



中国农业科学院研究生院 (CAAS)

# 茶树氮代谢中CsGS1和CsTS的功能研究

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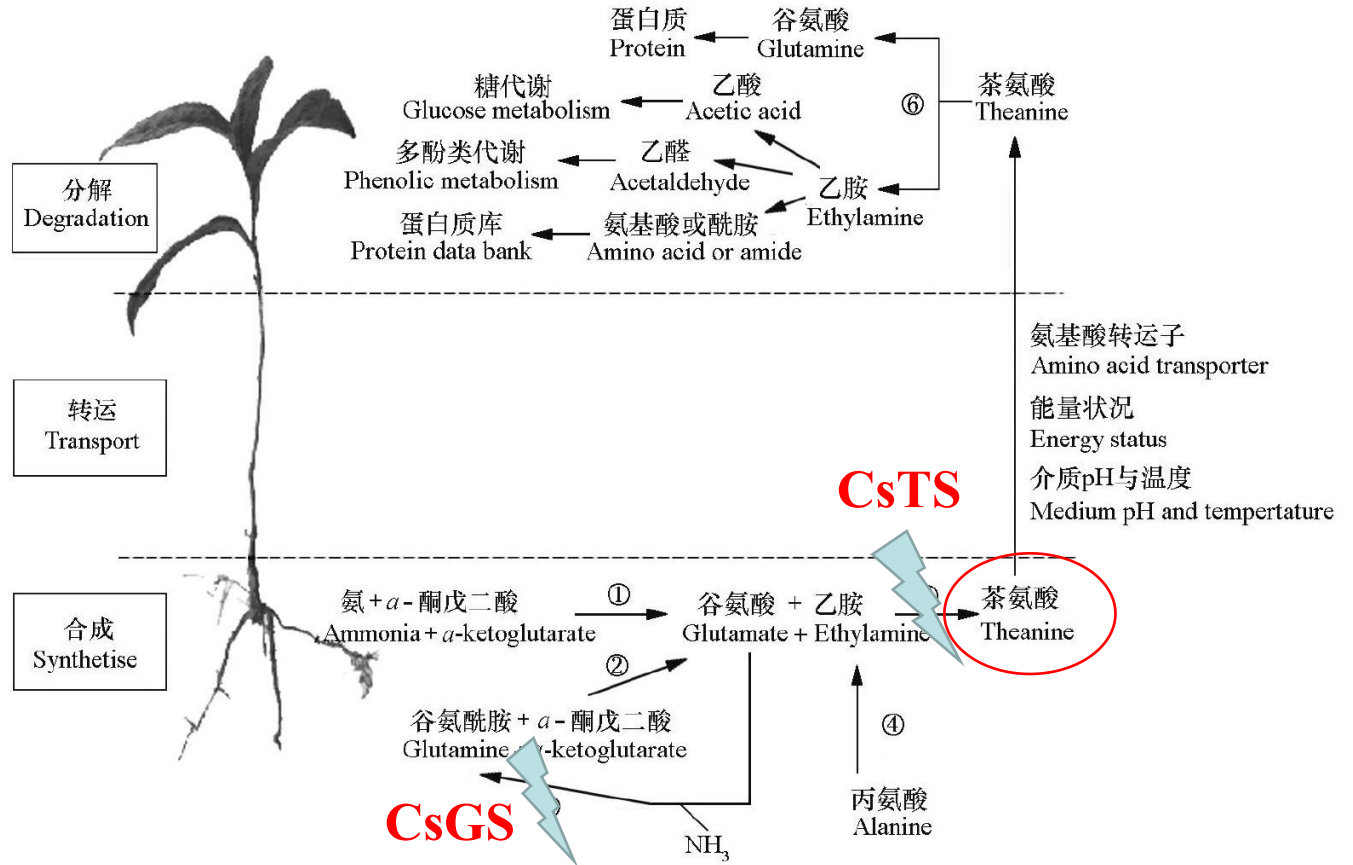
2018-11-11

# Content

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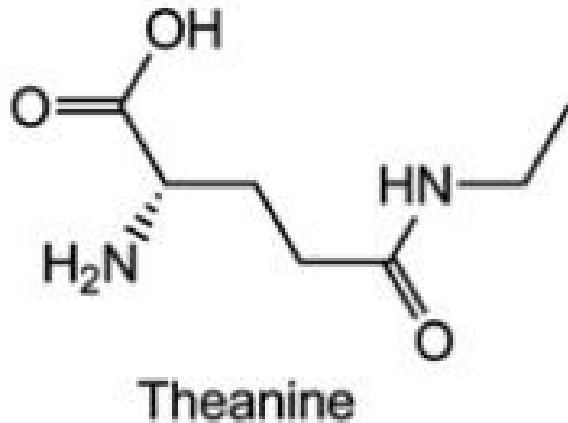
- Background
- Search and sequence alignment
- Protein sequence analysis and function prediction
- Protein modeling-3D structure
- Acknowledgement

# Background



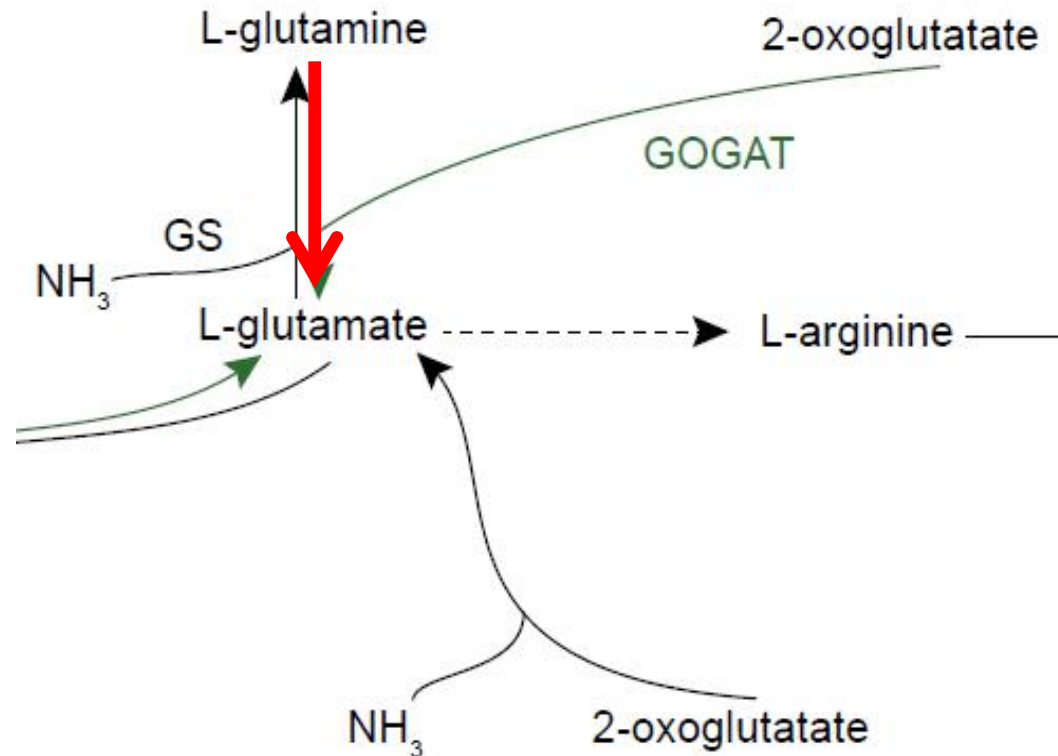
# Background

茶氨酸(Theanine)是茶树特征性非蛋白质氨基酸。在茶树新梢芽叶中,约占游离氨基酸总量的**70%**(宛晓春,2003)。与茶氨酸合成相关的酶有**茶氨酸合成酶**、**谷氨酸脱氢酶**、**谷氨酰胺合成酶**等(Mu et al.,2015)。



# Background

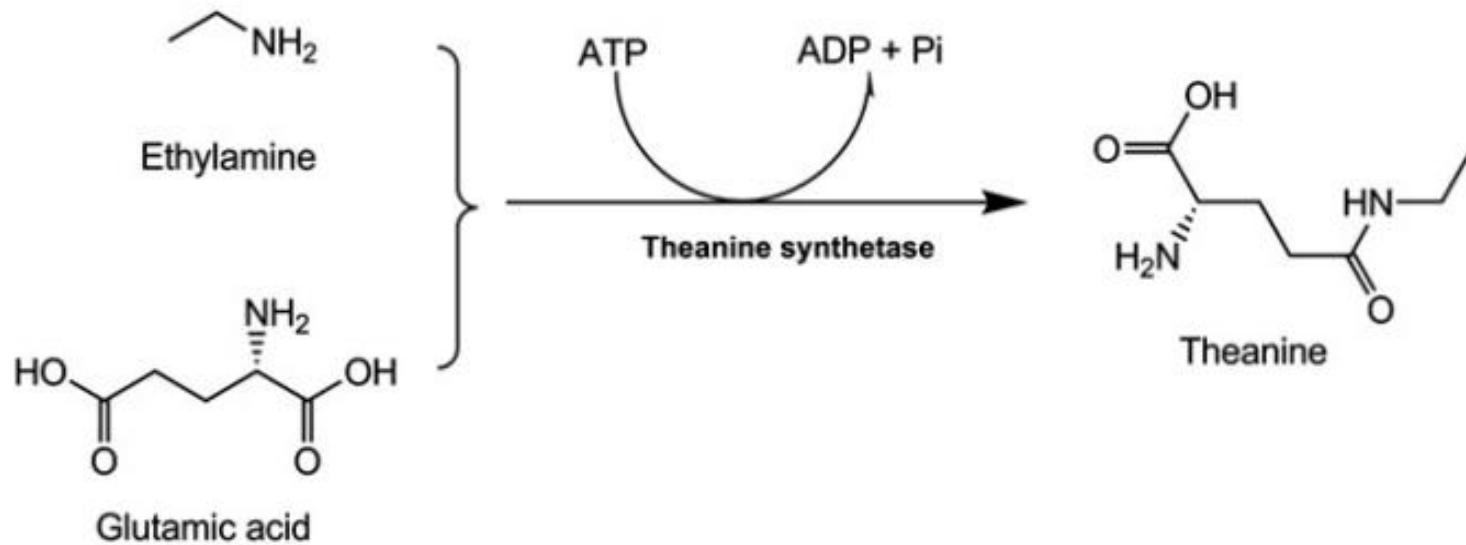
谷氨酰胺合成酶（Glutamine synthetase, GS）是高等植物氮同化的限速酶，存在植物的各个组织中，GS 是由 1 个小的基因家族编码，这些基因至少编码 1 种质体型和 3~5 种胞质型 GS 多肽（Bernard et al., 2008; Swarbreck et al., 2011）。



# Background

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## 茶氨酸合成酶 (Theanine synthetase, TS)



# Background

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- 史成颖（2011）和徐乾（2012）研究显示，*CsGS*与*CsTS*基因相似性极高。
- 陈琪（2011）通过原核表达试验和酶促反应，发现TS1不具备GS的催化活力，GS也不能催化谷氨酰胺和谷氨酸合成茶氨酸。



## 功能差异原因？

# Search

The image shows a composite of two web pages. The top page is the 'Annual Reviews' website, featuring a dark blue header with the 'AR' logo, the text 'ANNUAL REVIEWS', and subtext 'For Librarians & Agents' and 'For Authors'. It also includes 'Access Provided by Agricultural Information Institute' and navigation links for 'JOURNALS A-Z' and 'JOURNAL INFO'. Below the header is a breadcrumb trail 'Home / Search Results' and a large orange banner with the text 'Search Results'. A search bar is visible with a dropdown menu set to 'All Databases'. The bottom page is the Springer Link website, showing the Springer logo and 'Springer Link' text. It features a search bar with a magnifying glass icon and a settings gear icon. Below the search bar is a 'Home' link. A sidebar on the left offers 'Browse by discipline' with links for 'Biomedicine', 'Business and Management', and 'Chemistry'. A large text block on the right states: 'Providing researchers with access to millions of scientific documents from journals, books, series, protocols and reference works.' On the far right edge, a vertical list of links is partially visible: 'Popula', 'PubMed', 'Booksh', 'PubMed', and 'BLAST'.



# Search

- Molecule of the Month
- UniProtKB-glutamine synthetase

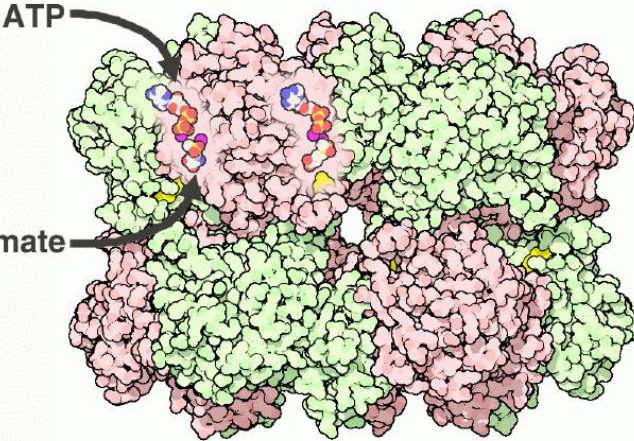
RCSB **PDB-101** Molecular explorations through biology and medicine

Educational portal of **PDB** PROTEIN DATA BANK

Molecule of the Month

## Glutamine Synthetase

*Glutamine synthetase monitors the levels of nitrogen-rich amino acids and decides when to make more*



The image shows a 3D surface representation of the Glutamine Synthetase protein, which is a large, multi-subunit enzyme. The protein is colored in shades of green, pink, and brown. Two specific binding sites are highlighted with arrows and labels: 'ATP' and 'glutamate'. The ATP site is located on the left side of the protein, and the glutamate site is located on the right side.

## UniProtKB - Q8LCE1 (GLN12\_ARATH)

### Display

[BLAST](#) [Align](#) [Format](#) [Add to basket](#) [History](#)

[Feedback](#) [Help video](#) [Other tutorials](#)

[Entry](#)

[Publications](#)

[Feature viewer](#)

[Feature table](#)

None

[Function](#)

[Names & Taxonomy](#)

[Subcell. location](#)

**Protein** | **Glutamine synthetase cytosolic isozyme 1-2**

**Gene** | **GLN1-2**

**Organism** | *Arabidopsis thaliana (Mouse-ear cress)*

### Status

[Reviewed](#) - Annotation score:       - Experimental evidence at level<sup>1</sup>

### Function<sup>1</sup>

Low-affinity glutamine synthetase. May contribute to the homeostatic of glutamine synthesis in roots.

### Catalytic activity<sup>1</sup>

# Sequences

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获得克隆序列CsGS1的氨基酸序列: Weblab → getorf

>Lj-CsGS1

```
MAQLSDLINMNLSDSTEKIIAEYIWIGGTGLDLRSKARTLSGPVNDPKKLPKWN  
DGSSTGQAPGEDSEVIIYPQAIFKDPFRKGNNILVMCDAYTPAGEPIPTNKRYNAE  
KIFSHTDVVAEEPWYGIEQEYTLLQKDVKWPIGWPKGGYPGPQGPYYCGAGAD  
KAFGRDIVDSHYKACLYAGINISGINGEVMPGQWEFQVGPAVGISAGDELWVARY  
ILERITEIAGVVVSFDPKPIEGDWNGAGAHTNYSTKSMRKGDDGGYQVIKKAIEKLG  
LKHKEHIAAYGEGNERRLTGRHETADINTFLWGVANRGASVRVGRDTEKAGKGY  
FEDRRPASNMDPYVVVTSMIAATTILWKP
```

>AEM44626.1 GsTS

```
MSLLSDLINLNLSDTTEKVI AEYIWIGGSGMDLRSKARTLSGPVSDPKKLPKWN  
DGSSTGQAPGEDSEVIIYPQAIFKDPFRRGNNILVMCDTYTPGGGEPIPTNKRFNAA  
KIFSHPDVVAEEPWYGIEQEYTLLQKQVKWPLGWPLGGFPGPQGPYYCGIGVDK  
AYGRDIVDSHYKACLYAGVNI SGINGEVMPGQWEFQVGPSVGISAGDELWVARY  
ILERITEIAGVVVSFDPKPIEGDWNGAGAHTNYSTKSTRSDGGFEVIKKAIEKLG  
RHKEHIAAYGEGNERRLTGRHETADINTFLWGVANRGASIRVGRDTEKAGKGYF  
EDRRPASNMDPYVVVTSMIAETTLLWKP
```

# Sequences

NCBI→Blastp

Enter query sequence: 输入氨基酸  
序列 (CsGS或CsTS)

Database: swissprot

Exclude Option: 勾选上后, blast  
不到任何序列, 取消勾选

Algorithm: blastp

Expect threshold: 0.01

Word size: 3

Matrix: BLUSOM62

	Description
<input type="checkbox"/>	<a href="#">RecName: Full=Glutamine synthetase cytosolic isozyme 1; AltName: Full=Glutamate--ammonia liqase</a>
<input type="checkbox"/>	<a href="#">RecName: Full=Glutamine synthetase cytosolic isozyme 2; AltName: Full=Glutamate--ammonia liqase</a>
<input type="checkbox"/>	<a href="#">RecName: Full=Glutamine synthetase nodule isozyme; Short=GS; AltName: Full=Glutamate--ammonia liqase</a>
<input type="checkbox"/>	<a href="#">RecName: Full=Glutamine synthetase cytosolic isozyme 2; AltName: Full=GS1-2; AltName: Full=Glutamate--ammonia liqase</a>
<input type="checkbox"/>	<a href="#">RecName: Full=Glutamine synthetase N-1; AltName: Full=Gln isozyme gamma; AltName: Full=Glutamate--ammonia liqase</a>
<input type="checkbox"/>	<a href="#">RecName: Full=Glutamine synthetase cytosolic isozyme; AltName: Full=Glutamate--ammonia liqase</a>
<input type="checkbox"/>	<a href="#">RecName: Full=Glutamine synthetase PR-1; AltName: Full=Gln isozyme beta; AltName: Full=Glutamate--ammonia liqase</a>
<input type="checkbox"/>	<a href="#">RecName: Full=Glutamine synthetase cytosolic isozyme; AltName: Full=GS1; AltName: Full=Glutamate--ammonia liqase</a>
<input type="checkbox"/>	<a href="#">RecName: Full=Glutamine synthetase nodule isozyme; AltName: Full=Glutamate--ammonia liqase</a>
<input type="checkbox"/>	<a href="#">RecName: Full=Glutamine synthetase cytosolic isozyme 1-1; AltName: Full=Glutamate--ammonia liqase GLN1;1; Short=OsGLN1</a>
<input type="checkbox"/>	<a href="#">RecName: Full=Glutamine synthetase PR-2; AltName: Full=Gln isozyme alpha; AltName: Full=Glutamate--ammonia liqase</a>
<input type="checkbox"/>	<a href="#">RecName: Full=Glutamine synthetase nodule isozyme; Short=GS; AltName: Full=Glutamate--ammonia liqase</a>
<input type="checkbox"/>	<a href="#">RecName: Full=Glutamine synthetase cytosolic isozyme 1-1; AltName: Full=Glutamate--ammonia liqase GLN1;1; Short=GLN1;</a>
<input type="checkbox"/>	<a href="#">RecName: Full=Glutamine synthetase; AltName: Full=Glutamate--ammonia liqase</a>
<input type="checkbox"/>	<a href="#">RecName: Full=Glutamine synthetase; AltName: Full=GS(1); AltName: Full=Glutamate--ammonia liqase</a>



# Sequence alignment

- Tools > Pairwise Sequence Alignment > EMBOSS Needle

```
# Length: 356
# Identity: 325/356 (91.3%)
# Similarity: 344/356 (96.6%)
# Gaps: 0/356 ( 0.0%)
# Score: 1802.0
#
#
#=====
```

**CsTS**  
(AEM44626.1)

**CsGS1**

Lj-CsGS1.2	1	MAQLSDLINMNLSDSTEKIIAEYIWIGGTGLDLRSKARTLSGPFVNDPKKL	50
AEM44626.1	1	MSLLSDLINLNLSDTTEKVIAEYIWIGGSGMDLRSKARTLSGPFVSDPKKL	50
Lj-CsGS1.2	51	PKWNYDGSSTGQAPGEDSEVIIYPQAI FKDPFRKGNNILVMCDAYTPAGE	100
AEM44626.1	51	PKWNYDGSSTGQAPGEDSEVIIYPQAI FKDPFRKGNNILVMCDTYTPGGE	100
Lj-CsGS1.2	101	PIPTNKRYNAEKIFSHTDVVAEEPWYGIEQEYTL LQKDVKWPIGWPKGGY	150
AEM44626.1	101	PIPTNKRFNAAKIFSHPDVVAEEPWYGIEQEYTL LQKQVKWPLGWPLGGF	150
Lj-CsGS1.2	151	PGPQGPYYCGAGADKAFGRDIVDSHYKACLYAGINIS GINGEVMPGQWEF	200
AEM44626.1	151	PGPQGPYYCGIGVDKAYGRDIVDSHYKACLYAGV NIS GINGEVMPGQWEF	200
Lj-CsGS1.2	201	QVGPAVGISAGDELWVARYILERITEIAGVVVS FDKPKPIEGDWNAGAAHT	250
AEM44626.1	201	QVGPSVGISAGDELWVARYILERITEIAGVVVS FDKPKPIEGDWNAGAAHT	250
Lj-CsGS1.2	251	NYSTKSMRKDGGYQVIKKAIEKLGLKHKEHIAAY GEGNERRLTGRHETAD	300
AEM44626.1	251	NYSTKSTRSDGGFEVIKKAIEKLGLRHKEHIAAY GEGNERRLTGRHETAD	300
Lj-CsGS1.2	301	INTFLWGVANRGASVRVGRDTEKAGKGYFEDRR PASNMDPYVVTSMIAAT	350
AEM44626.1	301	INTFLWGVANRGASIRVGRDTEKAGKGYFEDRR PASNMDPYVVTSMIAET	350
Lj-CsGS1.2	351	TILWKP	356
AEM44626.1	351	TLLWKP	356

# Protein sequence analysis and function prediction

➤ NetPhos 3.1 Server-predicts phosphorylation sites

CsGS1

#	MAQLSDLINMNLSDSTEKIIAEYIWIGGTGLDLRSKARTLSGPVNDPKKL	#	50
	PKWNYDGSSTGQAPGEDSEVIIYPQAIKDPFRKGNILVMCDAYTPAGE	#	100
	PIPTNKRYNAEKIFSHTDVVAEEPWYIEQEYLLQKDVKWPIGWPKGGY	#	150
	PGPQGPYYCGAGADKAFGRDIVDSHYKACLYAGINISGINGEVMPGQWEF	#	200
	QVGPVAVGISAGDELWVARYILERITEIAGVVVSFDPKPIEGDWNGAGAHT	#	250
	NYSTKSMRKDGGYQVIKKAIEKLGKHKHEIAAYGEGNERRLTGRHETAD	#	300
	INTFLWGVANRGASVRVGRDTEKAGKGYFEDRRPASNMDPYVVTSMIAAT	#	350
	TLLWKP	#	400
%1	.....S.S.....S..T.S.....	#	50
%1	.....S.....YT....	#	100
%1	...T.....S.T.....Y.....Y.....Y	#	150
%1	.....S.....S.....	#	200
%1	.....S.....S.....	#	250
%1	..ST.S.....Y.....Y.....T...T..	#	300
%1	..T.....S.....T.....S...Y..S...T	#	350
%1	T.....		

✓ 磷酸化位点的差异也可能是造成它们受到的调控途径与其活性存在一定的差异的原因

CsTS

#	MSLLSDLINLNLSDTTEKVIAEYIWIGGSGMDLRSKARTLSGPVSDPKKL	#	50
	PKWNYDGSSTGQAPGEDSEVIIYPQAIKDPFRGNNILVMCDYTPGGE	#	100
	PIPTNKRFNAEKIFSHPDVVAEEPWYIEQEYLLQKQVKWPLGWPLGGF	#	150
	PGPQGPYYCGIGVDKAYGRDIVDSHYKACLYAGVNISGINGEVMPGQWEF	#	200
	QVGPVAVGISAGDELWVARYILERITEIAGVVVSFDPKPIEGDWNGAGAHT	#	250
	NYSTKSTRSDGGFEVIKKAIEKGLRHKEHIAAYGEGNERRLTGRHETAD	#	300
	INTFLWGVANRGASIRVGRDTEKAGKGYFEDRRPASNMDPYVVTSMIAET	#	350
	TLLWKP	#	400
%1	..S.S.....S.T.....S.....S..T.S...S.....	#	50
%1	.....S.....YT....	#	100
%1	...T.....S.....Y.....Y.....	#	150
%1	.....Y.....S.....S.....	#	200
%1	.....S.....S.....	#	250
%1	..S.S.S.....Y.....T...T..	#	300
%1	..T.....S.....T.....S...Y..S...T	#	350
%1	T.....		

serine, threonine or tyrosine

# Protein sequence analysis and function prediction

➤ ExPASy → ProtParam

	CsGS1	CsTS
Number of amino acids	356	356
Molecular weight	39240.34	39250.31
<b>Theoretical pI</b>	<b>6.13</b>	<b>5.79</b>
Negatively charged residues	44	44
Positively charged residues	41	39
<b>Instability index</b>	<b>37.25</b>	<b>42.63</b>
Grand average of hydropathicity	-0.464	-0.414

➤ PredictProtein



CsGS1

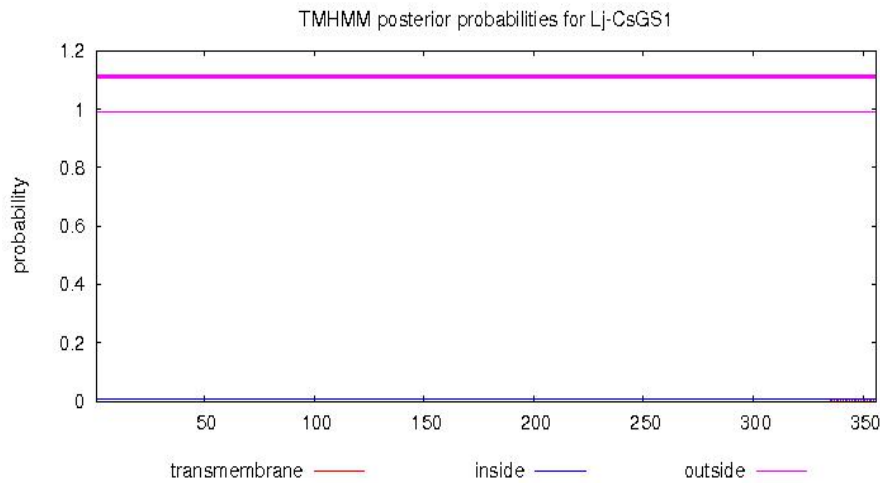


CsTS

# Transmembran structure

## ➤ 跨膜结构预测:

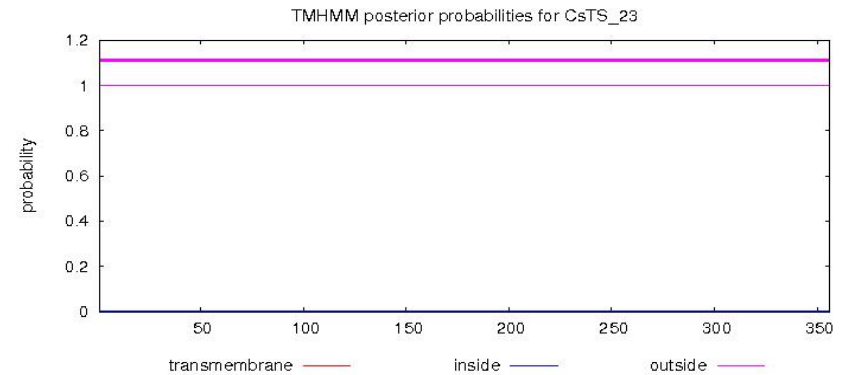
- PredictProtein
- TMHMM



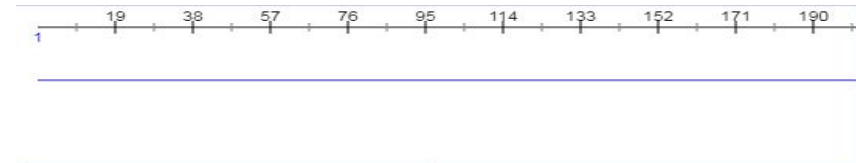
CsGS1



```
# CsTS_23 Length: 356
# CsTS_23 Number of predicted TMs: 0
# CsTS_23 Exp number of AAs in TMs: 0.00388
# CsTS_23 Exp number, first 60 AAs: 0.0017
# CsTS_23 Total prob of N-in: 0.00170
CsTS_23 TMHMM2.0      outside 1 356
```

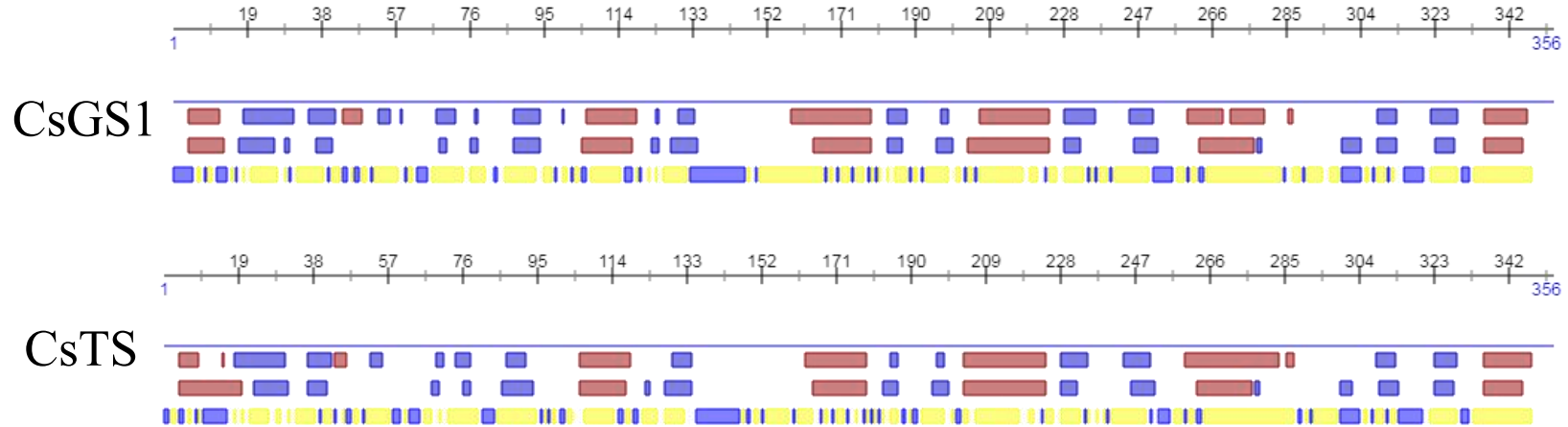


CsTS

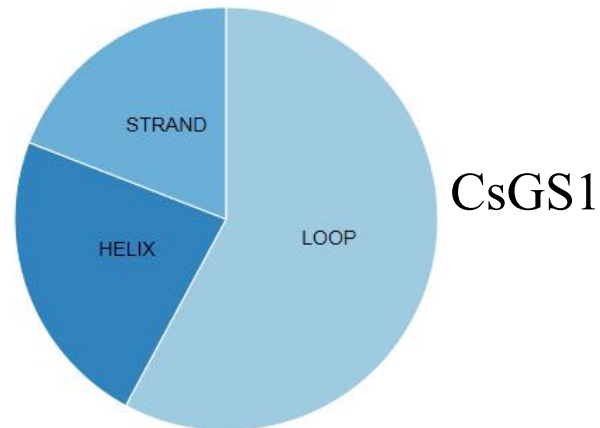


# Secondary Structure

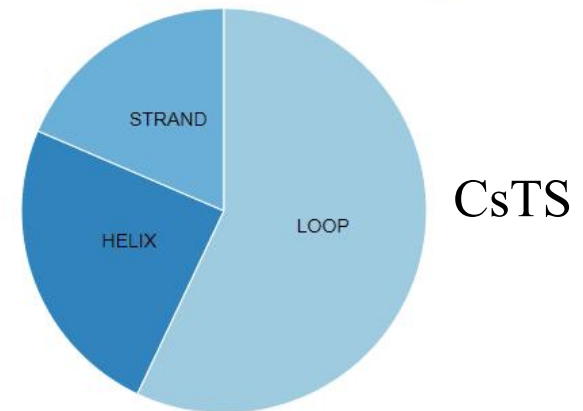
## ➤ PredictProtein



Secondary Structure Composition

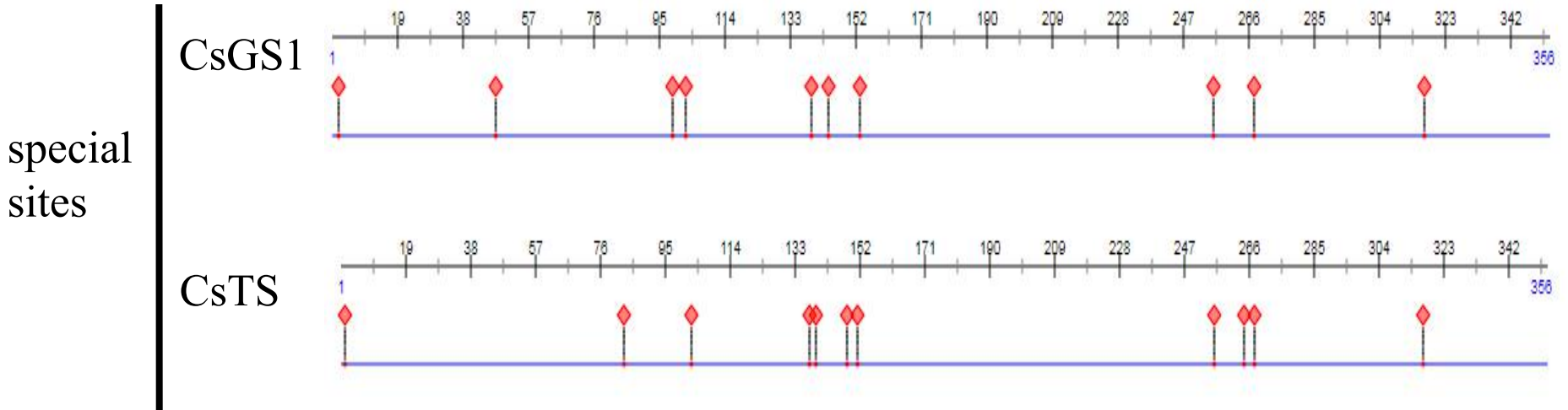


Secondary Structure Composition





# Secondary Structure

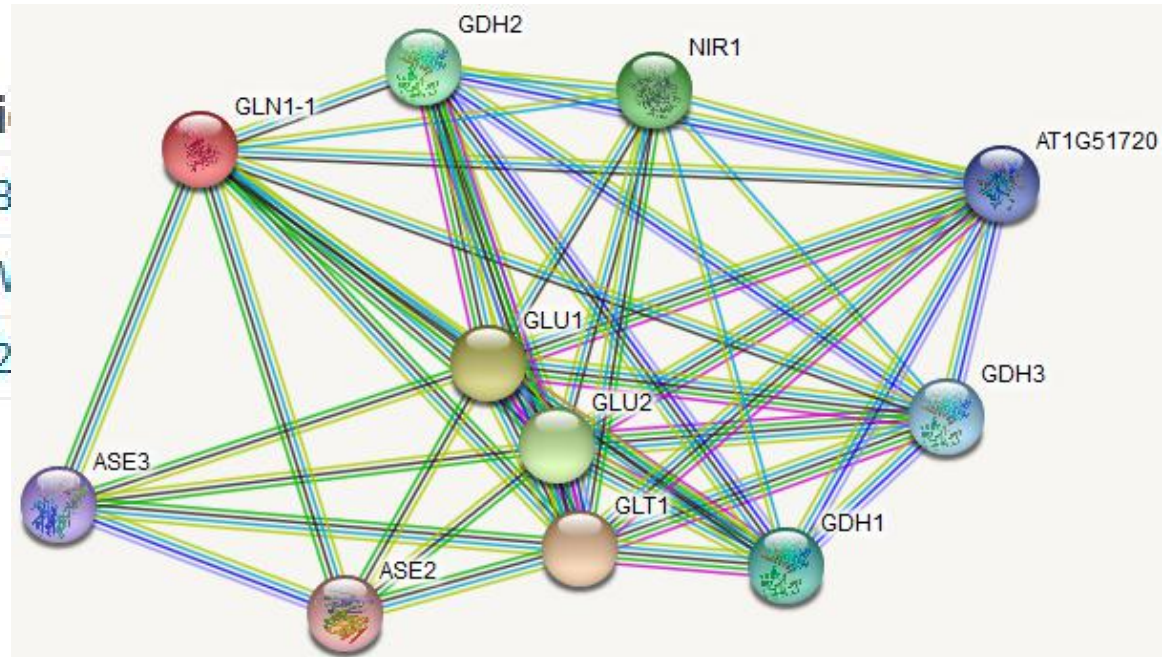


◆ protein binding region

Prediced protein interaction

## Protein-protein interacti

BioGrid <sup>i</sup>	1898
IntAct <sup>i</sup>	Q56V
STRING <sup>i</sup>	3702



# Database of Interacting Proteins

Search by:[\[protein\]](#) [\[sequence\]](#) [\[motif\]](#) [\[article\]](#) [\[IMEx\]](#) [\[pathBLAST\]](#)

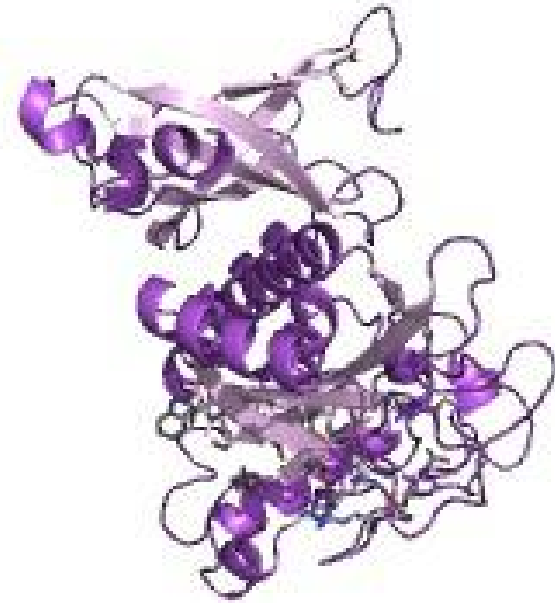
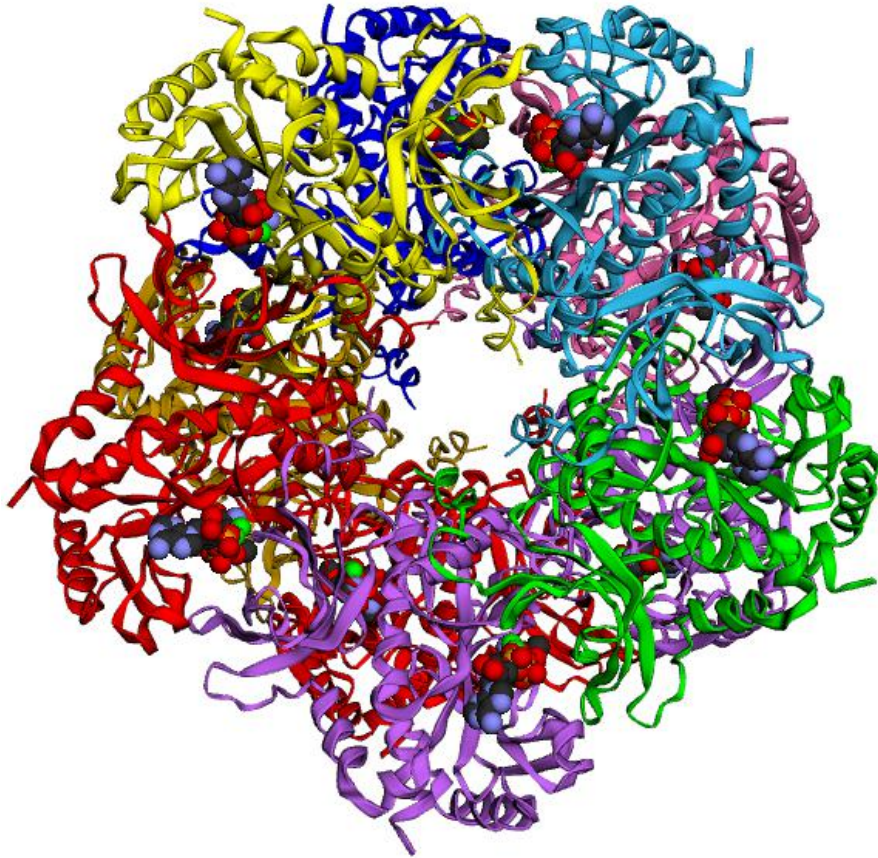
**THE DIP DATABASE**

# Protein modeling

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玉米 GS (已通过 X-射线晶体衍射法加核磁共振)

PDB id: 2d3a

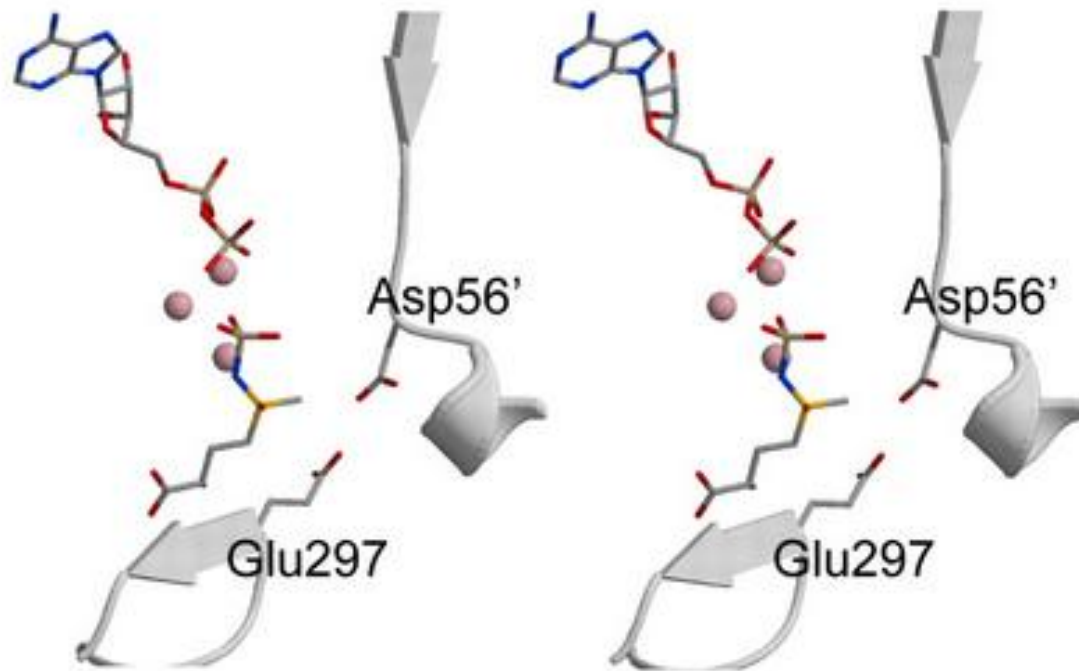


Structure: Glutamine synthetase. Chain: a, b, c, d, e, f, g, h, i, j. Engineered: yes

<https://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl?pdbcode=2d3a&template=3Dmoljsbox.html>

# Protein modeling

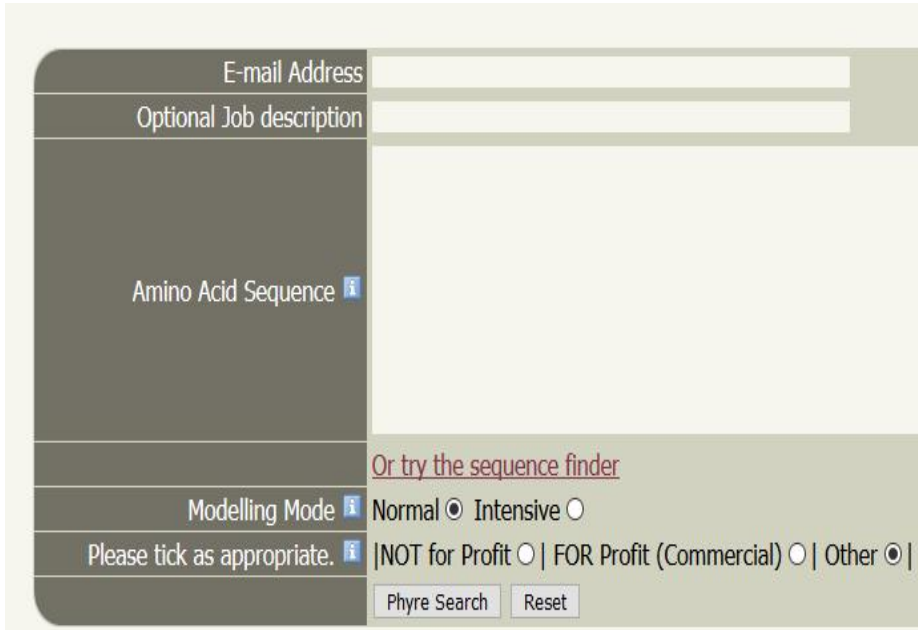
---



Active site structure formed by Asp-56 and Glu-297 of maize GS1a. a, stereo view of the side chain structures of Asp-56' and Glu-297 together with ADP, MetSox-P, and three Mn<sup>2+</sup> ions.

# Protein modeling

## ➤ Phyre2



The screenshot shows the Phyre2 web interface with the following fields and options:

- E-mail Address: [input field]
- Optional Job description: [input field]
- Amino Acid Sequence: [input field]
- Modelling Mode: Normal  Intensive
- Please tick as appropriate: NOT for Profit  FOR Profit (Commercial)  Other
- Buttons: Phyre Search, Reset
- Link: [Or try the sequence finder](#)



Phyre2 results for job description: Undefined  
Unique job identifier: 915fbd1105818e5b

Please find attached to this email a PDB formatted model of your protein.  
For help viewing your PDB file off-line, please see the FAQ here:  
<http://www.sbg.bio.ic.ac.uk/phyre2/html/help.cgi?id=help/faq>

NORMAL mode.  
Confidence in the model: 100.0%



CsGS1.pdb



CsTS.pdb



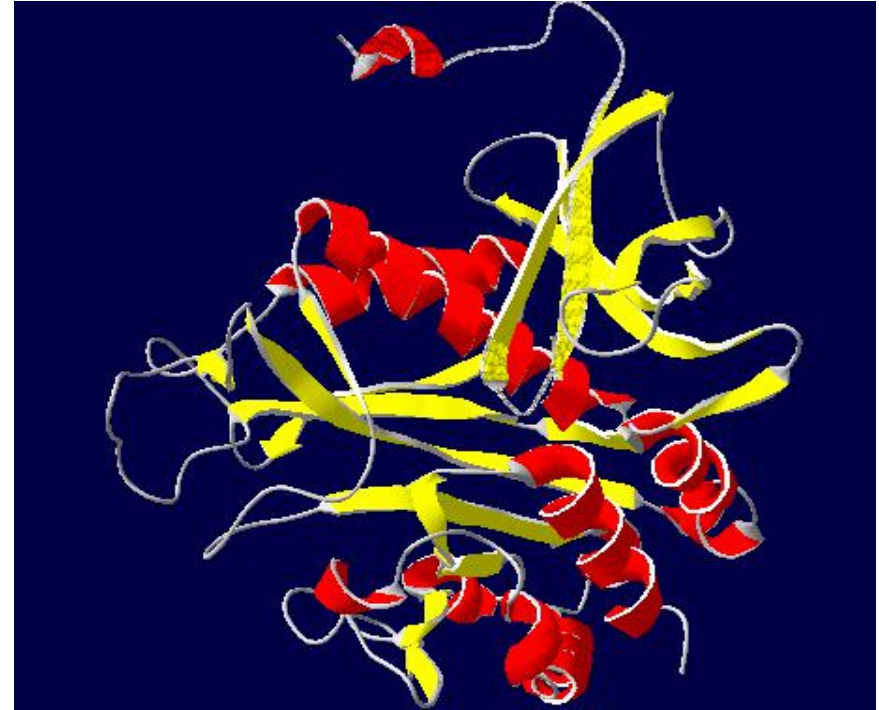
# Predicted 3D structure

---

CsGS1



CsTS



# Predicted 3D structure

➤ Fit



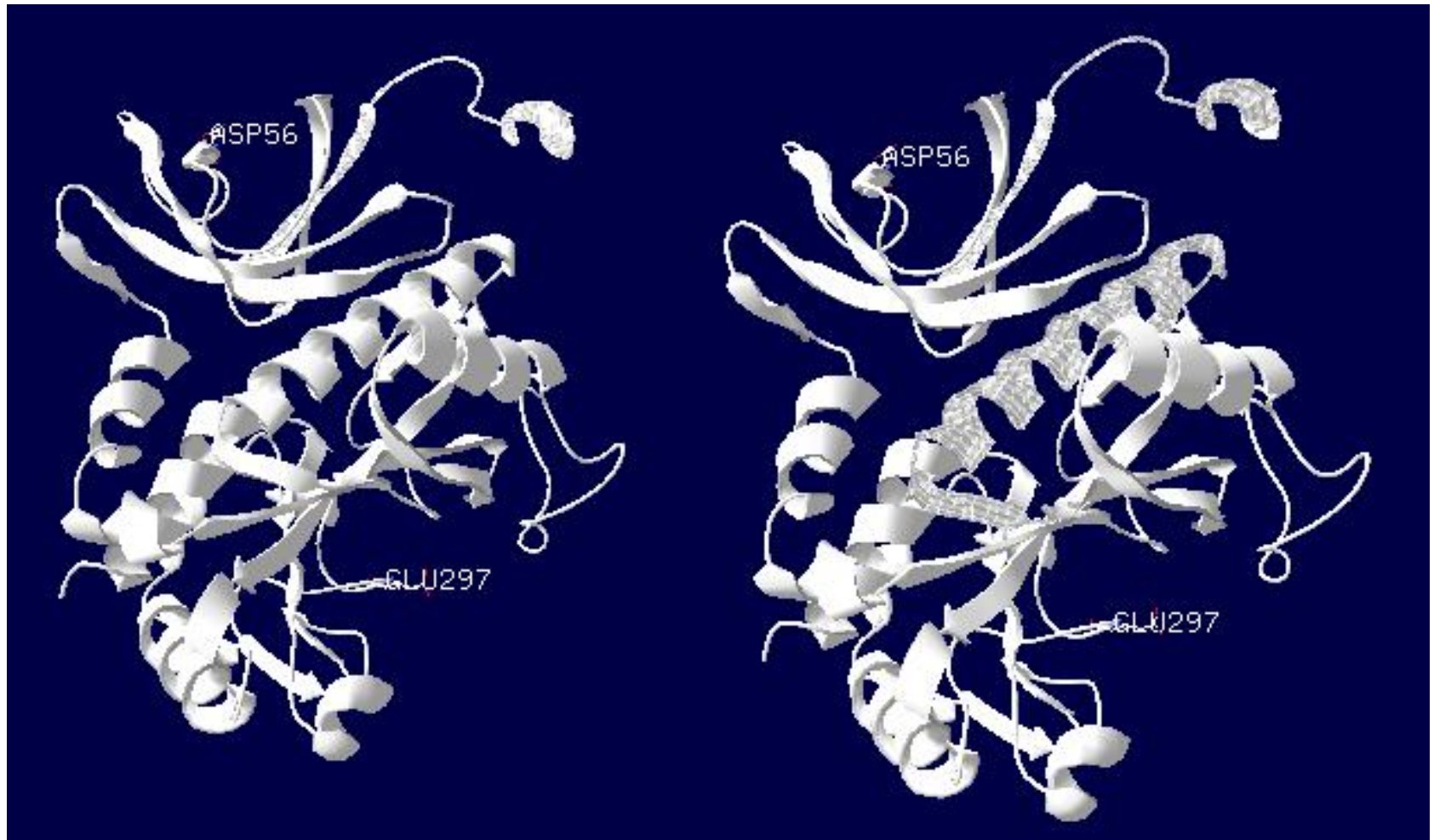
Alignment

CsGS1	91.8	100.0	S	D	L	I	N	L	N	L	S	D	S	T	E	I	I	A	E	Y	I	W	I	G	T	S	D	L	R	S	K	A	R	T	L	S	G	P	V	S	D	P	K	K	L	P	K	W	N	Y	D	G	S	S	T	G	Q	A	P	G	E	D	S	E	V	I			
CsTS	100.0	100.0	L	S	D	L	I	N	L	N	L	S	D	S	T	E	I	I	A	E	Y	I	W	I	G	G	S	G	M	D	L	R	S	K	A	R	T	L	S	G	P	V	S	D	P	K	K	L	P	K	W	N	Y	D	G	S	S	T	G	Q	A	P	G	E	D	S	E	V	I

CsTS: THR39 rms: 0.000

# Predicted 3D structure

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# Acknowledgement

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- 真挚地感谢罗老师为我们讲授实用生物信息学，以及指导我们学习
- 感谢小组成员，相互合作努力
- 谢谢同学们的聆听

# Reference

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列出部分参考文献：

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# 中国农业科学院研究生院 (CAAS)

 *Thank you*

请老师、同学们批评指正

