Structural and functional analysis of the HSP90 heat shock protein family in Blumeria graminis f.sp.tritici

小麦白粉菌热激蛋白家族HSP90结构和功能预测

G07小组成员： 王振花 吕斌娜
刘炳花 阎维巍

汇报人： 王振花

目 录

1、简述小组成员所做的课题

G07B_吕斌娜:

粉红螺旋聚孢霉 内切几丁质酶基因CHI67 的生物信息学分析及功能验证。

粉红螺旋聚孢霉是一类重要的菌寄生菌（生防菌），能够侵染多种植物病原真菌，对多种作物真菌病害具有良好的生防效果。通过对粉红螺旋聚孢霉高效菌株67-1寄生核盘菌转录组测序，获得了一个明显上调表达的内切几丁质酶基因CHI67， CHI67可能参与了粉红螺旋聚孢霉菌寄生过程。后期对该基因进行功能验证和生物信息学分析。

G07C_刘炳花：

链霉菌 产生武夷菌素的 竞争基因簇的竞争机制和作用方式。

武夷菌素是一种由不吸水链霉菌产生的高效、低毒、无残留的新型农用抗生素，可以对多种病原真菌具有很好的抑制作用。通过三代测序得到武夷菌素产生菌CK-15全基因组序列，结合生物信息学分析初步明确武夷菌素次级代谢竞争基因簇，通过对武夷菌素竞争簇的敲除等方法看对产量是否有影响，并且明确竞争簇的竞争机制和作用方式。

G07D_阎维巍：

主要研究c-di-GMP信号受体（PilZ）介导的水稻白叶枯病菌毒性调控途径。

c-di-GMP信号系统是新近发现的细菌第二信号传导系统，通过对c-di-GMP信号受体与互作蛋白的功能分析，鉴定和发掘出新的PilZ受体介导的c-di-GMP信号通路，揭示其抑制病菌毒性表达的调控机制。

G07A_王振花

小麦白粉菌耐高温相关基因的生物信息学分析及功能验证

对小麦白粉菌进行温度敏感性测试，出现耐高温菌株。筛选温度敏感性不同的小麦白粉菌进行转录组分析，筛选耐高温基因进行后续的生物信息学分析和功能验证。

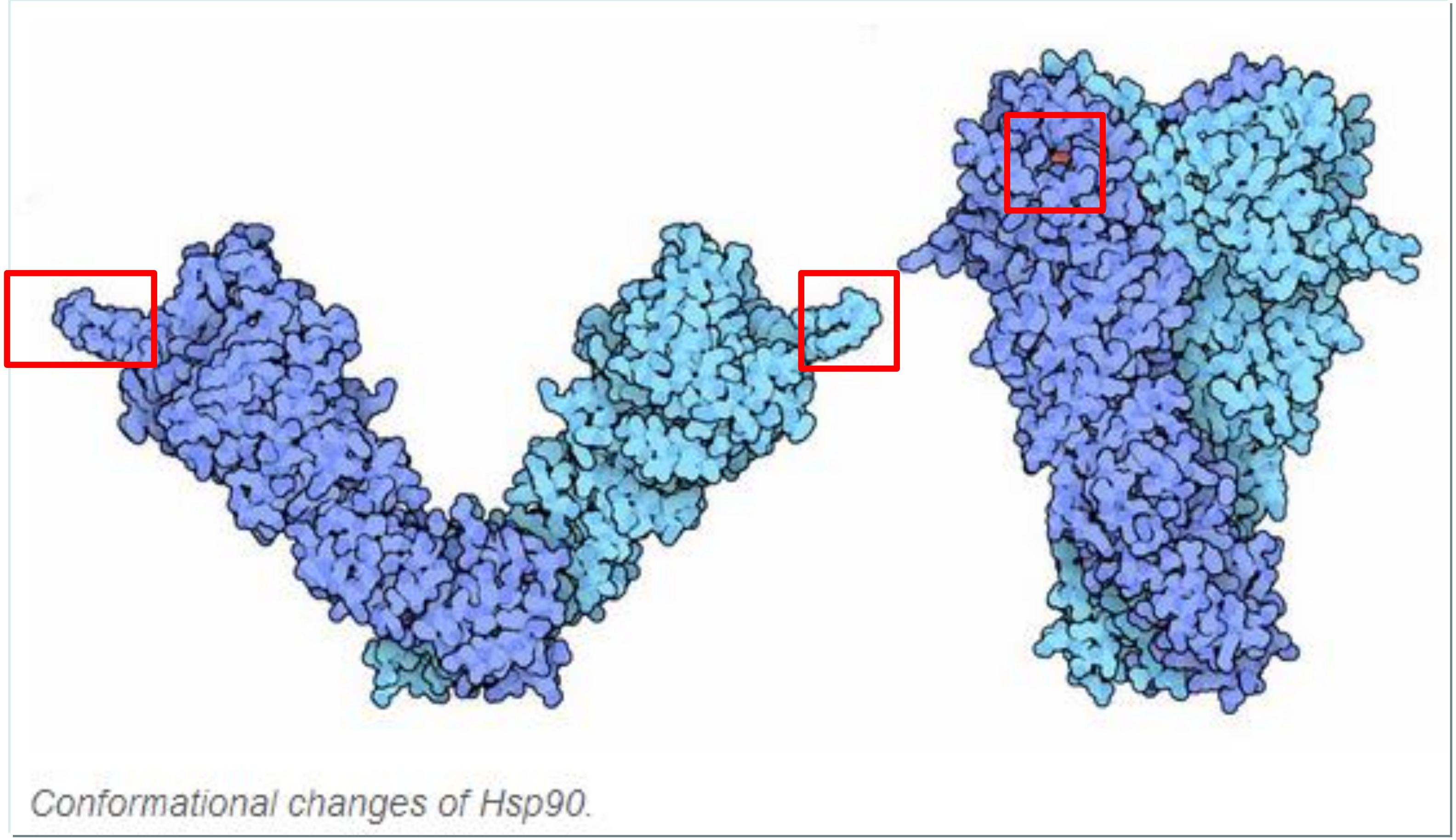
以热激蛋白家族基因HSP90为例进行结构和功能分析。

2、简述热激蛋白HSP90

热激蛋白在生物体中普遍存在，是一组高度保守的蛋白质，按照其分子质量大小可分为HSP110、HSP90、HSP70、HSP60、HSP（25、27、28）五大家族。HSP90分子量约为82—90KDa

利用分子半月报及文献查找HSP90相关信息。

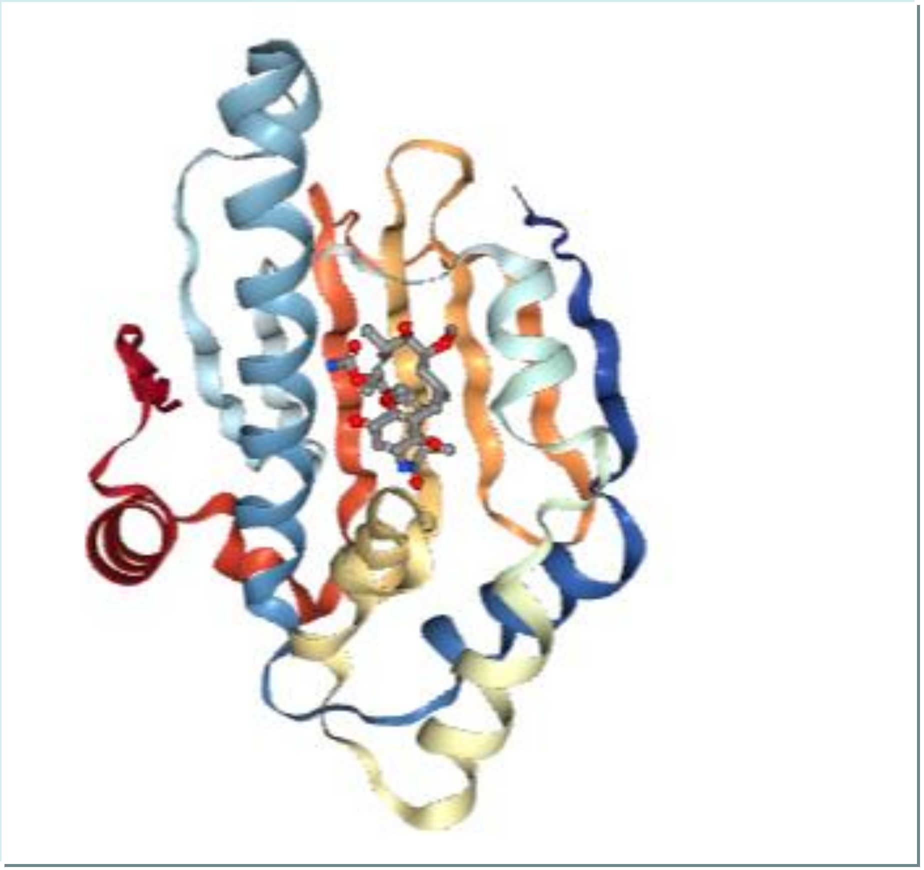
HSP90是一类存在于细胞质中高度保守的同源二聚体分子伴侣。1、当细胞感受到环境变化时，Hsp90阻止一些不成熟蛋白的错误折叠，协助底物蛋白折叠成正确的构像。2、Hsp90与其他的底物蛋白会形成比较大的复合体，参与蛋白质的正确折叠。

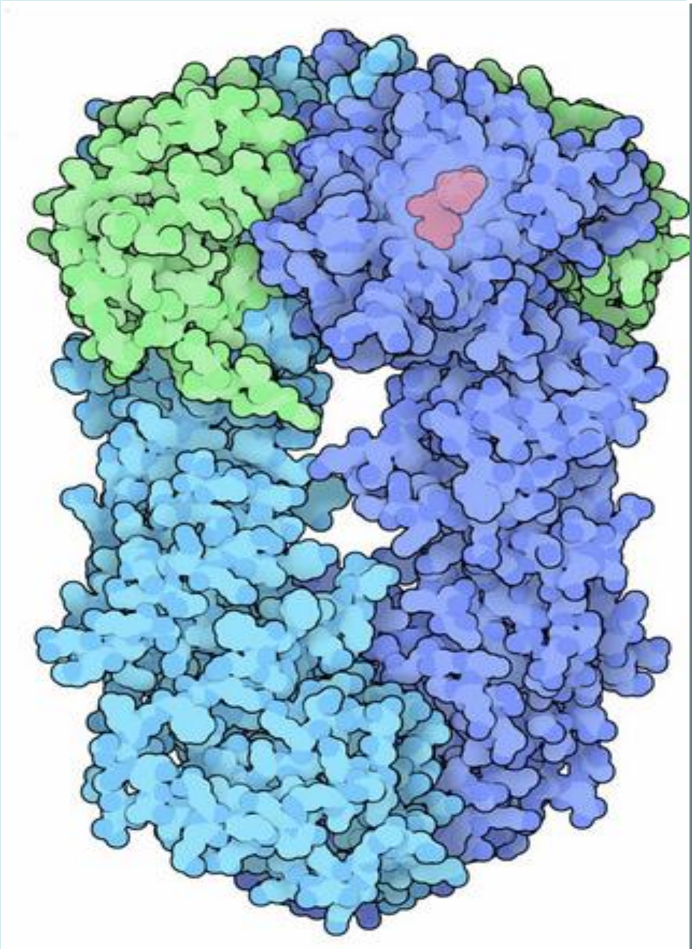


Conformational changes of Hsp90.

左图：HSP90非活性形式，未结合ATP 开放构像，环状结构

右图：HSP90活性形式， 结合ATP后结构紧密的构像



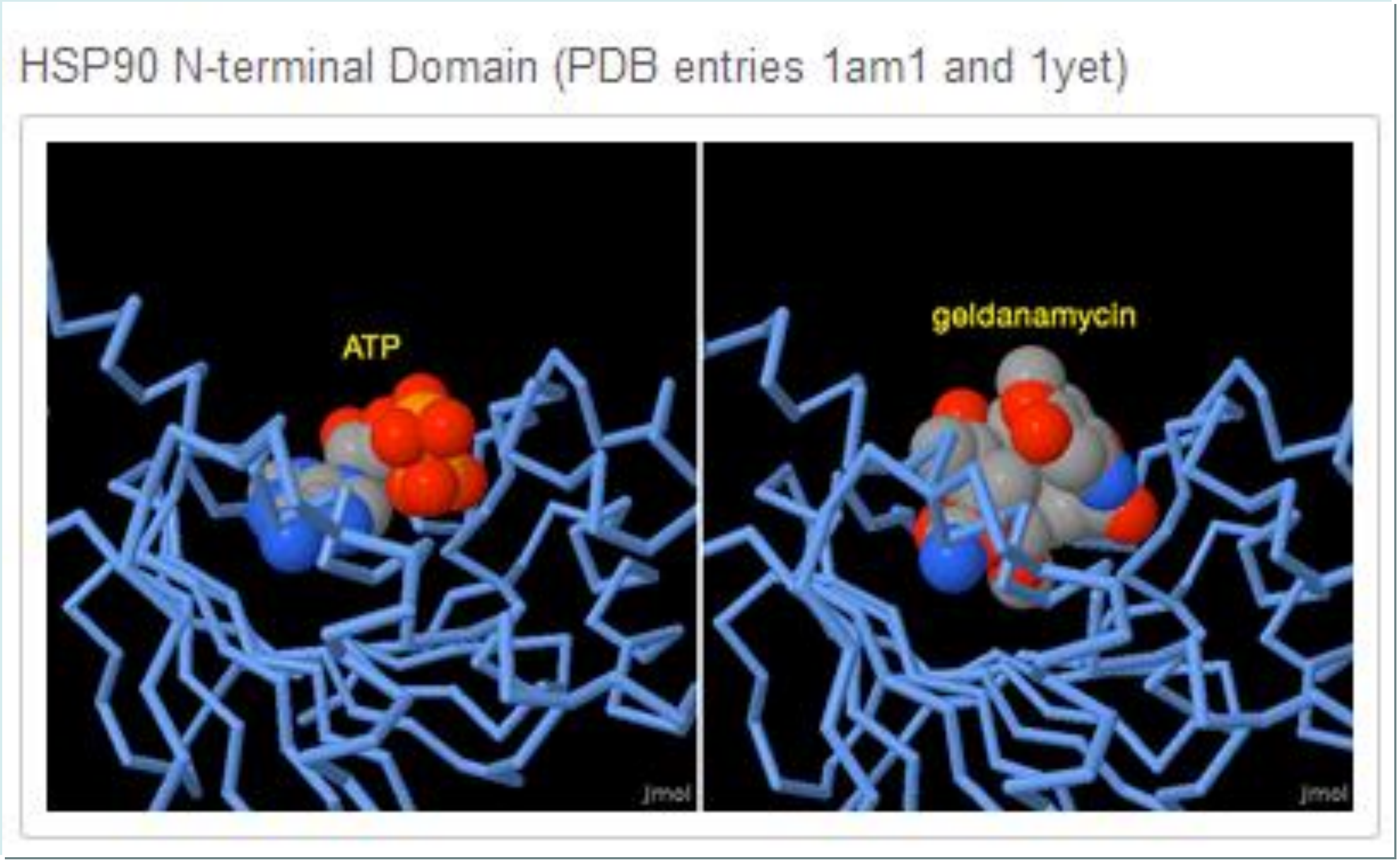


Hsp90 (blue) and cochaperone Sba1 (green), with bound ATP (red).

HSP90：蓝色和蓝绿色
底物蛋白：绿色
ATP：红色

医学上，许多由Hsp90协助合成的蛋白质可以参与细胞的生长。

一般认为Hsp90对正常细胞来说是很重要的，医学上研究癌细胞相对于正常细胞来说更需要Hsp90，Hsp90抑制剂可以抑制癌细胞的发展。从结构上看HSP90 N端区域有ATP结合位点，在癌细胞中，HSP90抑制剂格尔德霉素可以锁定ATP的结合区，防止ATP与HSp90结合，导致形成HSP90的复合体和错误蛋白质。最后通过破坏控制细胞生长的信号通路杀死癌细胞。



HSP90 N-terminal Domain (PDB entries 1am1 and 1yet)

ATP结合区

格尔德霉素与ATP结合区结合

3、对HSP90进行生物信息学分析

3.1、利用NCBI、Uniprot查找HSP90序列信息

3.2、利用MEGA7进行多序列比对

3.3、利用MEGA7构建进化树

3.4、利用NCBI 对HSP90 预测ORF

3.5、利用CBS、Predict protein、Smart 、DNAMAN 对HSP90进行结构预测

3.6、利用Phyre2预测HSP90模型

3.7、利用Swiss-PdbViewer对HSP90分析

3.8、利用NCBI 进行引物设计

3.1、利用NCBI、Uniprot查找HSP90序列信息

NCBI查找HSP90序列信息

Protein

(hsp90) AND blumeria graminis f.sp.tritici[Organism]

Species clear
✓ Fungi (12)
Customize ...

Source databases
Customize ...

Sequence length
Custom range...

Molecular weight
Custom range...

Release date
Custom range...

Revision date
Custom range...

Clear all

Show additional filters

See the results of this search (2 items) in our new Identical Protein Groups database.

Items: 12

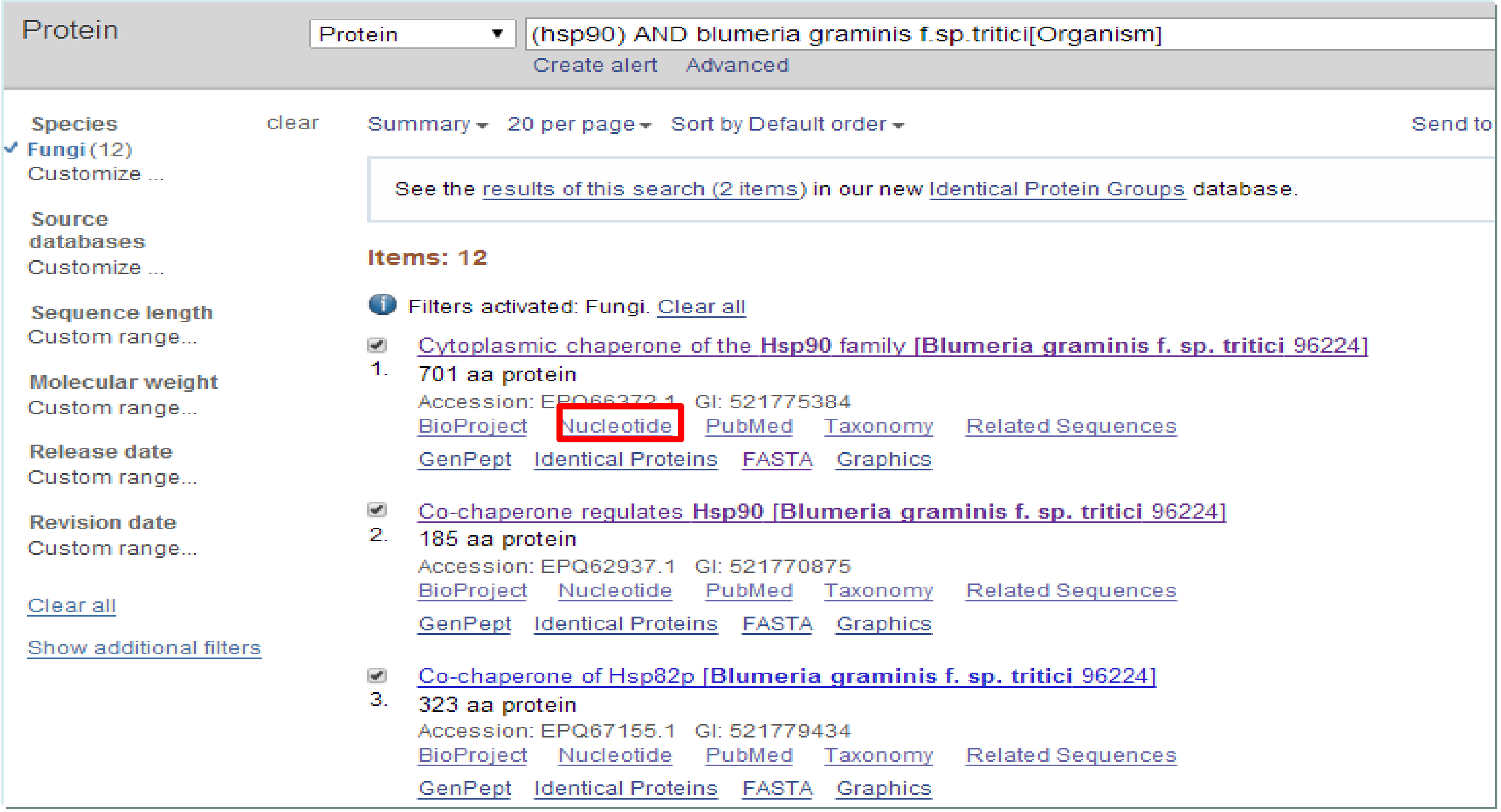
Filters activated: Fungi. Clear all

☑ 1. Cytoplasmic chaperone of the Hsp90 family [Blumeria graminis f. sp. tritici 96224]
701 aa protein
Accession: EPQ66372.1 GI: 521775384
BioProject Nucleotide PubMed Taxonomy Related Sequences
GenPept Identical Proteins FASTA Graphics

☑ 2. Co-chaperone regulates Hsp90 [Blumeria graminis f. sp. tritici 96224]
185 aa protein
Accession: EPQ62937.1 GI: 521770875
BioProject Nucleotide PubMed Taxonomy Related Sequences
GenPept Identical Proteins FASTA Graphics

☑ 3. Co-chaperone of Hsp82p [Blumeria graminis f. sp. tritici 96224]
323 aa protein
Accession: EPQ67155.1 GI: 521779434
BioProject Nucleotide PubMed Taxonomy Related Sequences
GenPept Identical Proteins FASTA Graphics

NCBI查找HSP90核酸序列信息



Protein

Protein ▼ (hsp90) AND blumeria graminis f.sp.tritici[Organism]

Create alert Advanced

Species clear
✓ Fungi (12)
Customize ...

Source databases
Customize ...

Sequence length
Custom range...

Molecular weight
Custom range...

Release date
Custom range...

Revision date
Custom range...

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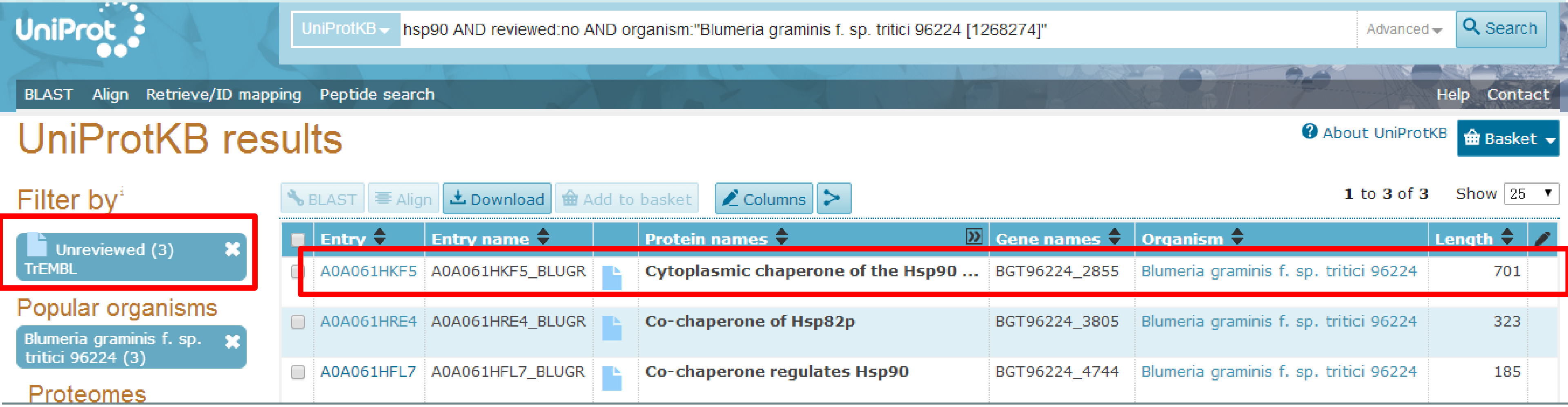
BioProject Nucleotide PubMed Taxonomy Related Sequences
GenPept Identical Proteins FASTA Graphics

Uniprot查找HSP90蛋白序列信息



hsp90 AND reviewed:no AND organism:"Blumeria graminis f. sp. tritici 96224 [1268274]"

UniProtKB results

Filter by

Unreviewed (3) TrEMBL

Popular organisms

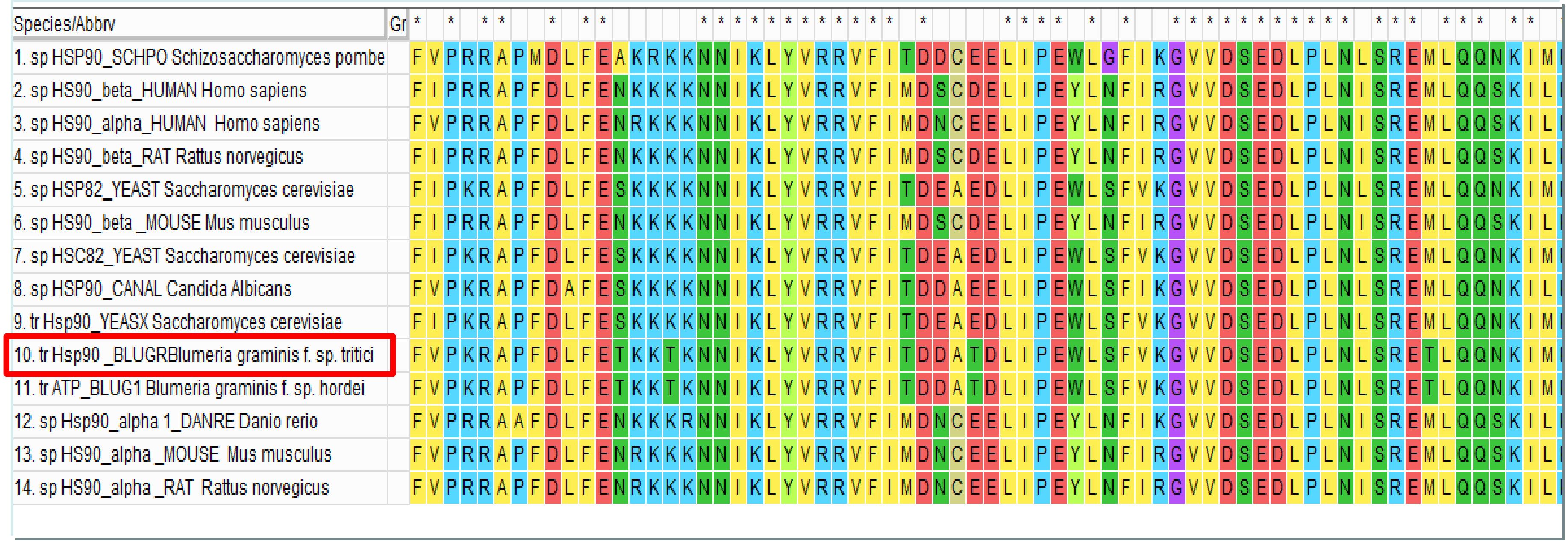
Blumeria graminis f. sp. tritici 96224 (3)

Proteomes

1 to 3 of 3 Show 25

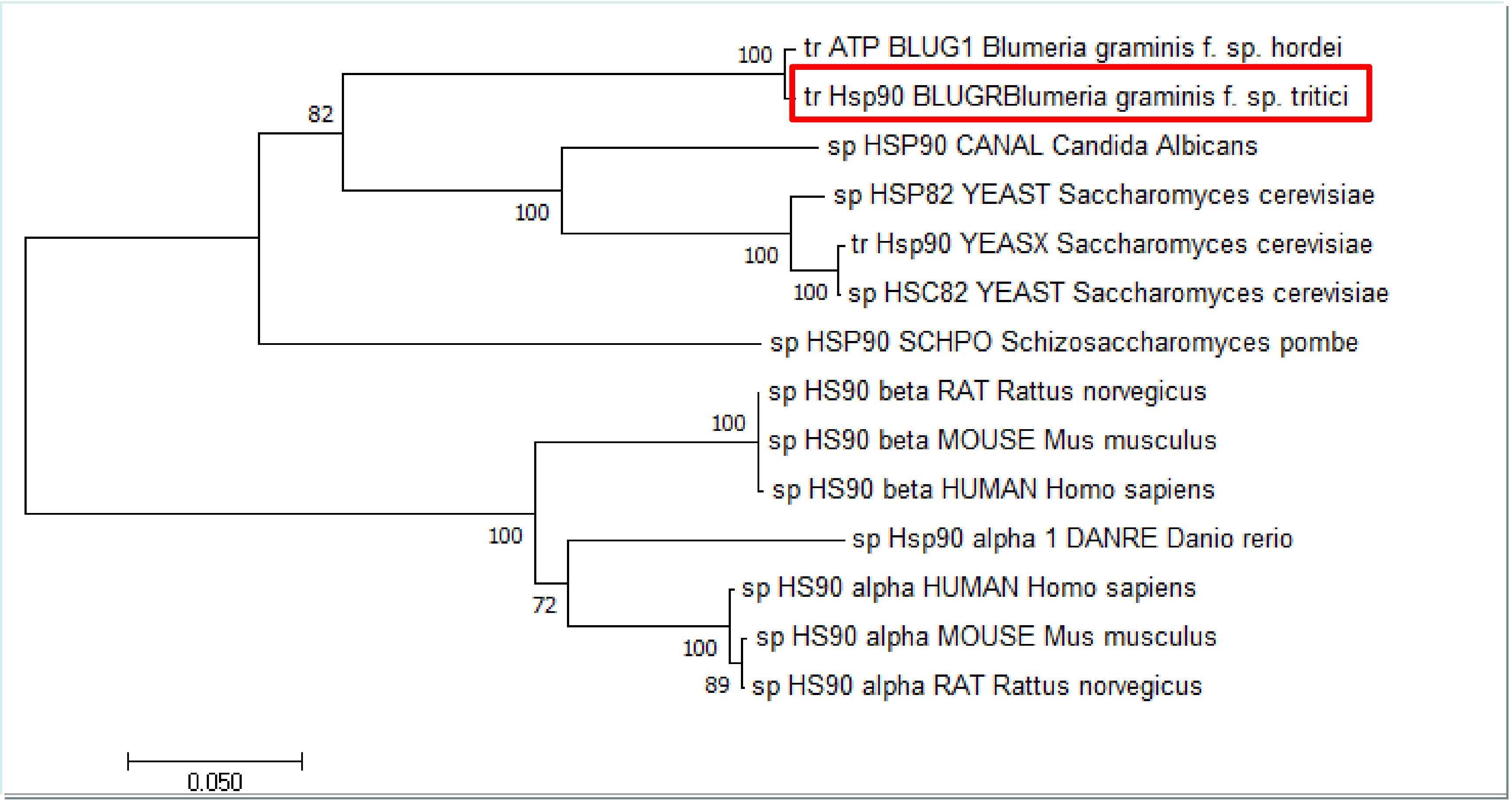
	Entry	Entry name		Protein names	Gene names	Organism	Length
☐	A0A061HKF5	A0A061HKF5_BLUGR		**Cytoplasmic chaperone of the Hsp90 ...**	BGT96224_2855	Blumeria graminis f. sp. tritici 96224	701
☐	A0A061HRE4	A0A061HRE4_BLUGR		**Co-chaperone of Hsp82p**	BGT96224_3805	Blumeria graminis f. sp. tritici 96224	323
☐	A0A061HFL7	A0A061HFL7_BLUGR		**Co-chaperone regulates Hsp90**	BGT96224_4744	Blumeria graminis f. sp. tritici 96224	185

利用MEGA7进行多序列比对

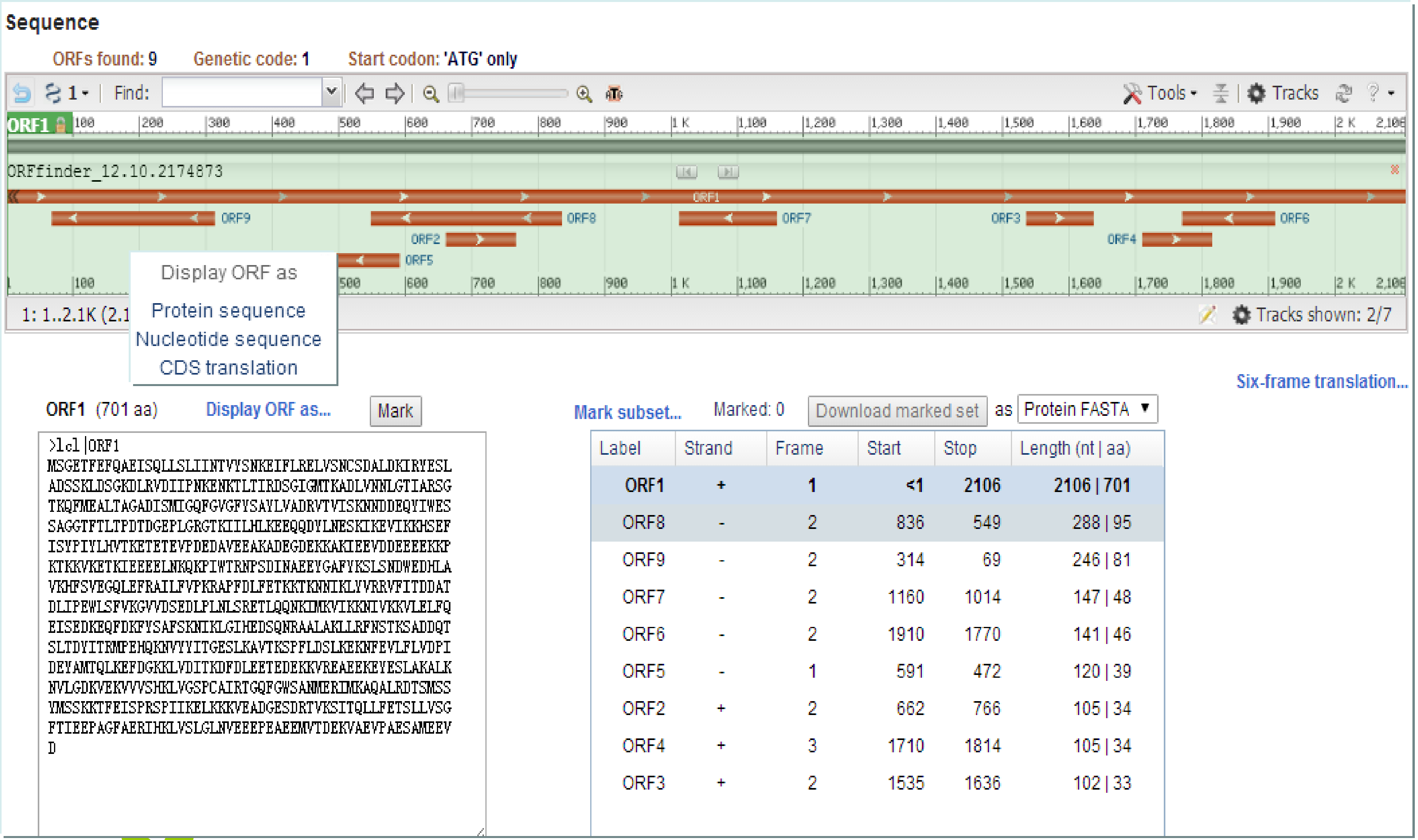


Species/Abbrv	Gr	*		*		*	*			*		*	*						*	*	*	*	*	*	*	*	*	*	*	*		*					*	*	*	*		*		*			*	*	*	*	*	*	*	*	*	*	*		*	*	*		*	*	*		*	*	
1. sp HSP90_SCHPO Schizosaccharomyces pombe		F	V	P	R	R	A	P	M	D	L	F	E	A	K	R	K	K	N	N	I	K	L	Y	V	R	R	V	F	I	T	D	D	C	E	E	L	I	P	E	W	L	G	F	I	K	G	V	V	D	S	E	D	L	P	L	N	L	S	R	E	M	L	Q	Q	N	K	I	M
2. sp HS90_beta_HUMAN Homo sapiens		F	I	P	R	R	A	P	F	D	L	F	E	N	K	K	K	K	N	N	I	K	L	Y	V	R	R	V	F	I	M	D	S	C	D	E	L	I	P	E	Y	L	N	F	I	R	G	V	V	D	S	E	D	L	P	L	N	I	S	R	E	M	L	Q	Q	S	K	I	L
3. sp HS90_alpha_HUMAN Homo sapiens		F	V	P	R	R	A	P	F	D	L	F	E	N	R	K	K	K	N	N	I	K	L	Y	V	R	R	V	F	I	M	D	N	C	E	E	L	I	P	E	Y	L	N	F	I	R	G	V	V	D	S	E	D	L	P	L	N	I	S	R	E	M	L	Q	Q	S	K	I	L
4. sp HS90_beta_RAT Rattus norvegicus		F	I	P	R	R	A	P	F	D	L	F	E	N	K	K	K	K	N	N	I	K	L	Y	V	R	R	V	F	I	M	D	S	C	D	E	L	I	P	E	Y	L	N	F	I	R	G	V	V	D	S	E	D	L	P	L	N	I	S	R	E	M	L	Q	Q	S	K	I	L
5. sp HSP82_YEAST Saccharomyces cerevisiae		F	I	P	K	R	A	P	F	D	L	F	E	S	K	K	K	K	N	N	I	K	L	Y	V	R	R	V	F	I	T	D	E	A	E	D	L	I	P	E	W	L	S	F	V	K	G	V	V	D	S	E	D	L	P	L	N	L	S	R	E	M	L	Q	Q	N	K	I	M
6. sp HS90_beta _MOUSE Mus musculus		F	I	P	R	R	A	P	F	D	L	F	E	N	K	K	K	K	N	N	I	K	L	Y	V	R	R	V	F	I	M	D	S	C	D	E	L	I	P	E	Y	L	N	F	I	R	G	V	V	D	S	E	D	L	P	L	N	I	S	R	E	M	L	Q	Q	S	K	I	L
7. sp HSC82_YEAST Saccharomyces cerevisiae		F	I	P	K	R	A	P	F	D	L	F	E	S	K	K	K	K	N	N	I	K	L	Y	V	R	R	V	F	I	T	D	E	A	E	D	L	I	P	E	W	L	S	F	V	K	G	V	V	D	S	E	D	L	P	L	N	L	S	R	E	M	L	Q	Q	N	K	I	M
8. sp HSP90_CANAL Candida Albicans		F	V	P	K	R	A	P	F	D	A	F	E	S	K	K	K	K	N	N	I	K	L	Y	V	R	R	V	F	I	T	D	D	A	E	E	L	I	P	E	W	L	S	F	I	K	G	V	V	D	S	E	D	L	P	L	N	L	S	R	E	M	L	Q	Q	N	K	I	L
9. tr Hsp90_YEASX Saccharomyces cerevisiae		F	I	P	K	R	A	P	F	D	L	F	E	S	K	K	K	K	N	N	I	K	L	Y	V	R	R	V	F	I	T	D	E	A	E	D	L	I	P	E	W	L	S	F	V	K	G	V	V	D	S	E	D	L	P	L	N	L	S	R	E	M	L	Q	Q	N	K	I	M
10. tr Hsp90 _BLUGRBlumeria graminis f. sp. tritici		F	V	P	K	R	A	P	F	D	L	F	E	T	K	K	T	K	N	N	I	K	L	Y	V	R	R	V	F	I	T	D	D	A	T	D	L	I	P	E	W	L	S	F	V	K	G	V	V	D	S	E	D	L	P	L	N	L	S	R	E	T	L	Q	Q	N	K	I	M
11. tr ATP_BLUG1 Blumeria graminis f. sp. hordei		F	V	P	K	R	A	P	F	D	L	F	E	T	K	K	T	K	N	N	I	K	L	Y	V	R	R	V	F	I	T	D	D	A	T	D	L	I	P	E	W	L	S	F	V	K	G	V	V	D	S	E	D	L	P	L	N	L	S	R	E	T	L	Q	Q	N	K	I	M
12. sp Hsp90_alpha 1_DANRE Danio rerio		F	V	P	R	R	A	A	F	D	L	F	E	N	K	K	K	R	N	N	I	K	L	Y	V	R	R	V	F	I	M	D	N	C	E	E	L	I	P	E	Y	L	N	F	I	K	G	V	V	D	S	E	D	L	P	L	N	I	S	R	E	M	L	Q	Q	S	K	I	L
13. sp HS90_alpha _MOUSE Mus musculus		F	V	P	R	R	A	P	F	D	L	F	E	N	R	K	K	K	N	N	I	K	L	Y	V	R	R	V	F	I	M	D	N	C	E	E	L	I	P	E	Y	L	N	F	I	R	G	V	V	D	S	E	D	L	P	L	N	I	S	R	E	M	L	Q	Q	S	K	I	L
14. sp HS90_alpha _RAT Rattus norvegicus		F	V	P	R	R	A	P	F	D	L	F	E	N	R	K	K	K	N	N	I	K	L	Y	V	R	R	V	F	I	M	D	N	C	E	E	L	I	P	E	Y	L	N	F	I	R	G	V	V	D	S	E	D	L	P	L	N	I	S	R	E	M	L	Q	Q	S	K	I	L

利用MEGA7构建进化树



利用NCBI 对HSP90 预测ORF



ORFs found: 9 Genetic code: 1 Start codon: 'ATG' only

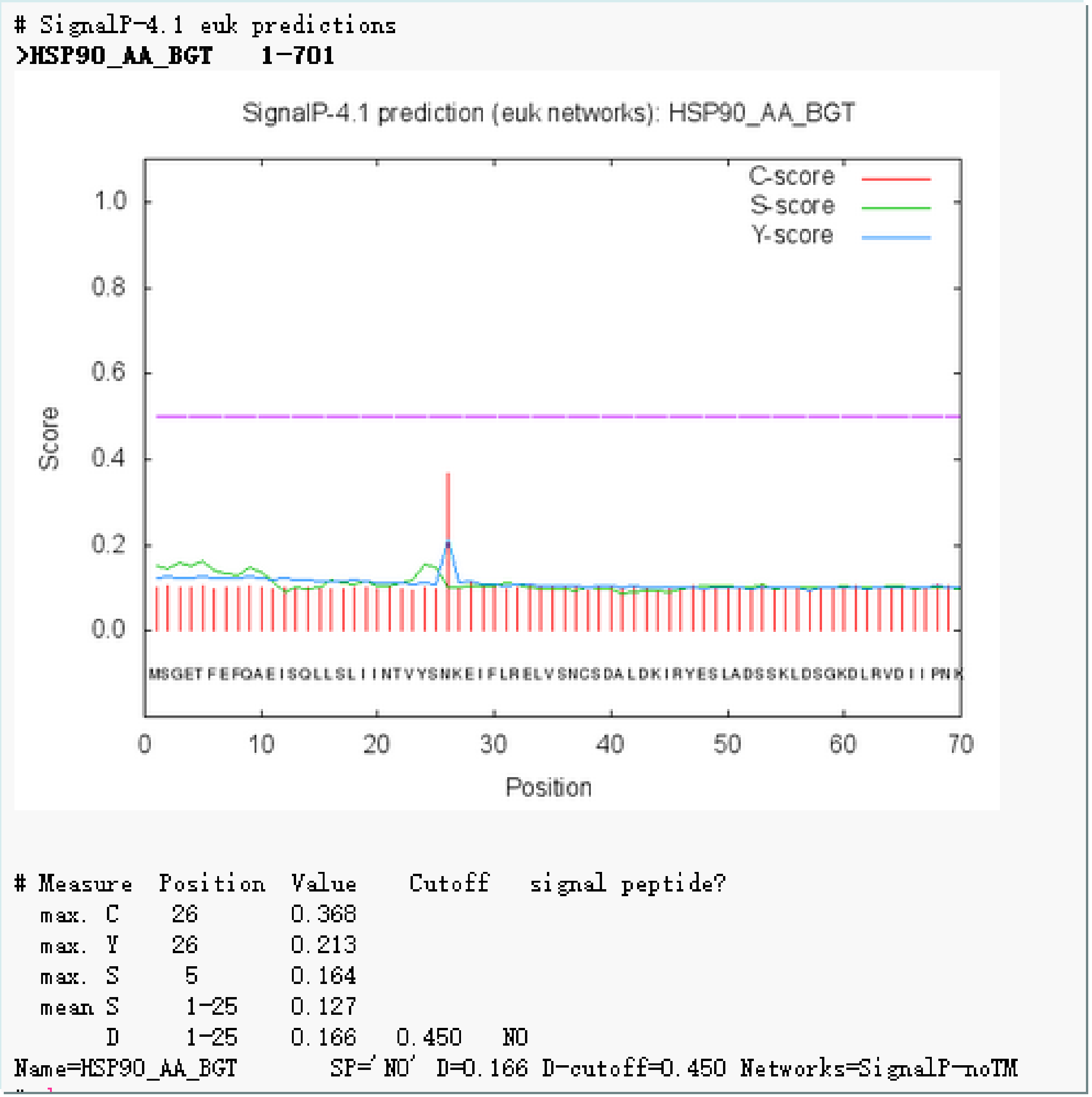
ORF1 (701 aa)

```
>lcl|ORF1
MSGETFEFQAEISQLLSLIINTVYSNKEIFLRELVSNCSDALDKIRYESL
ADSSKLDSGKDLRVDIIPNKENKTLTIRDSGIGMTKADLVNNLGTIARSG
TKQFMEALTAGADISMIGQFGVGFYSAYLVADRVTVISKNNDDEQYIWES
SAGGTFTLTPDTDGEPLGRGTKIILHLKEEQQDYLNESKIKEVIKKHSEF
ISYPIYLHVTKETETEVPDEDAVEEAKADEGDEKKAKIEEVDDEEEEKKP
KTKKVKETKIEEEELNKQKPIWTRNPSDINAEEYGAFYKSLSNDWEDHLA
VKHFSVEGQLEFRAILFVPKRAPFDLFETKKTKNNIKLYVRRVFITDDAT
DLIPEWLSFVKGVVDSEDLPLNLSRETLQQNKIMKVIKKNIVKKVLELFQ
EISEDKEQFDKFYSAFSKNIKLGIHEDSQNRAALAKLLRFNSTKSADDQT
SLTDYITRMPEHQKNVYYITGESLKAVTKSPFLDSLKEKNFEVLFLVDPI
DEYAMTQLKEFDGKKLVDITKDFDLEETEDEKKVREAEEKEYESLAKALK
NVLGDKVEKVVVSHKLVGSPCAIRTGQFGWSANMERIMKAQALRDTSMSS
YMSSKKTFEISPRSPIIKELKKKVEADGESDRTVKSITQLLFETSLLVSG
FTIEEPAGFAERIHKLVSLGLNVEEEPEAEEMVTDEKVAEVPAESAMEEV
D
```

Marked: 0

Label	Strand	Frame	Start	Stop	Length (nt | aa)
ORF1	+	1	<1	2106	2106 | 701
ORF8	-	2	836	549	288 | 95
ORF9	-	2	314	69	246 | 81
ORF7	-	2	1160	1014	147 | 48
ORF6	-	2	1910	1770	141 | 46
ORF5	-	1	591	472	120 | 39
ORF2	+	2	662	766	105 | 34
ORF4	+	3	1710	1814	105 | 34
ORF3	+	2	1535	1636	102 | 33

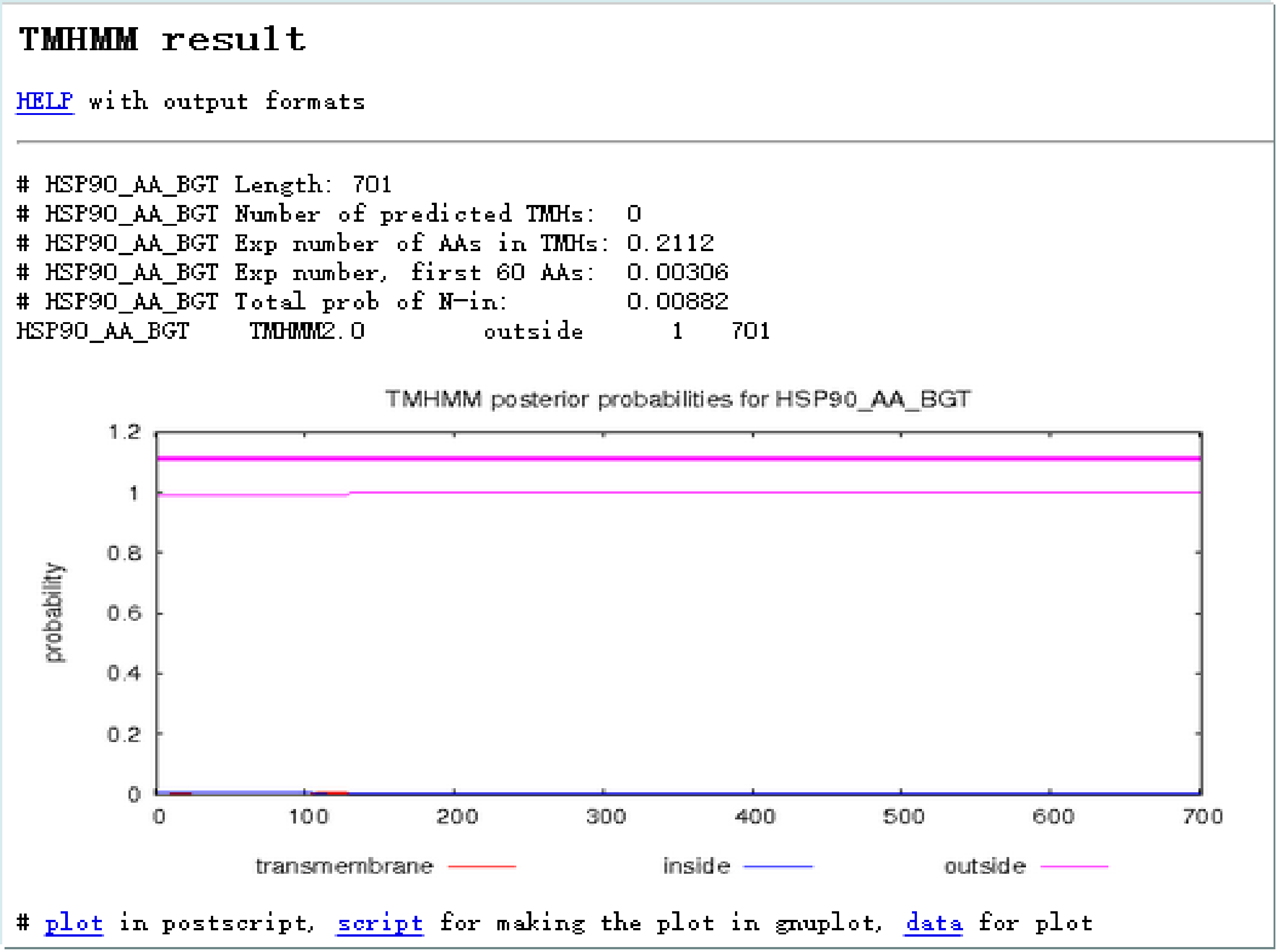
利用CBS 对HSP90进行信号肽预测。



```
# SignalP-4.1 euk predictions
>HSP90_AA_BGT    1-701
```

```
# Measure  Position  Value    Cutoff   signal peptide?
  max. C    26       0.368
  max. Y    26       0.213
  max. S     5       0.164
  mean S     1-25    0.127
       D     1-25    0.166   0.450   NO
Name=HSP90_AA_BGT      SP='NO'  D=0.166 D-cutoff=0.450 Networks=SignalP-noTM
```

利用CBS 对HSP90跨膜结构预测。



TMHMM result

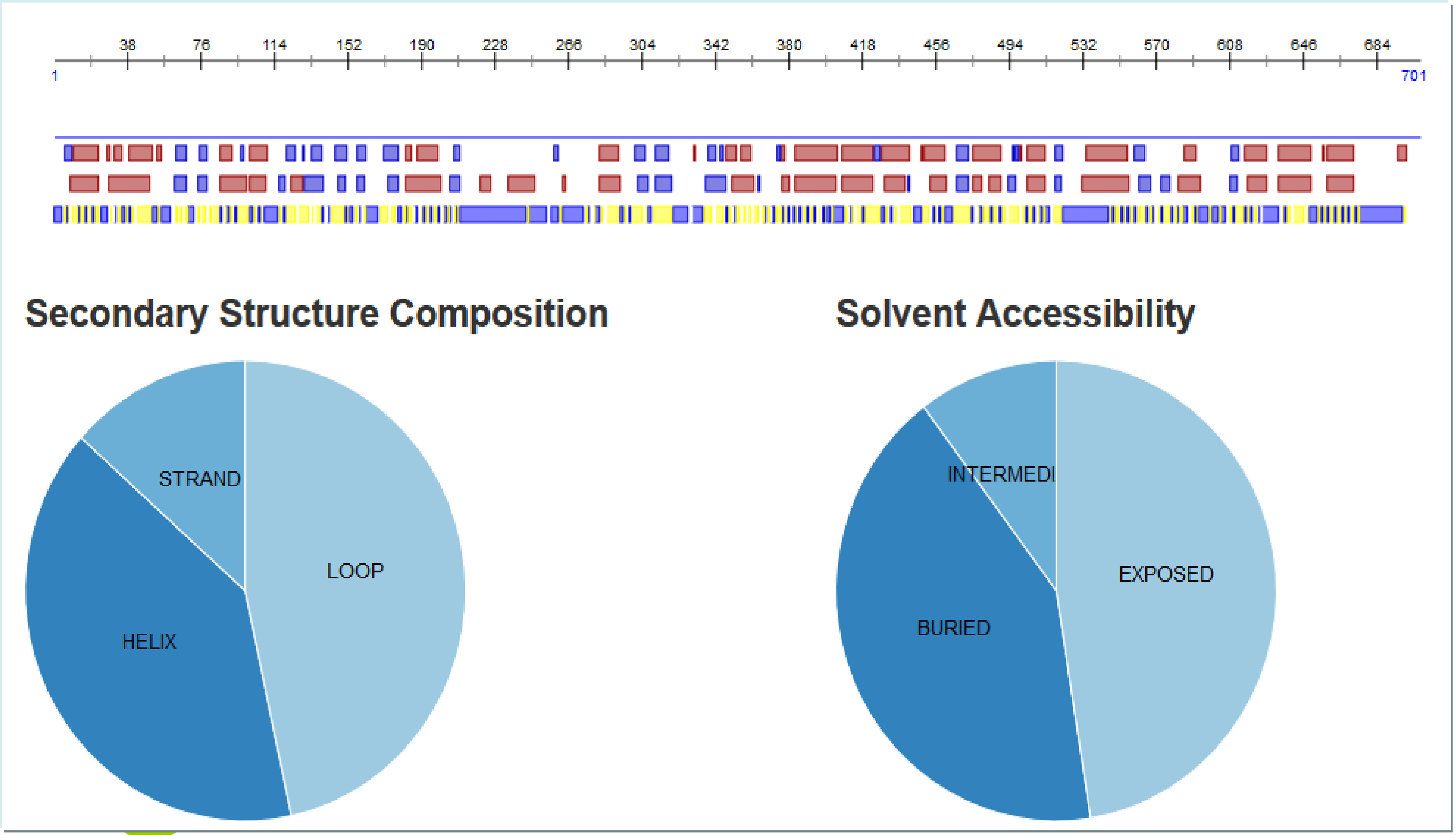
HELP with output formats

```
# HSP90_AA_BGT Length: 701
# HSP90_AA_BGT Number of predicted TMHs:  0
# HSP90_AA_BGT Exp number of AAs in TMHs: 0.2112
# HSP90_AA_BGT Exp number, first 60 AAs:  0.00306
# HSP90_AA_BGT Total prob of N-in:        0.00882
HSP90_AA_BGT    TMHMM2.0        outside      1   701
```

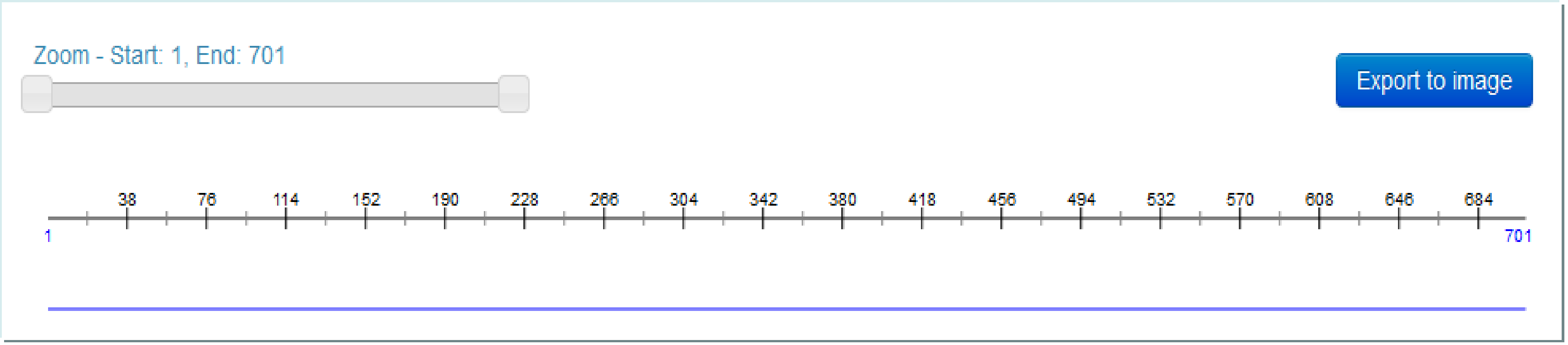
plot in postscript, script for making the plot in gnuplot, data for plot

利用Predict protein对HSP90进行蛋白结构预测。

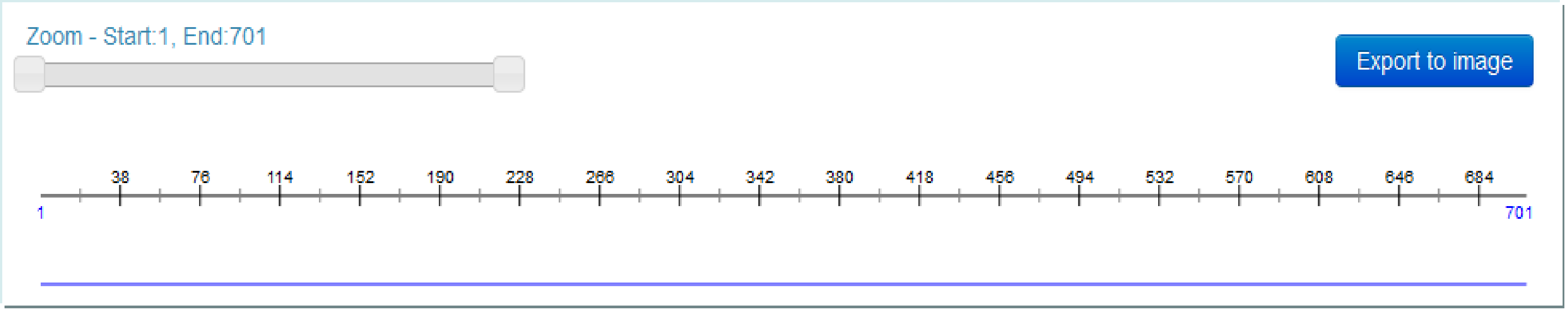
Secondary Structure and Solvent Accessibility Prediction



Transmembrane Helices Prediction

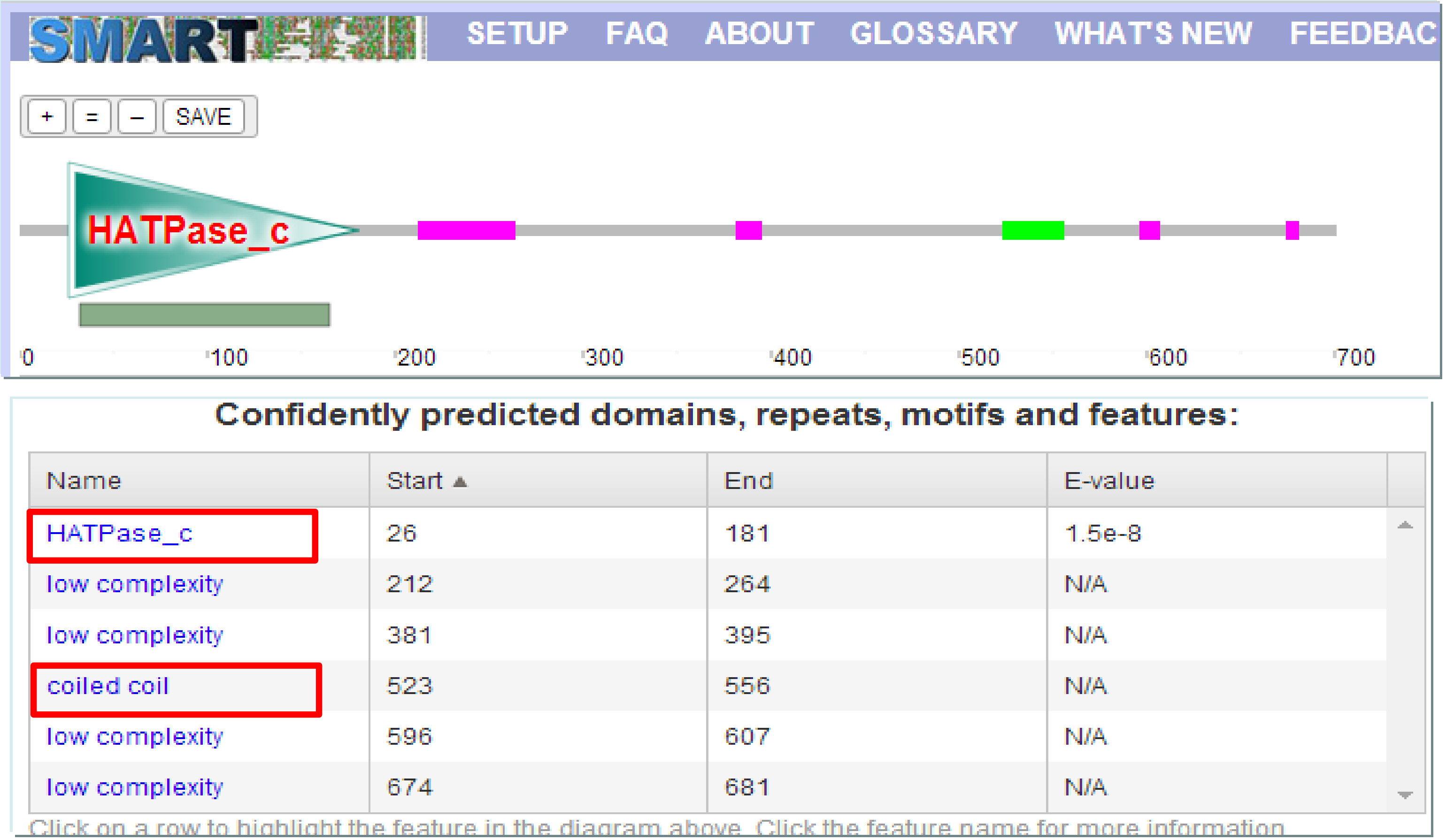

Zoom - Start: 1, End: 701

Disulphide Bridges Prediction


Zoom - Start:1, End:701

亚细胞定位

利用Smart对HSP90进行结构域预测。

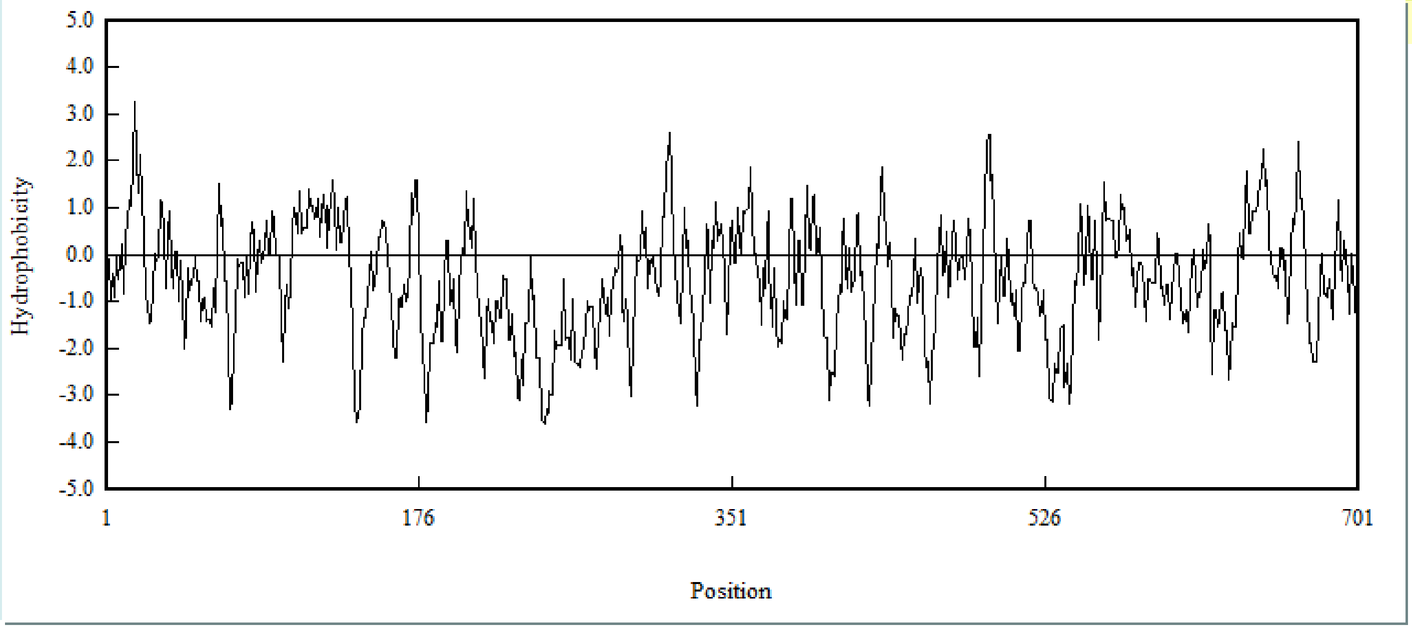


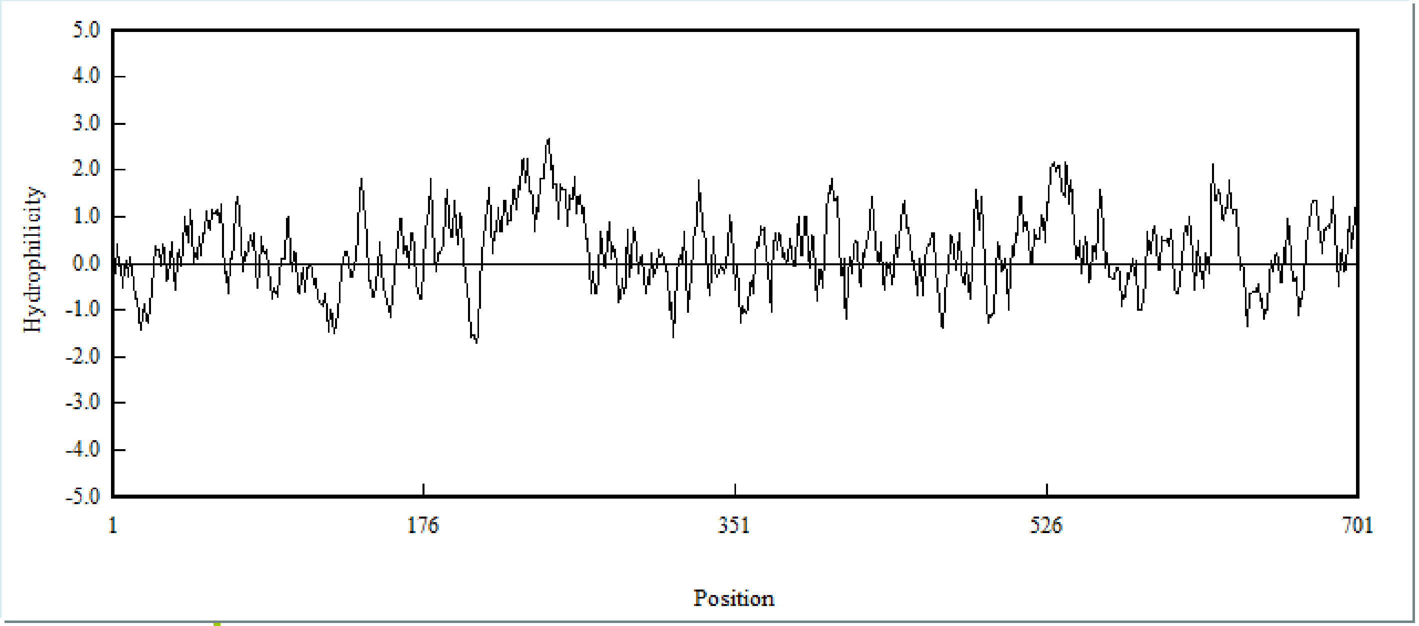
Confidently predicted domains, repeats, motifs and features:

Name	Start ▲	End	E-value
HATPase_c	26	181	1.5e-8
low complexity	212	264	N/A
low complexity	381	395	N/A
coiled coil	523	556	N/A
low complexity	596	607	N/A
low complexity	674	681	N/A

Click on a row to highlight the feature in the diagram above. Click the feature name for more information.

利用（ ProtScale ）DNAMAN对氨基酸的亲水性预测。





蛋白质的疏水性图谱，数值越高表明该区域多肽链的疏水性越强，hsp90存在数段较为显著的疏水区域。

利用Phyre2预测HSP90模型

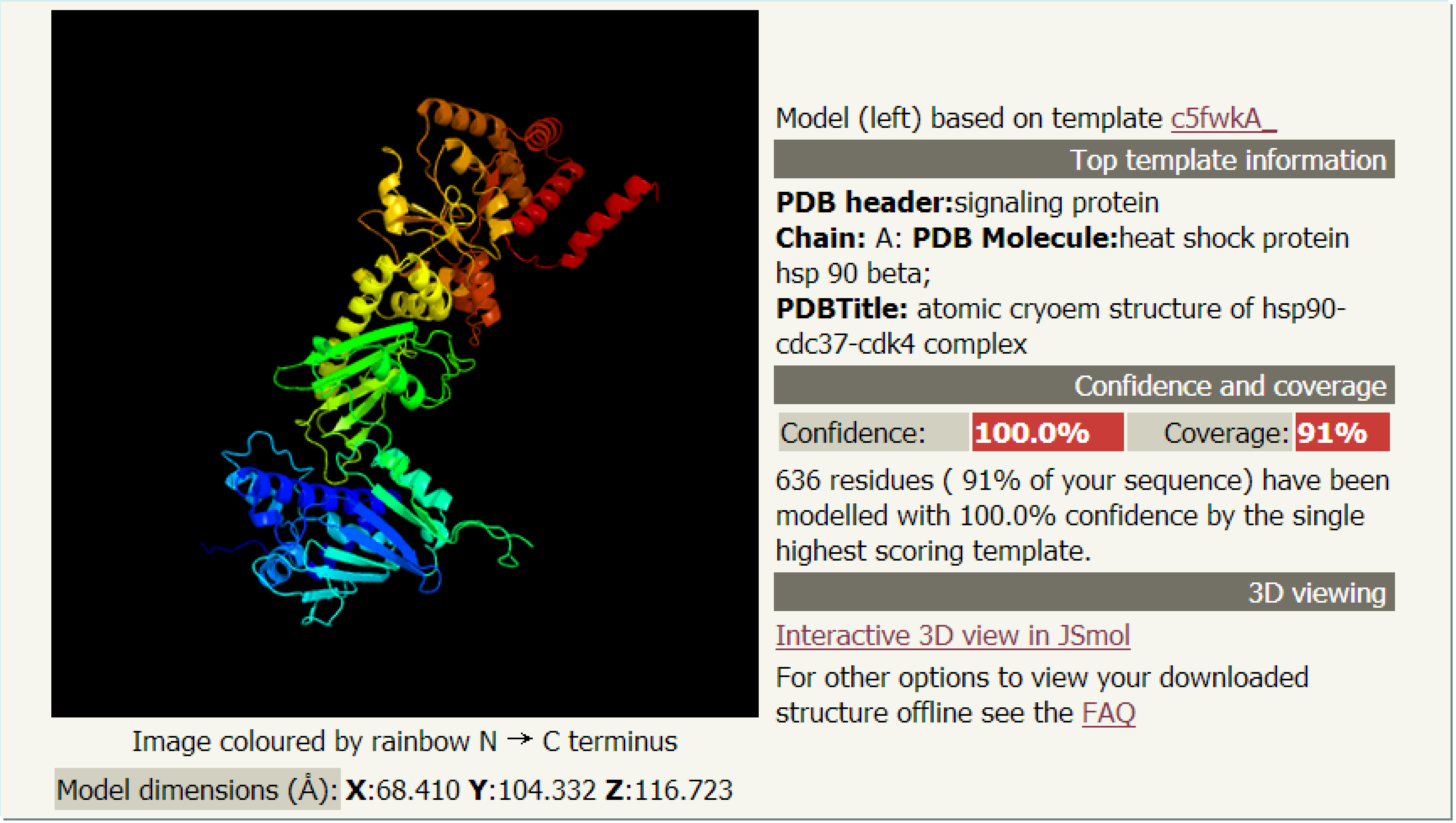


Image coloured by rainbow N → C terminus

Model dimensions (Å): **X**:68.410 **Y**:104.332 **Z**:116.723

Model (left) based on template c5fwkA_

Top template information

PDB header:signaling protein
Chain: A: **PDB Molecule:**heat shock protein hsp 90 beta;
PDBTitle: atomic cryoem structure of hsp90-cdc37-cdk4 complex

Confidence and coverage

Confidence:	**100.0%**	Coverage:	**91%**

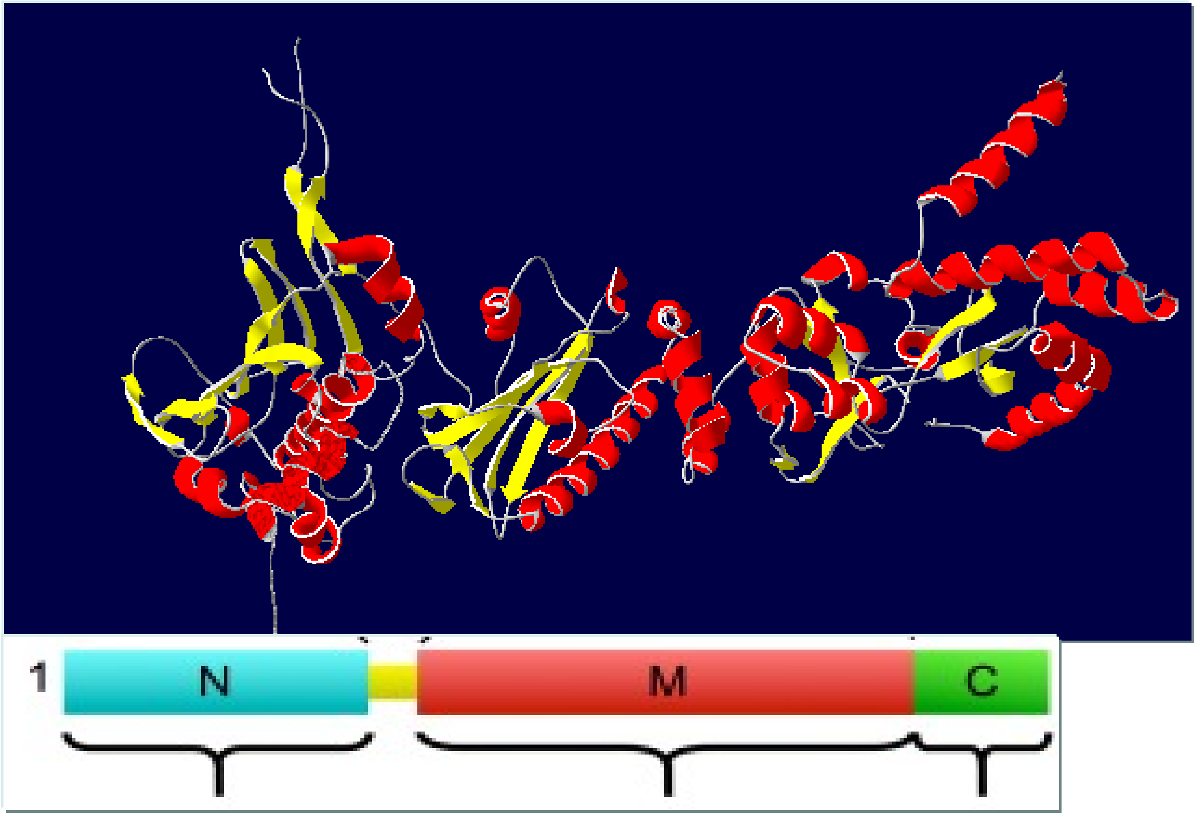
636 residues (91% of your sequence) have been modelled with 100.0% confidence by the single highest scoring template.

3D viewing

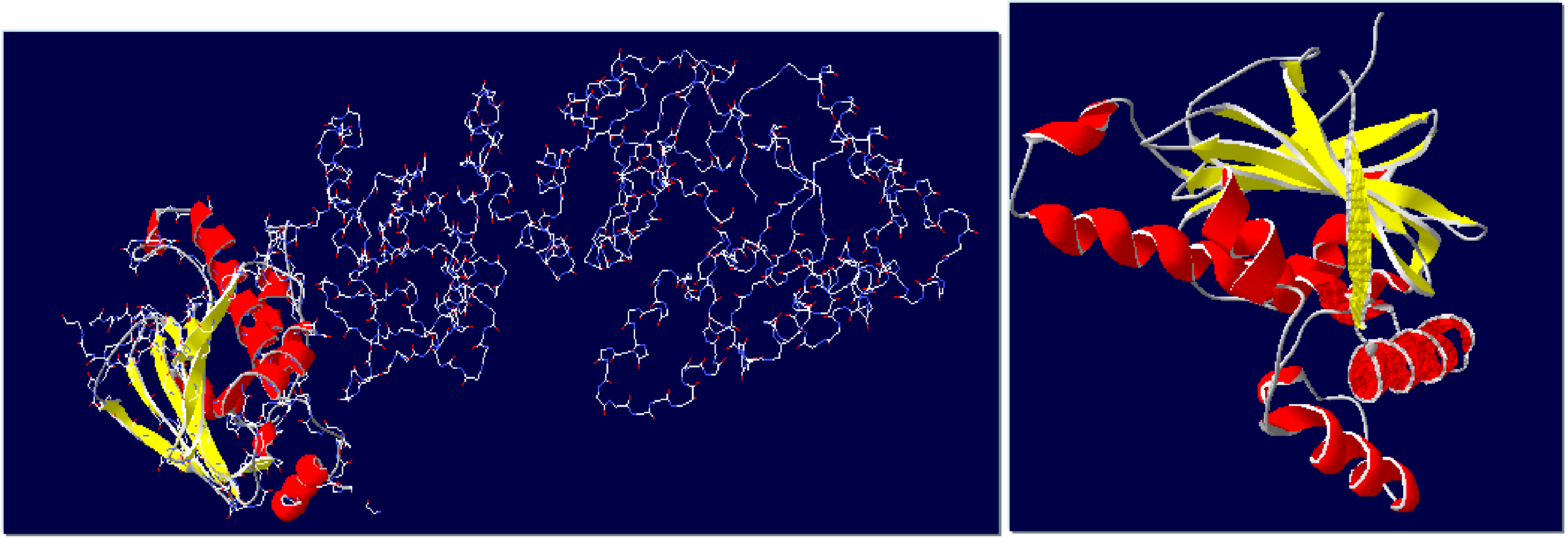
Interactive 3D view in JSmol

For other options to view your downloaded structure offline see the FAQ

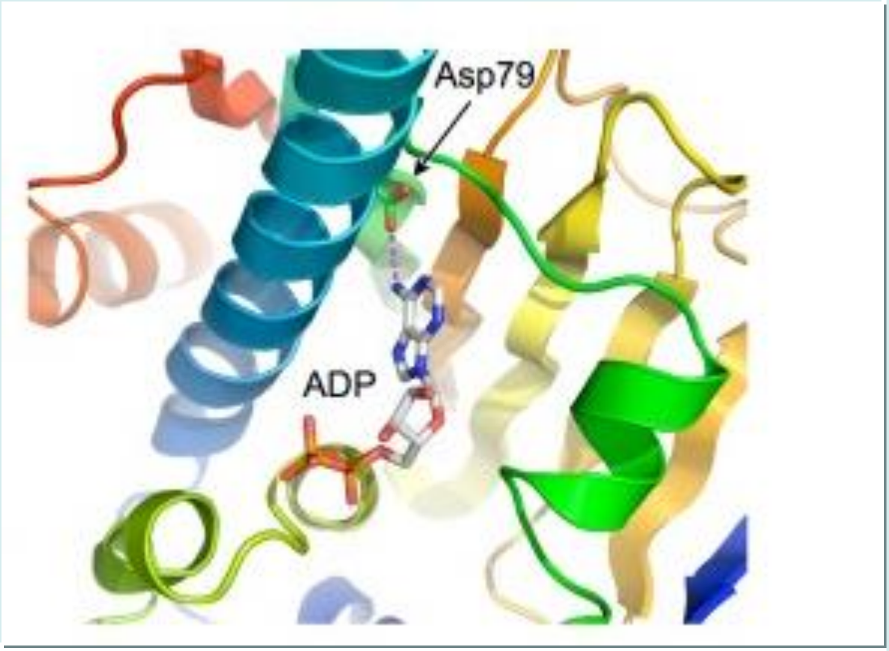
利用Swiss-PdbViewer对HSP90分析

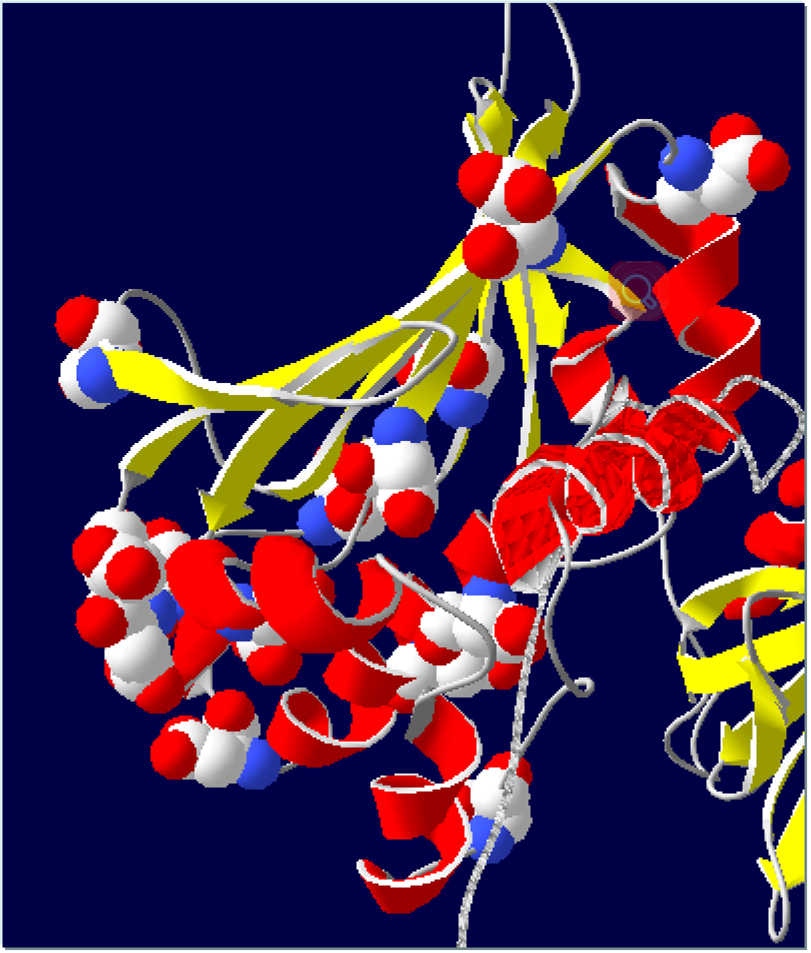


ATP结合区　　底物蛋白结合区　　二聚作用



HSP90 N端有α螺旋（位于一侧）和β折叠（位于另一侧），中间形成的“口袋”为ATP结合区。HSP90 与ATP结合改变自身构像，辅助底物蛋白形成正确的构像。

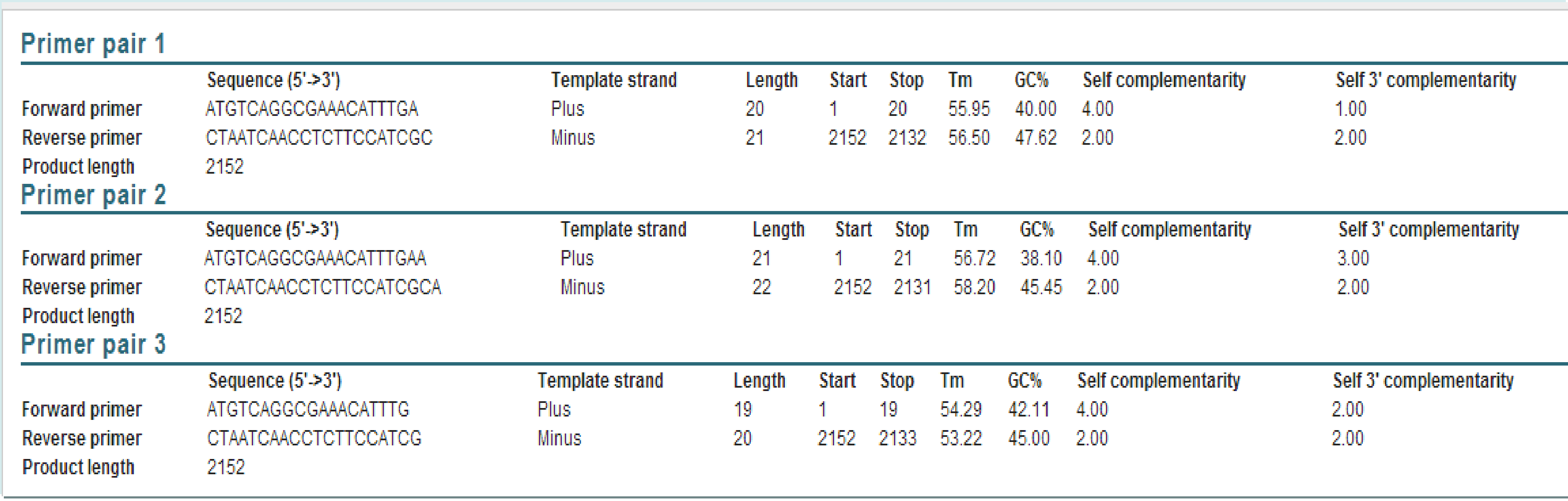




ATP腺嘌呤深入口袋和输水氨基酸ASP天冬氨酸侧链以氢键结合，但具体与哪一位天冬氨酸结合还需研究

分析：小麦白粉菌在受到高温的压力时，体内的一些酶或转录因子可能会发生错误的折叠，HSP90表达量会增加，协助错误折叠的蛋白形成正确的构像，抵抗高温。

利用NCBI 进行引物设计（扩全长）



Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ATGTCAGGCGAAACATTTGA	Plus	20	1	20	55.95	40.00	4.00	1.00
Reverse primer	CTAATCAACCTCTTCCATCGC	Minus	21	2152	2132	56.50	47.62	2.00	2.00
Product length	2152								

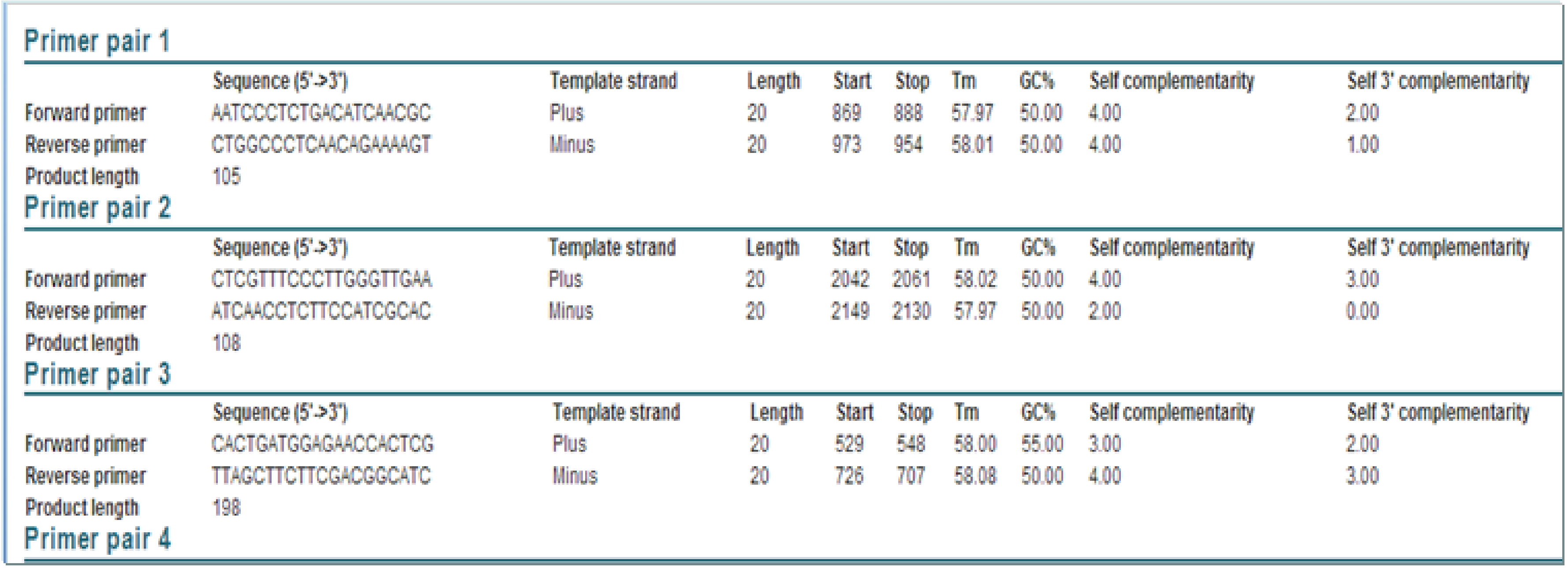
Primer pair 2

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ATGTCAGGCGAAACATTTGAA	Plus	21	1	21	56.72	38.10	4.00	3.00
Reverse primer	CTAATCAACCTCTTCCATCGCA	Minus	22	2152	2131	58.20	45.45	2.00	2.00
Product length	2152								

Primer pair 3

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ATGTCAGGCGAAACATTTG	Plus	19	1	19	54.29	42.11	4.00	2.00
Reverse primer	CTAATCAACCTCTTCCATCG	Minus	20	2152	2133	53.22	45.00	2.00	2.00
Product length	2152								

利用NCBI 进行引物设计（荧光定量PCR）



Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AATCCCTCTGACATCAACGC	Plus	20	869	888	57.97	50.00	4.00	2.00
Reverse primer	CTGGCCCTCAACAGAAAAGT	Minus	20	973	954	58.01	50.00	4.00	1.00
Product length	105								

Primer pair 2

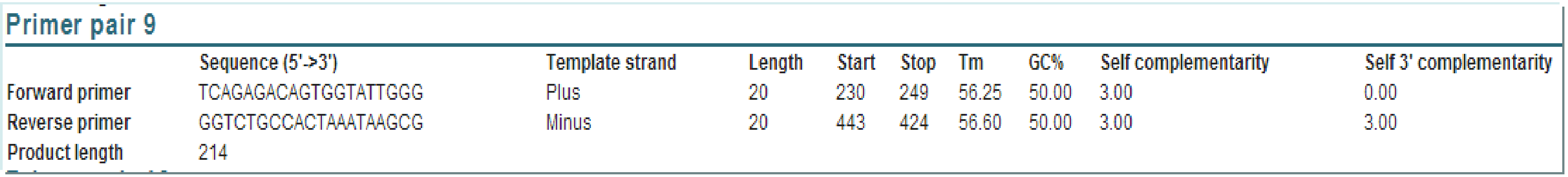
	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CTCGTTTCCCTTGGGTTGAA	Plus	20	2042	2061	58.02	50.00	4.00	3.00
Reverse primer	ATCAACCTCTTCCATCGCAC	Minus	20	2149	2130	57.97	50.00	2.00	0.00
Product length	108								

Primer pair 3

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CACTGATGGAGAACCACTCG	Plus	20	529	548	58.00	55.00	3.00	2.00
Reverse primer	TTAGCTTCTTCGACGGCATC	Minus	20	726	707	58.08	50.00	4.00	3.00
Product length	198								

Primer pair 4

引物跨越内含子（内含子262bp-307bp）



Primer pair 9

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TCAGAGACAGTGGTATTGGG	Plus	20	230	249	56.25	50.00	3.00	0.00
Reverse primer	GGTCTGCCACTAAATAAGCG	Minus	20	443	424	56.60	50.00	3.00	3.00
Product length	214								

1、对HSP90基因进行克隆，利用生物信息学方法对克隆的基因进行分析。

2、对高温处理的小麦白粉菌进行表达量分析。

3、利用HIGS技术对HSP90进行功能验证。

Thank You !