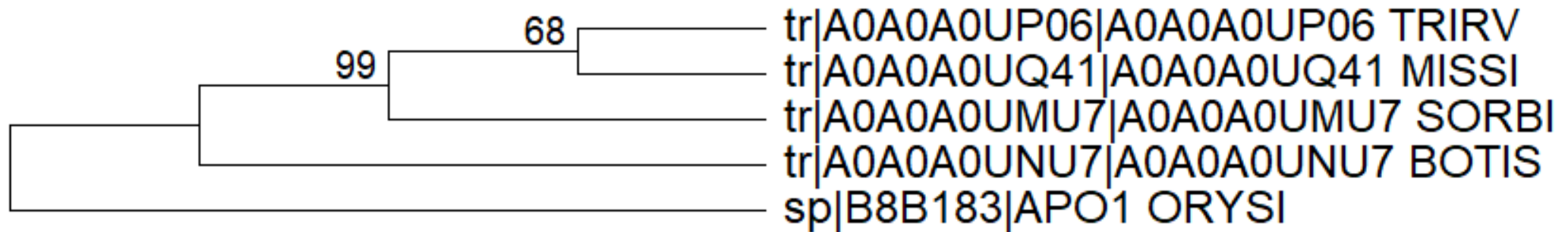
Tool: UniProt



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Species	Scientific name	Accession	AA Length
Rice	<i>Oryza sativa</i>	B8B183	429
Sorghum	<i>Sorghum bicolor</i>	A0A0A0UMU7	196
Yellow bluestem	<i>Bothriochloa ischaemum</i>	A0A0A0UNU7	194
Ravanna grass	<i>Triplidium ravennae</i>	A0A0A0UP06	193
Chinese silver grass	<i>Miscanthus sinensis</i>	A0A0A0UQ41	196



系统发育分析

APO2系统发育分析

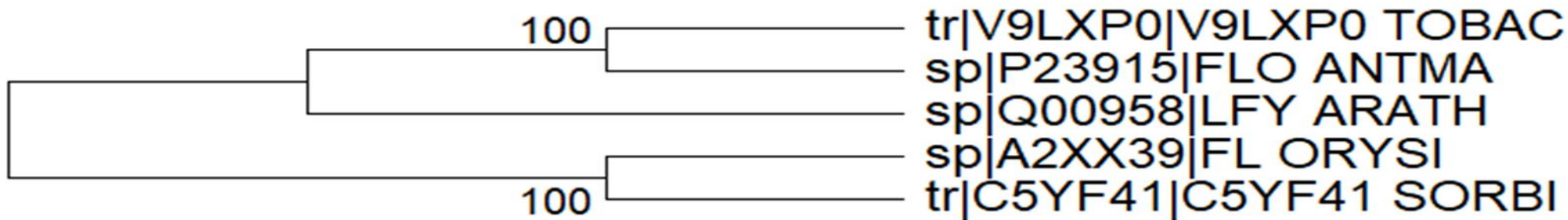
Tool: UniProt



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Species	Scientific name	Accession	AA Length
Rice	<i>Oryza sativa</i>	A2XX39	389
Sorghum	<i>Sorghum bicolor</i>	C5YF41	391
Tobacco	<i>Nicotiana tabacum</i>	V9LXP0	413
Snapdragon	<i>Antirrhinum majus</i>	P23915	396
Mouse-ear cress	<i>Arabidopsis thaliana</i>	Q00958	420



结果分析

INTERPRETATION OF RESULT

1

APO1

荻草与中国芒草同属一个分支（支持率 68%），二者遗传关系最接近；
上述 2 条序列，与高粱、黄须芒草共同聚为一支（支持率 99%），说明这 4 条序列的亲缘关系较近；

2

APO2

烟草与金鱼草聚为一支（支持率 100%）；
水稻与高粱聚为一支（支持率 100%）；
拟南芥 处于上述两个子分支的上游分支中，与这 4 种蛋白的亲缘关系介于两者之间。



Part **4**

潜在互作区域结构预测

APO1与APO2二级结构分析



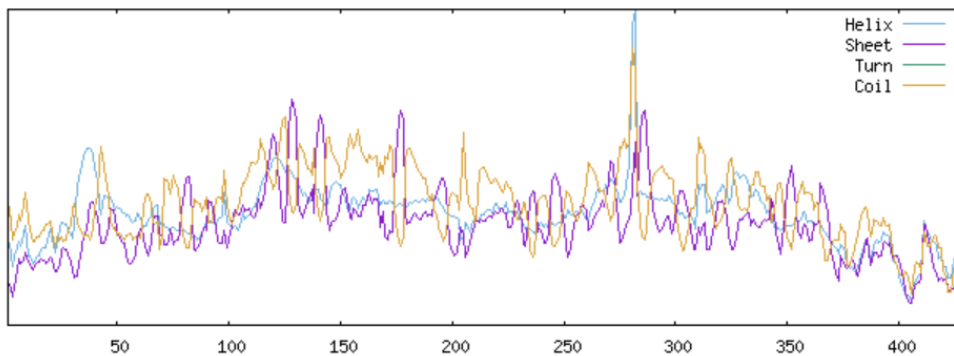
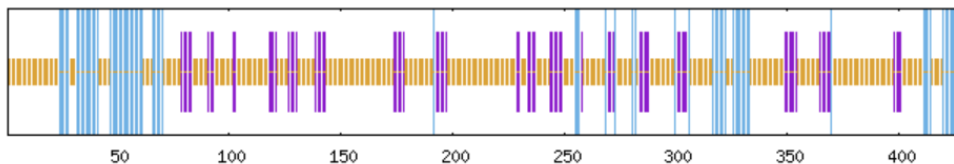
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ENCES

Sequence length : 429

SOPMA :

Alpha helix (Hh) :	73 is	17.02%
3 ₁₀ helix (Gg) :	0 is	0.00%
Pi helix (Ii) :	0 is	0.00%
Beta bridge (Bb) :	0 is	0.00%
Extended strand (Ee) :	74 is	17.25%
Beta turn (Tt) :	0 is	0.00%
Bend region (Ss) :	0 is	0.00%
Random coil (Cc) :	282 is	65.73%
Ambiguous states (?) :	0 is	0.00%
Other states :	0 is	0.00%



Parameters :

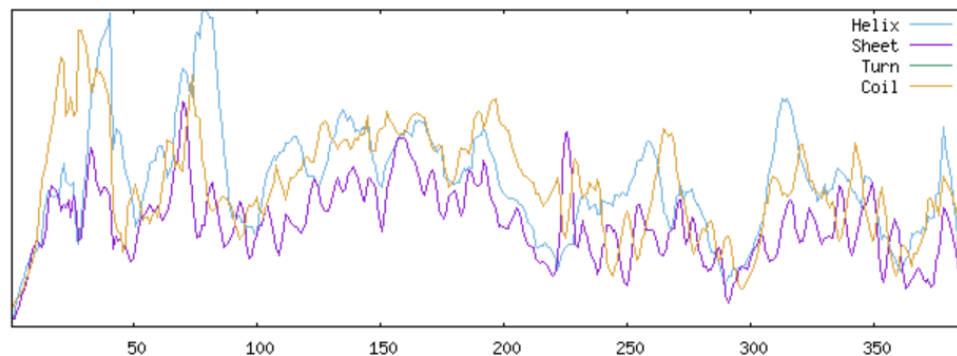
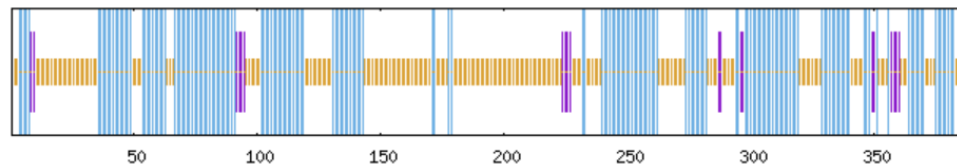
Window width :	17
Similarity threshold :	8
Number of states :	3

APO1

Sequence length : 389

SOPMA :

Alpha helix (Hh) :	179 is	46.02%
3 ₁₀ helix (Gg) :	0 is	0.00%
Pi helix (Ii) :	0 is	0.00%
Beta bridge (Bb) :	0 is	0.00%
Extended strand (Ee) :	20 is	5.14%
Beta turn (Tt) :	0 is	0.00%
Bend region (Ss) :	0 is	0.00%
Random coil (Cc) :	190 is	48.84%
Ambiguous states (?) :	0 is	0.00%
Other states :	0 is	0.00%



Parameters :

Window width :	17
Similarity threshold :	8
Number of states :	3

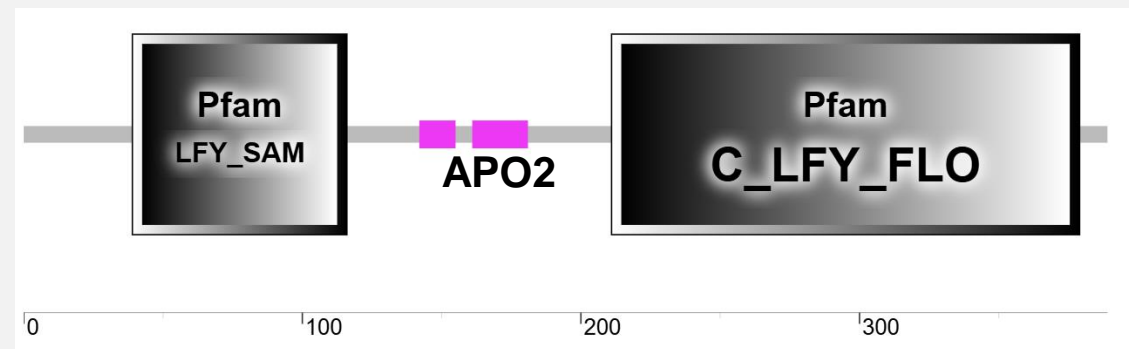
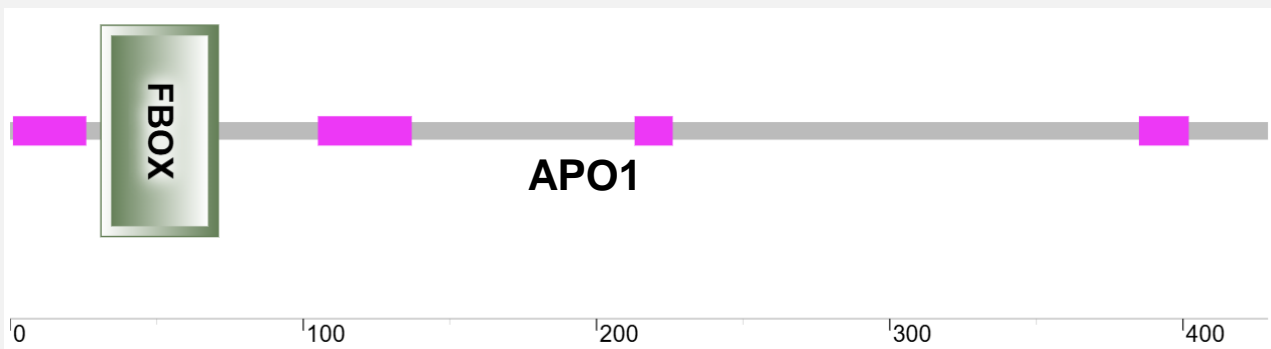
APO2

APO1与APO2蛋白互作的预测



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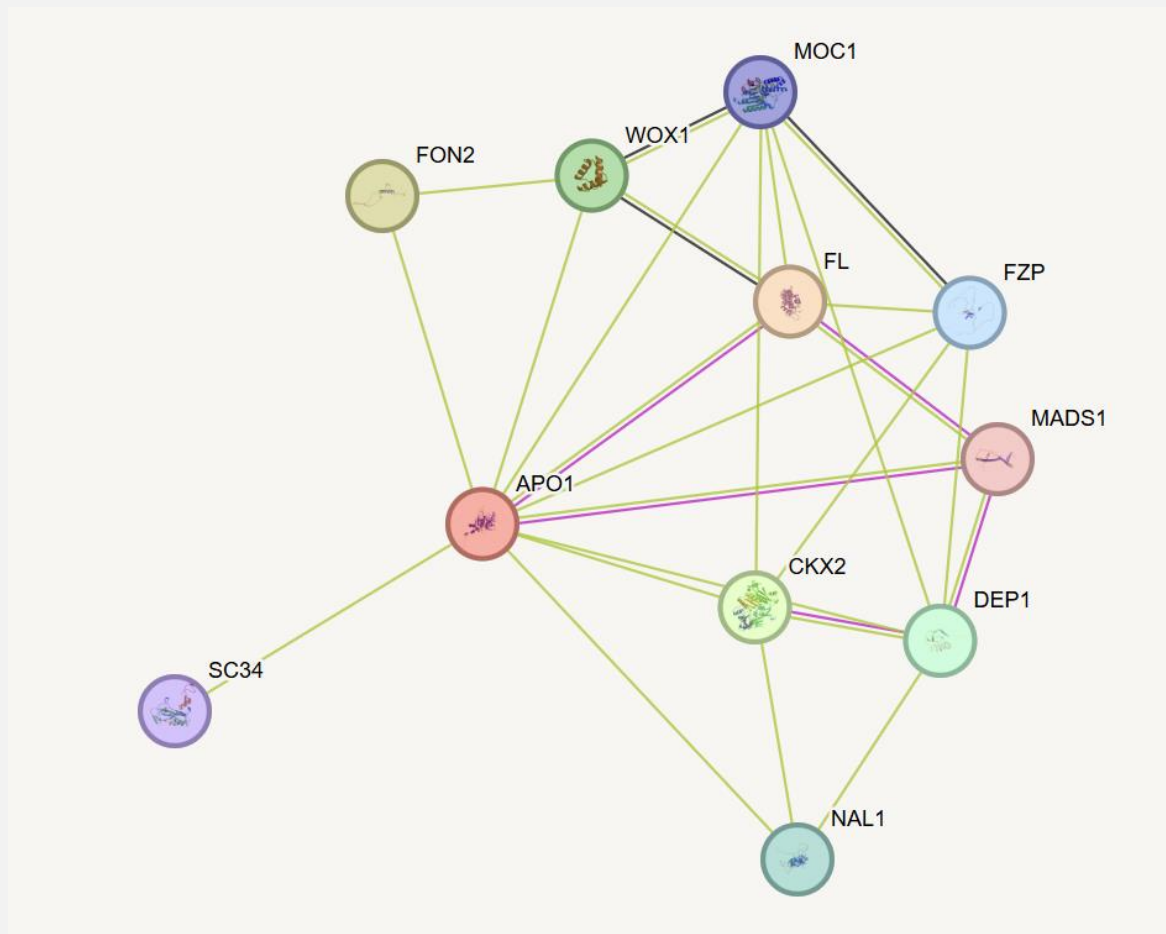


APO1与APO2蛋白互作的预测



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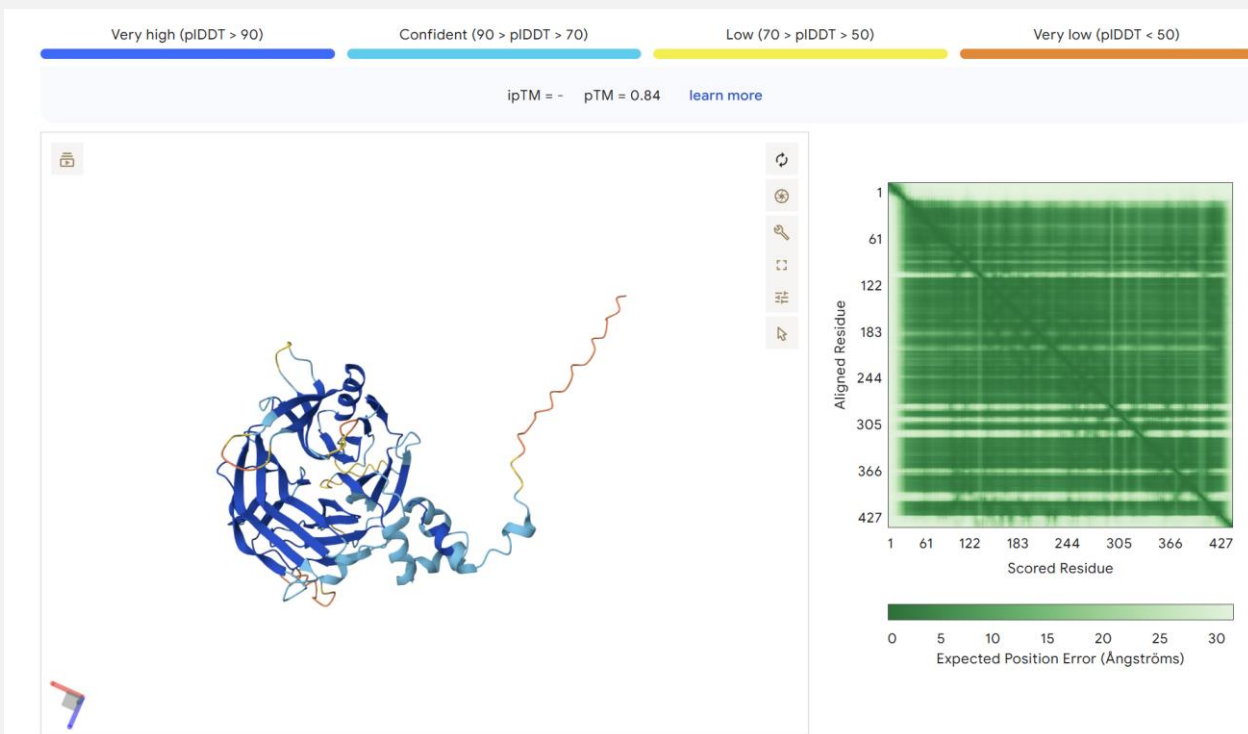


APO1与APO2的蛋白质三维结构预测

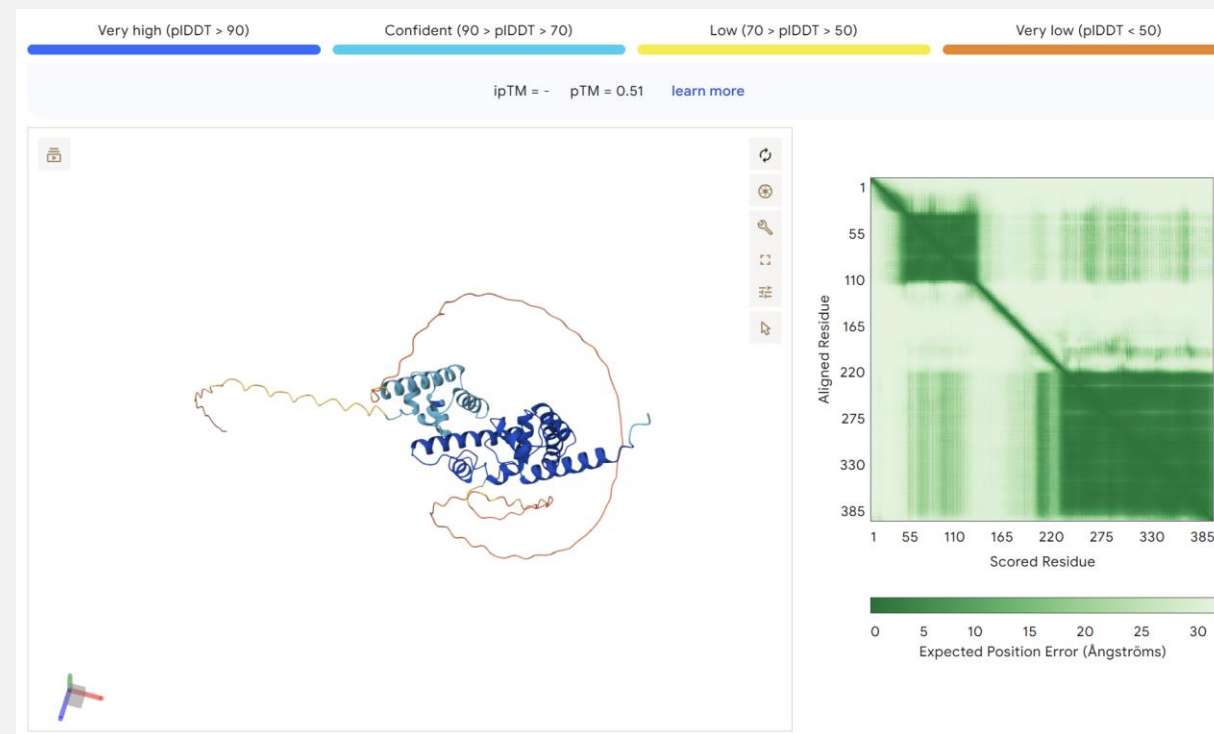


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APO1



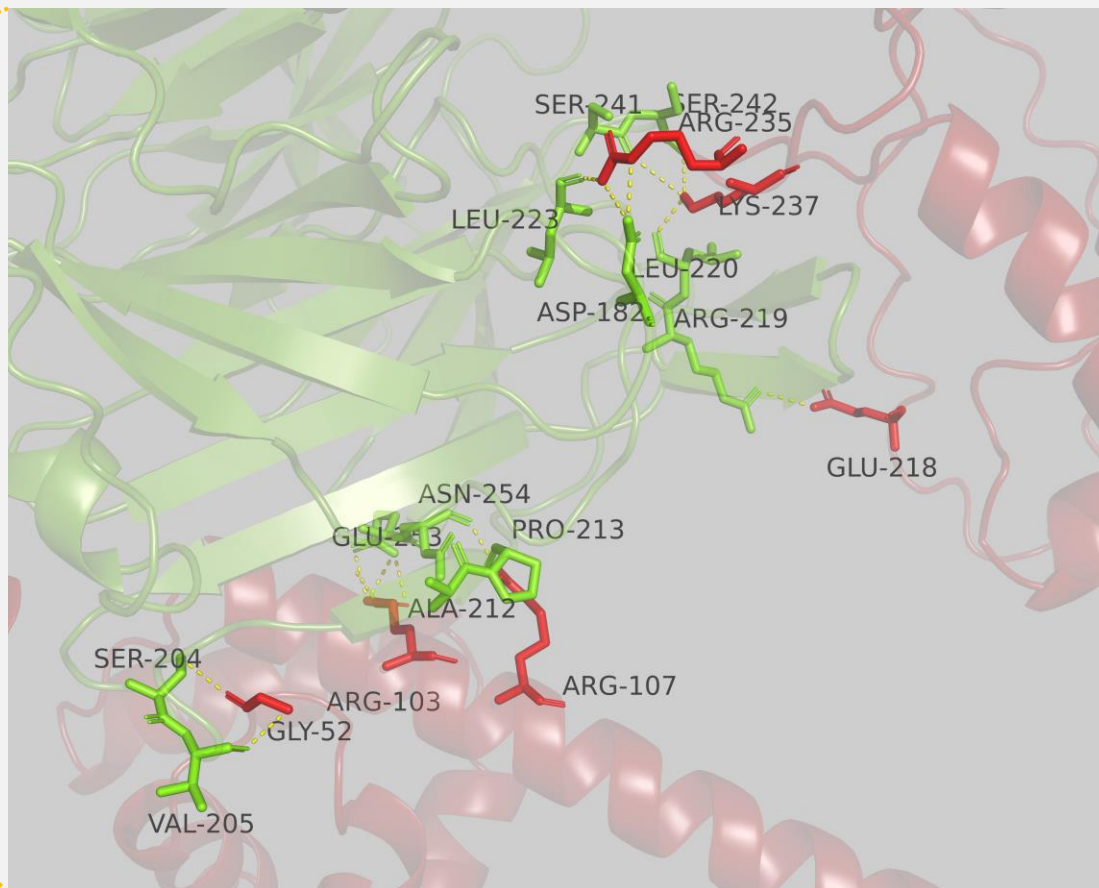
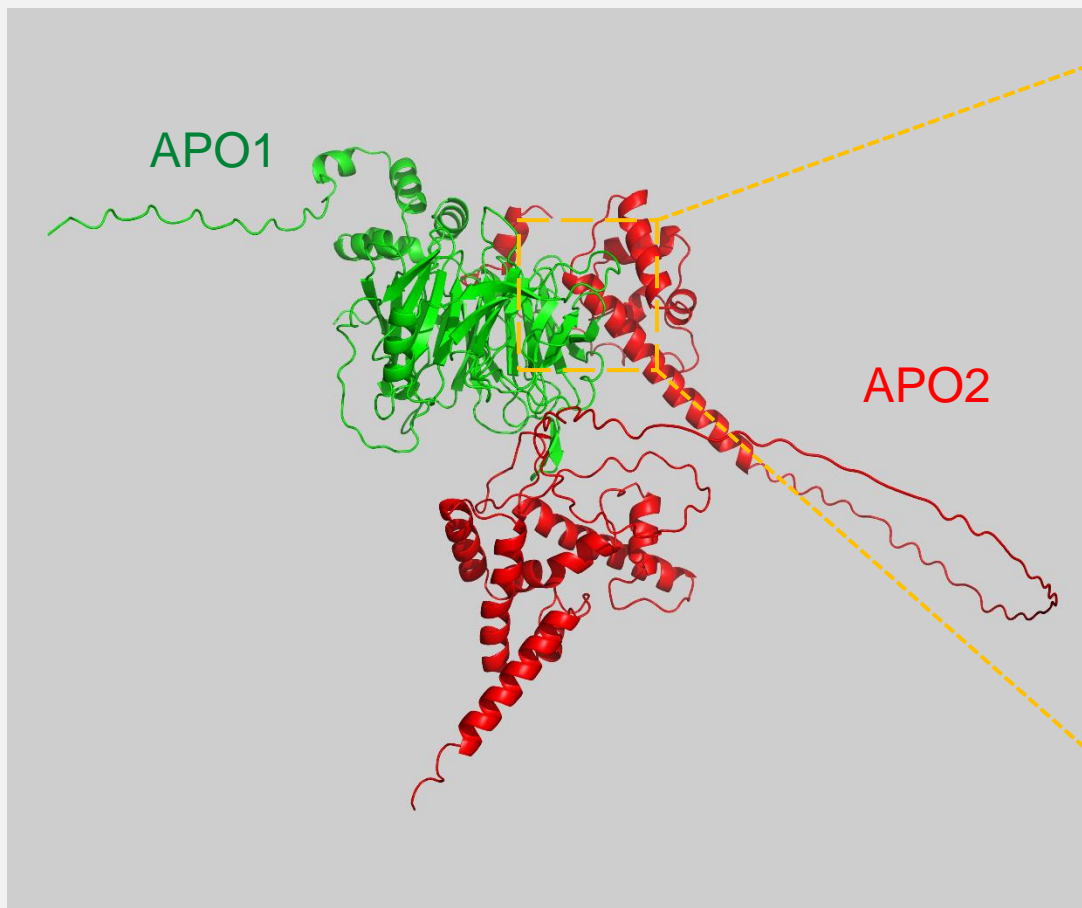
APO2

Pymol可视化互作位点



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Part **5**

讨论与总结

(1) 从生信角度看 APO1–APO2 互作的合理性

- APO1 与 APO2 在表达部位与亚细胞定位上具有重叠
- 均为非跨膜、非分泌蛋白，为直接或复合体互作提供空间基础

(2) 进化保守性支持互作功能的重要性

- 系统发育分析显示 APO1 / APO2 在禾本科中高度保守
- 保守区域与预测互作区域存在重叠，提示该互作机制可能在进化中受到选择

(3) 结构层面对互作位点的支持

- AlphaFold 预测结构显示候选互作区域位于蛋白表面
- 二级结构与疏水性分析表明该区域具备形成稳定蛋白–蛋白界面的可能性

(4) 本研究的局限性

- 生信预测不能替代实验验证
- 尚无法区分直接互作或通过复合物间接互作

APO1与APO2蛋白互作的预测



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通过生物信息学分析，我们预测了 APO1–APO2 潜在的互作区域，并发现这些区域在进化上具有较高保守性，因此推测其在穗发育调控中具有功能意义。下一步工作将重点放在互作关系的实验验证以及关键互作位点的功能解析，从而将生信预测结果转化为可验证的生物学结论。

小组成员照片



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演示完毕 感谢聆听!

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