茶树咖啡碱合成酶序列和结构分析

Sequence and structure analysis of Tea Caffeine Synthase

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主要内容

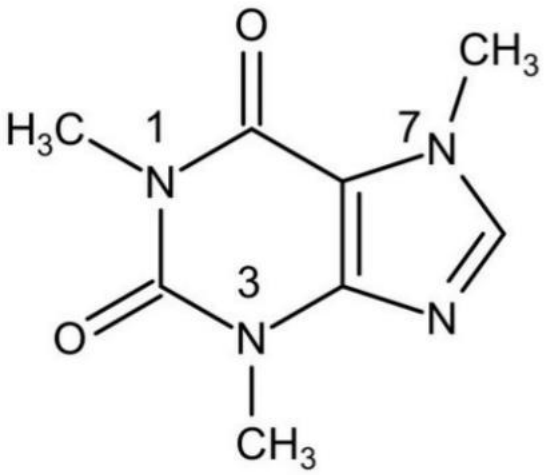
1、 研究背景

2、蛋白质序列分析

3、蛋白质结构预测

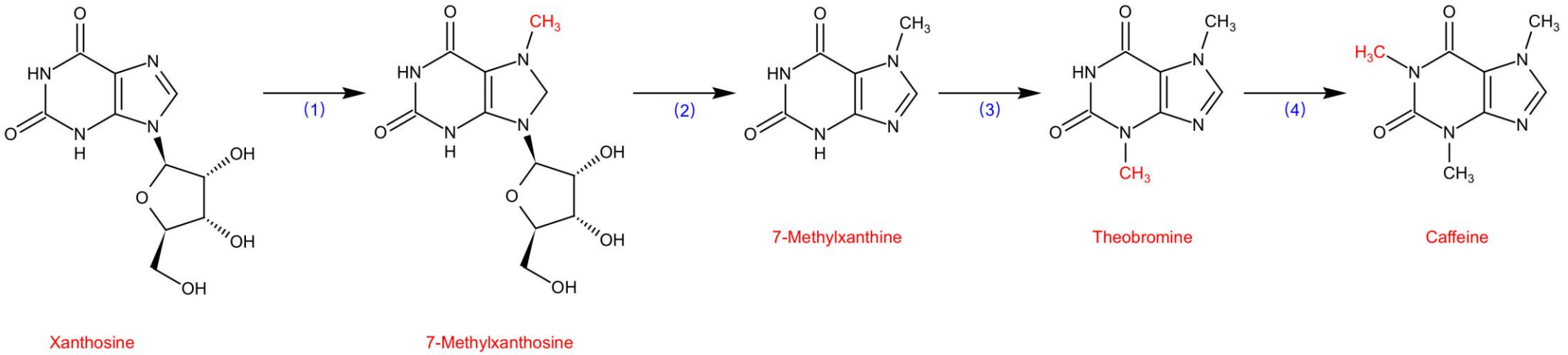
1、研究背景

茶、咖啡和可可是世界上最受欢迎的三大无酒精饮料，它们都含有嘌呤生物碱，如咖啡碱、可可碱等。嘌呤生物碱是茶树重要的次级代谢产物，参与一些生物和非生物胁迫。大部分茶树资源咖啡碱含量较高，一般占干物质含量的2-5%。咖啡碱不仅有利于茶叶风味形成，还有很好的保健功效，能够刺激中枢神经系统兴奋，有助于提高记忆和识别能力，还可以用于治疗和防御一些疾病。具有如此多生理活性的咖啡碱一直是茶树研究的一个重点和热点。



$C_8H_{10}N_4O_2$
1,3,7-trimethylxanthine
Caffeine

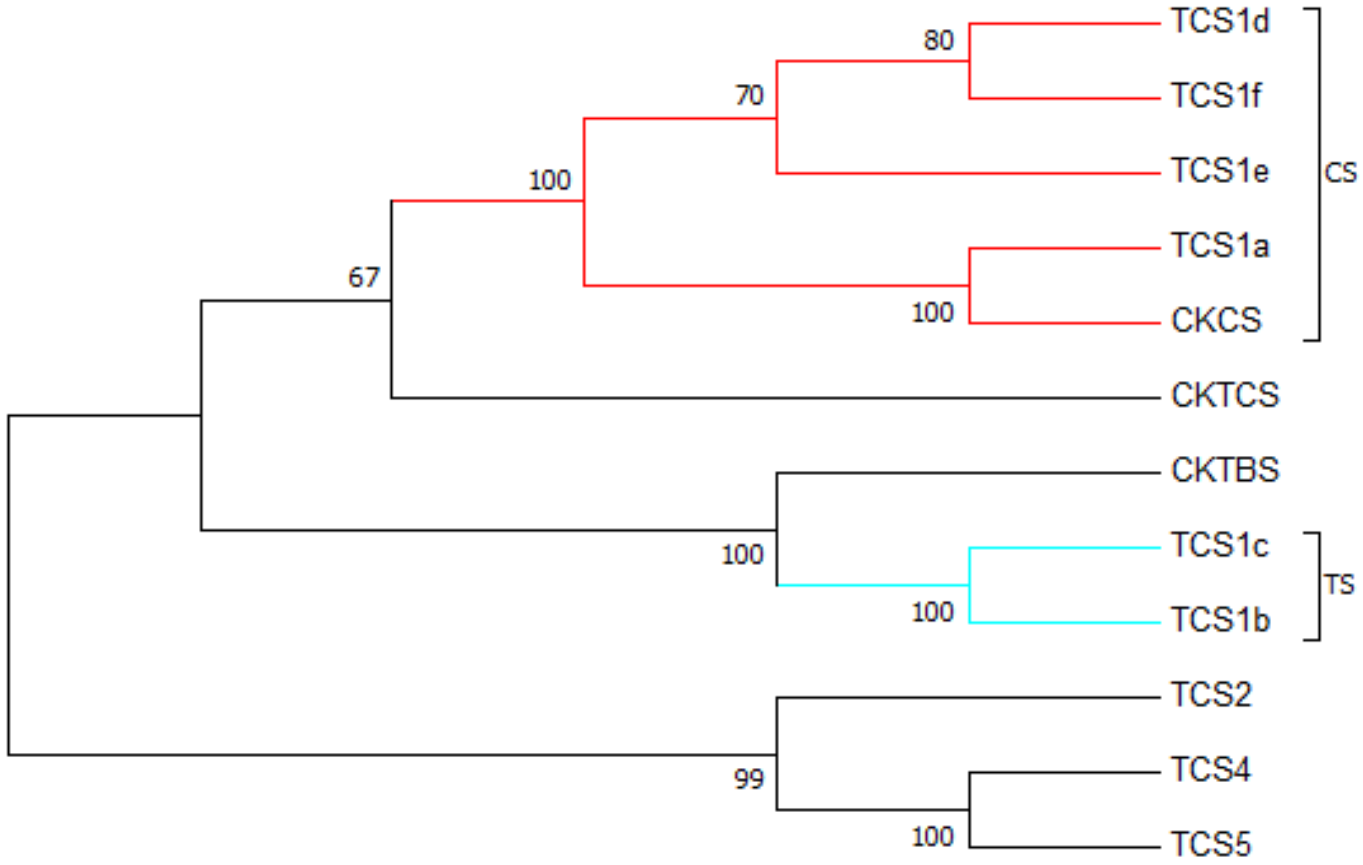
以往研究发现，可以合成咖啡碱等嘌呤生物碱的植物（茶、咖啡和可可）都具有如下图所示的咖啡碱核心合成途径，即由黄嘌呤核苷通过三步甲基化和一步脱核糖反应生成咖啡碱。其中，三步甲基化所需要的氮甲基转移酶（NMT）是咖啡碱等嘌呤生物碱合成的关键酶类。



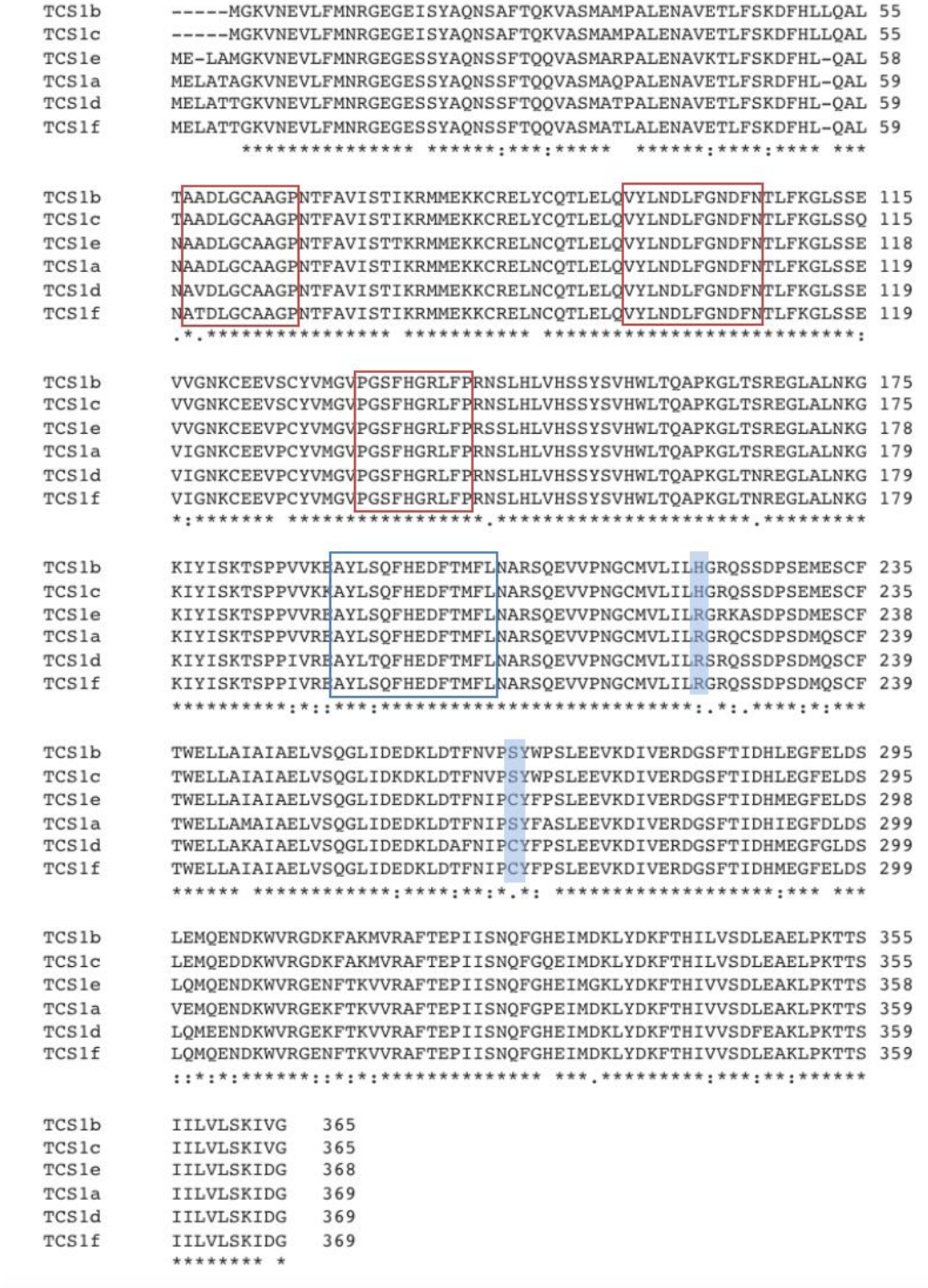
2、蛋白质序列分析

2.1、山茶属植物*NMTs*

现已从山茶属中分离克隆到20条左右的NMT基因。茶树中有多个NMT基因，研究表明茶树中咖啡碱合成的关键基因是*TCS1*，其他的基因无生物学功能或表达水平极低。现已克隆出6种NMT基因（*TCS1-TCS6*），并发现*TCS1*在茶组植物中存在变异，克隆出6种*TCS1*等位基因。



2.2、*TCS1*稀有等位变异氨基酸序列分析



3、蛋白质结构预测

3.1 咖啡碱合成酶TCS1一级结构分析
Protparam分析序列理化性质

Number of amino acids: 369

Molecular weight: 41272.07

Theoretical pI: 5.04

Amino acid composition: CSV format

Ala (A)	23	6.2%
Arg (R)	14	3.8%
Asn (N)	18	4.9%
Asp (D)	20	5.4%
Cys (C)	8	2.2%
Gln (Q)	15	4.1%
Glu (E)	29	7.9%
Gly (G)	22	6.0%
His (H)	8	2.2%
Ile (I)	19	5.1%
Leu (L)	37	10.0%
Lys (K)	20	5.4%
Met (M)	12	3.3%
Phe (F)	22	6.0%
Pro (P)	14	3.8%
Ser (S)	31	8.4%
Thr (T)	19	5.1%
Trp (W)	3	0.8%
Tyr (Y)	8	2.2%
Val (V)	27	7.3%
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): 49
Total number of positively charged residues (Arg + Lys): 34

Atomic composition:

Carbon	C	1834
Hydrogen	H	2871
Nitrogen	N	483
Oxygen	O	559
Sulfur	S	20

Formula: $C_{1834}H_{2871}N_{483}O_{559}S_{20}$
Total number of atoms: 5767

Extinction coefficients:

Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water.

Ext. coefficient 28920
Abs 0.1% (=1 g/l) 0.701, assuming all pairs of Cys residues form cystines

Ext. coefficient 28420
Abs 0.1% (=1 g/l) 0.689, assuming all Cys residues are reduced

Estimated half-life:

The N-terminal of the sequence considered is M (Met).

The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro).
>20 hours (yeast, in vivo).
>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 35.83
This classifies the protein as stable.

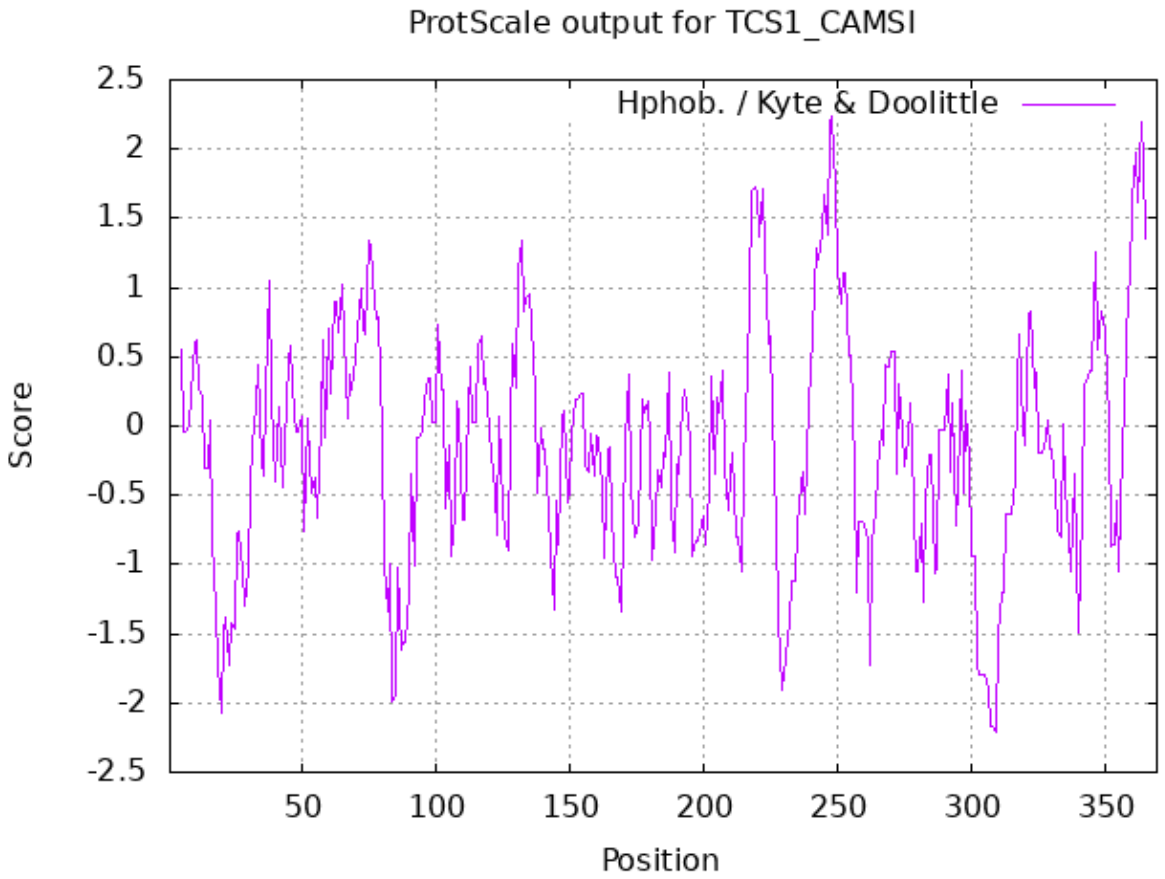
Aliphatic index: 86.64

Grand average of hydropathicity (GRAVY): -0.137

Protparam分析序列理化性质

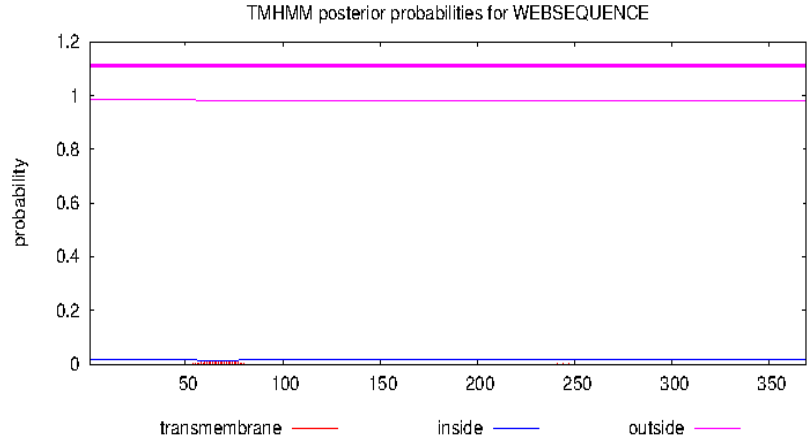
Number of amino acids	369
Molecular weight	41272.07
Theoretical pI	5.04
Total number of negatively charged residues （Asp+Glu）	49
Total number of positively charged residues （Arg+Lys）	34
Aliphatic index	86.64
Grand average of hydropathicity （GRAVY）	-0.137

Protscale分析亲疏水性、TMHMM分析跨膜螺旋



TMHMM result

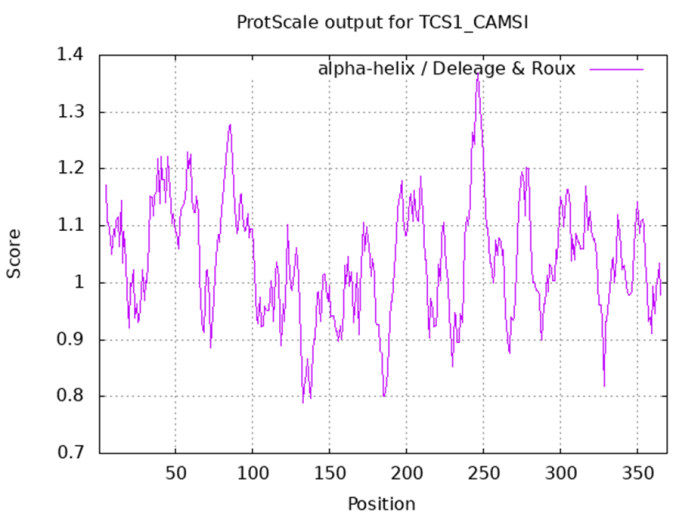
```
# WEBSEQUENCE Length: 369
# WEBSEQUENCE Number of predicted TMHs:  0
# WEBSEQUENCE Exp number of AAs in TMHs: 0.16553
# WEBSEQUENCE Exp number, first 60 AAs:  0.02844
# WEBSEQUENCE Total prob of N-in:        0.01544
WEBSEQUENCE     TMHMM2.0        outside      1   369
```



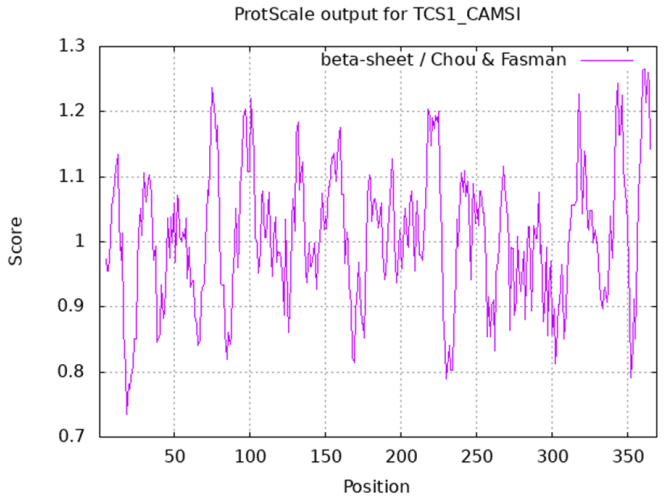
EMBOSS Explorer分析密码子偏好

Codon adaptation index, CAI	0.645
Effective number of codon, ENC	48.288
Observed number of occurrences of codon 'i', Obsi	Frequency
TTT	72.653
AAA	67.690
AAT	48.736

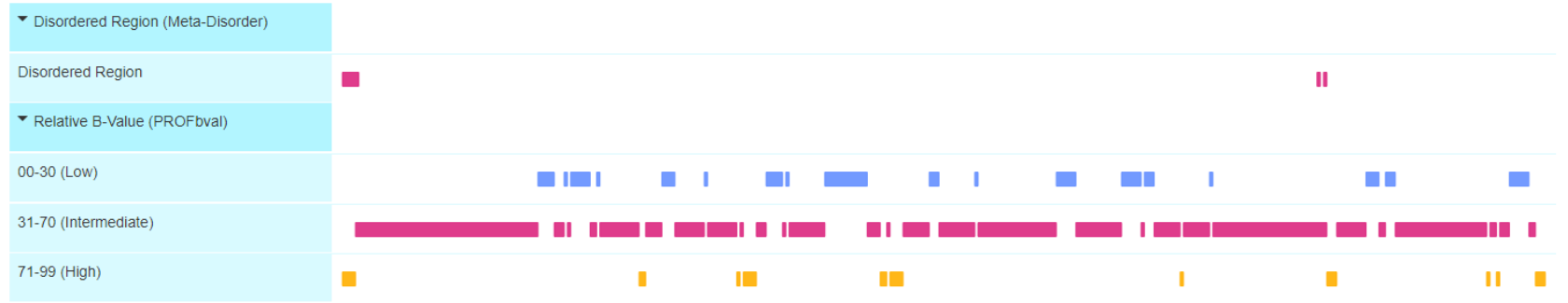
3.2 咖啡碱合成酶TCS1二级结构分析
PredictProtein预测蛋白质二级结构



A



B



PredictProtein预测亚细胞定位

细胞质

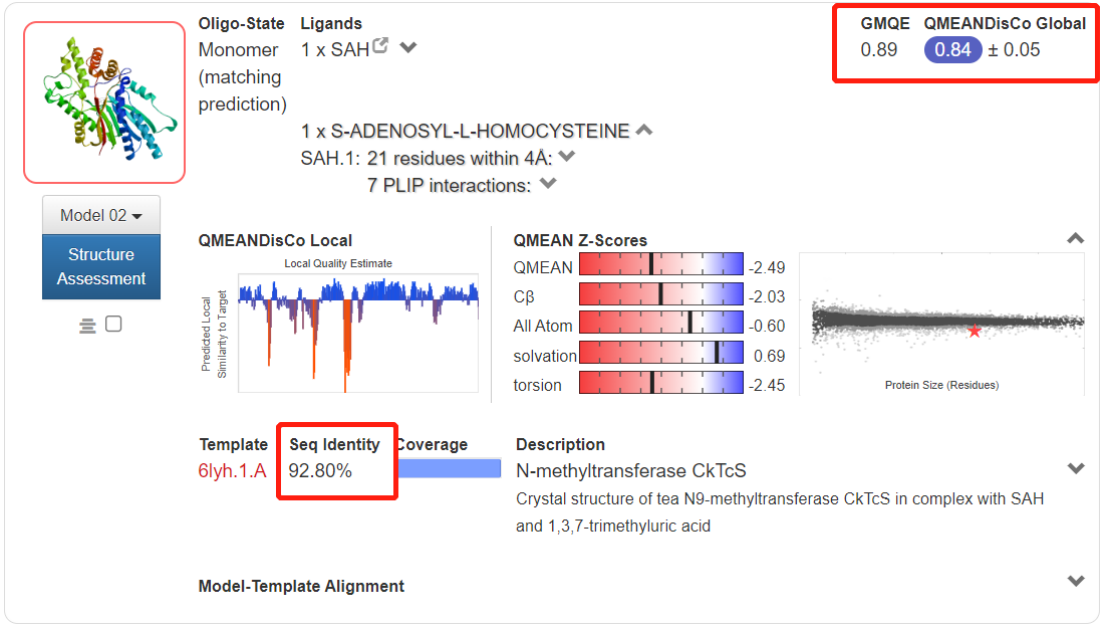
Predicted localization for the Eukarya domain: Cytoplasm (GO term ID: GO:0005737) Prediction confidence 26

3.2 咖啡碱合成酶TCS1三级结构预测
Swiss-Model查找蛋白质结构模型

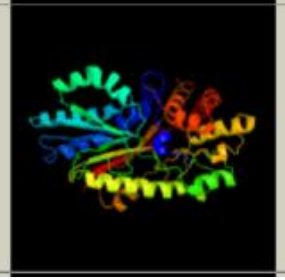
Template Results

Templates | Quaternary Structure | Sequence Similarity | Alignment | More

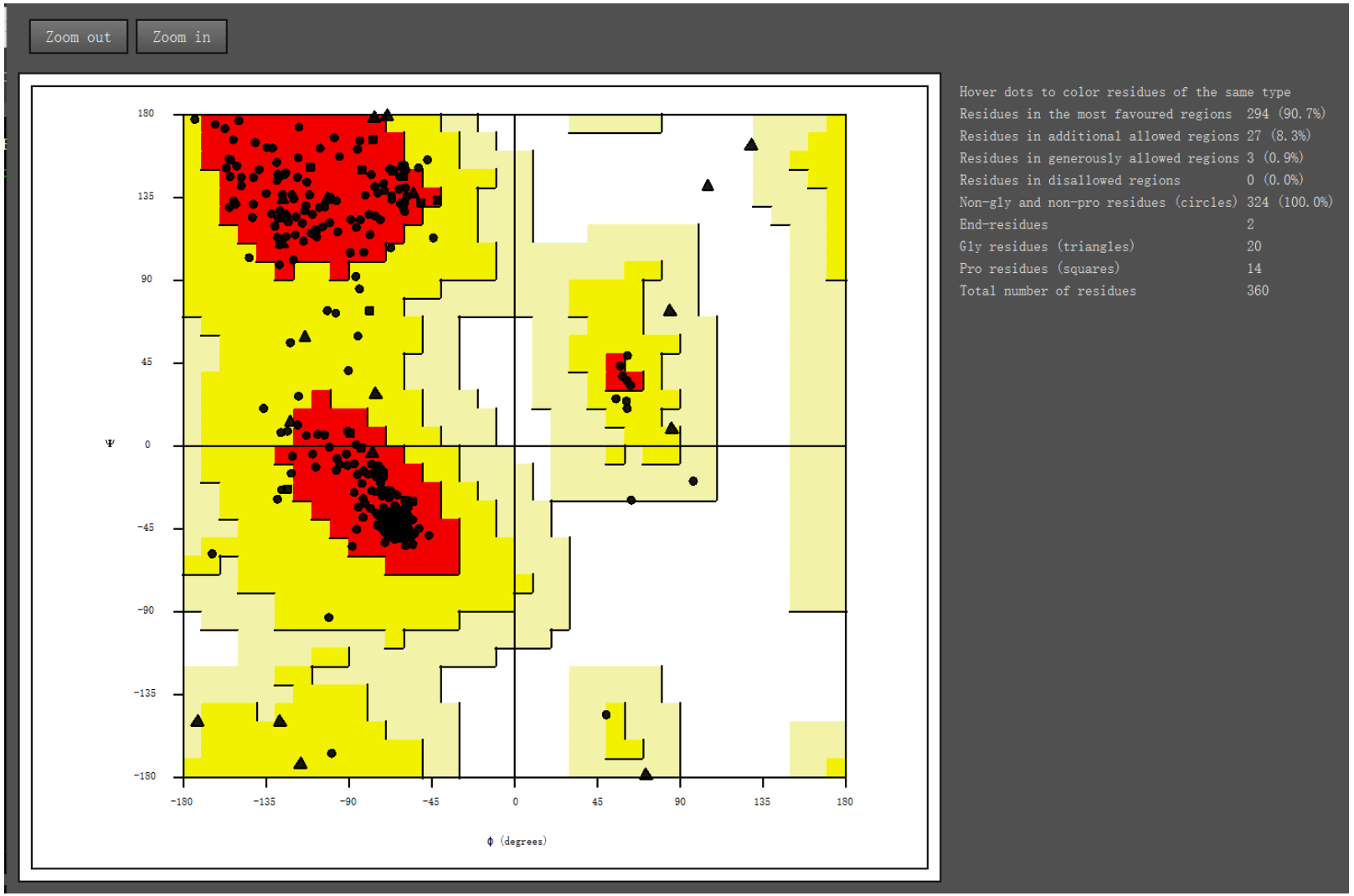
Sort	Coverage	GMQE	QSQE	Identity	Method	Oligo State	Ligands
6lyh.1.A N-methyltransferase CkTcS *Crystal structure of tea N9-methyltransferase CkTcS in complex with SAH and 1,3,7-trimethyluric acid*		0.90	0.61	91.97	X-ray, 3.2Å	homo-dimer	2 x SAH, 2 x EXU
6lyh.1.A N-methyltransferase CkTcS *Crystal structure of tea N9-methyltransferase CkTcS in complex with SAH and 1,3,7-trimethyluric acid*		0.90	0.61	92.80	X-ray, 3.2Å	homo-dimer	2 x SAH, 2 x EXU
6lyi.1.B N-methyltransferase CkTbS *Crystal structure of a N-methyltransferase CkTbS from Camellia assamica var. kucha*		0.90	0.62	91.06	X-ray, 2.5Å	homo-dimer	None
6lyi.1.B N-methyltransferase CkTbS *Crystal structure of a N-methyltransferase CkTbS from Camellia assamica var. kucha*		0.89	0.62	91.60	X-ray, 2.5Å	homo-dimer	None
6lyi.1.A N-methyltransferase CkTbS *Crystal structure of a N-methyltransferase CkTbS from Camellia assamica var. kucha*		0.88	0.60	91.06	X-ray, 2.5Å	homo-dimer	None
6lyi.1.A N-methyltransferase CkTbS *Crystal structure of a N-methyltransferase CkTbS from Camellia assamica var. kucha*		0.88	0.60	91.60	X-ray, 2.5Å	homo-dimer	None



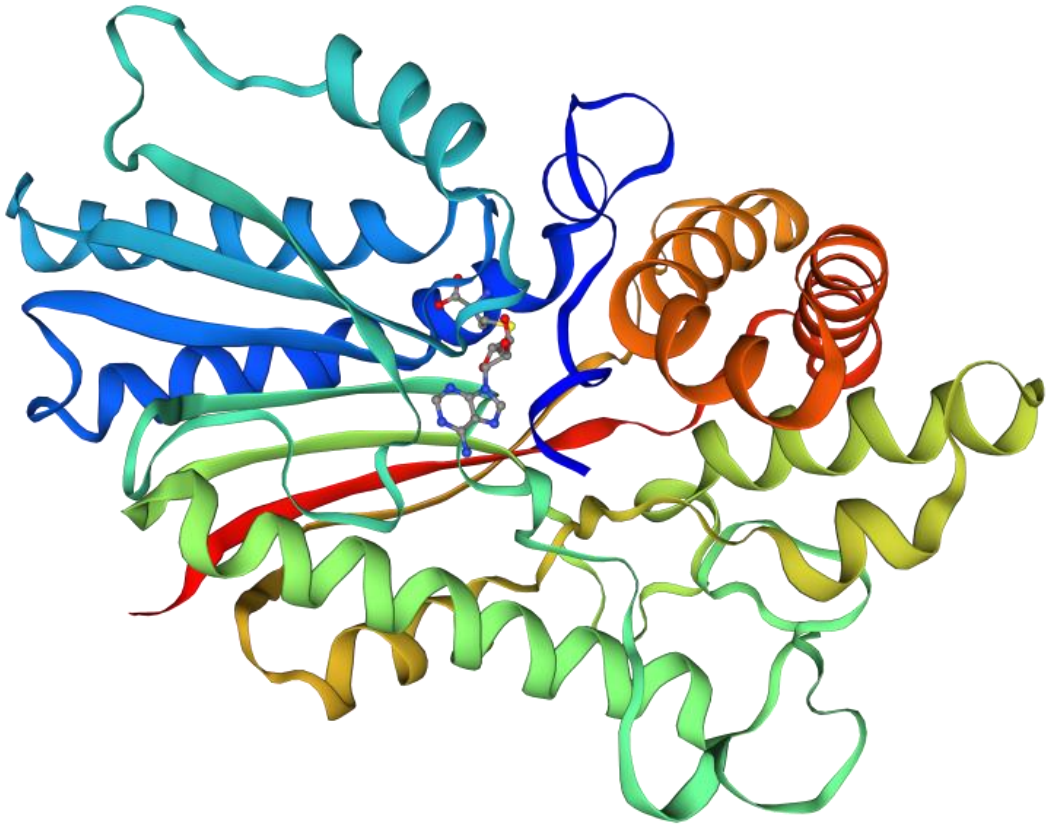
Phyre²网站建模

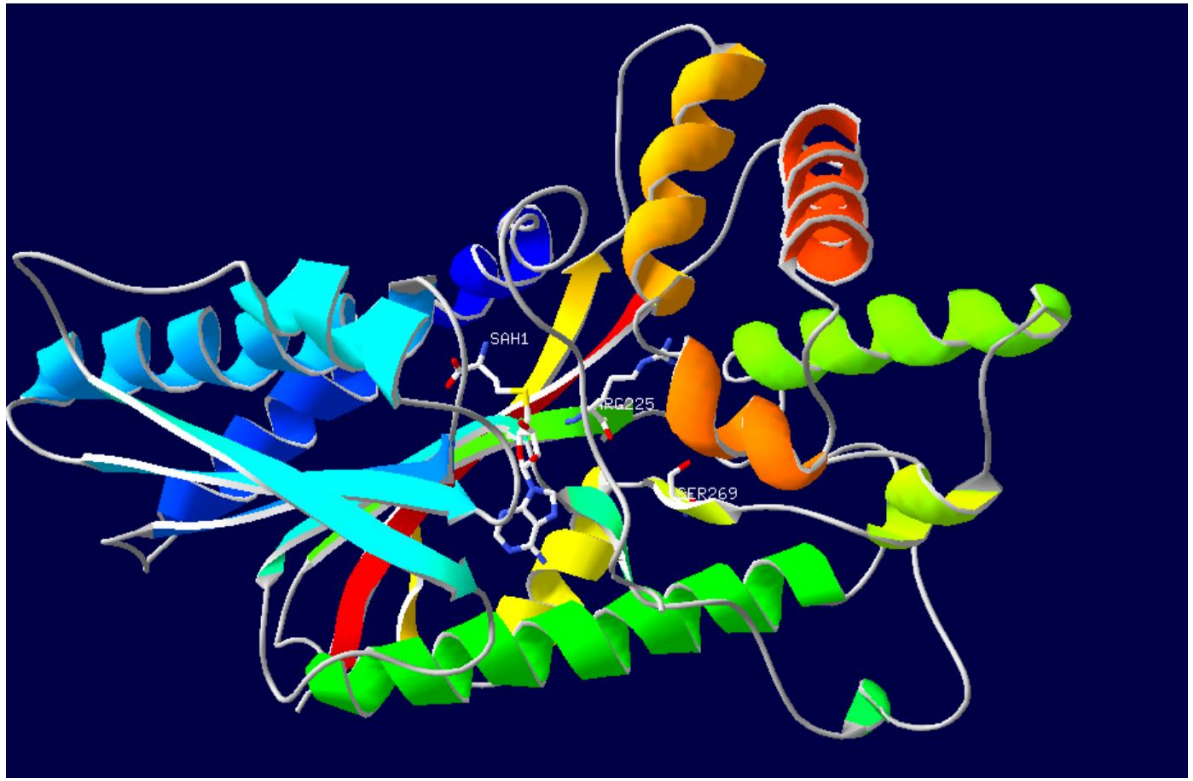
#	Template	Alignment Coverage	3D Model	Confidence	% i.d.	Template Information
1	c6lyhE	Alignment		100.0	91	**PDB header:**transferase **Chain:** E: **PDB Molecule:**n-methyltransferase cktcs; **PDBTitle:** crystal structure of tea n9-methyltransferase cktcs in com with2 sah and 1,3,7-trimethyluric acid **PDB Entry:** PDBe RCSB PDBj
2	d1m6ex	Alignment		100.0	41	**Fold:** S-adenosyl-L-methionine-dependent methyltransferases **Superfamily:** S-adenosyl-L-methionine-dependent methyltransferas **Family:** Salicylic acid carboxyl methyltransferase (SAMT) **PDB entry:** PDBe RCSB PDBj

Ramachandran Plot（拉氏图）

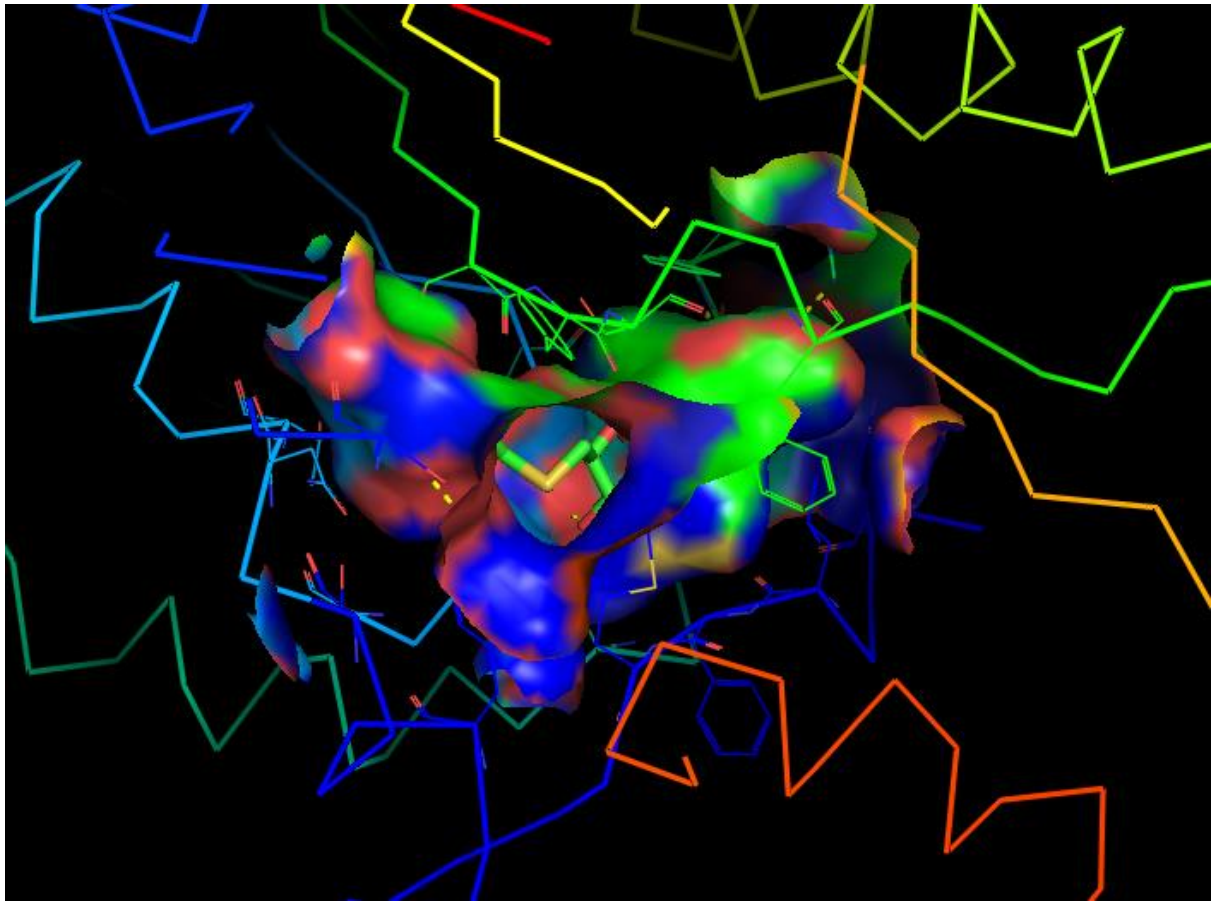


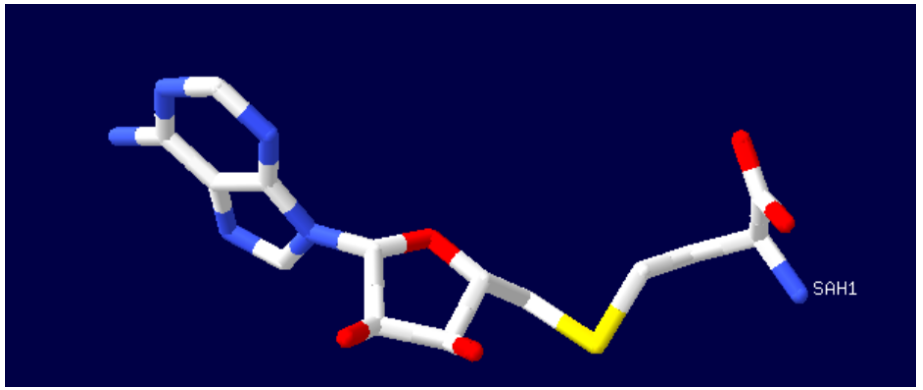
TCS1三级结构预测结果





TCS1特殊三级结构

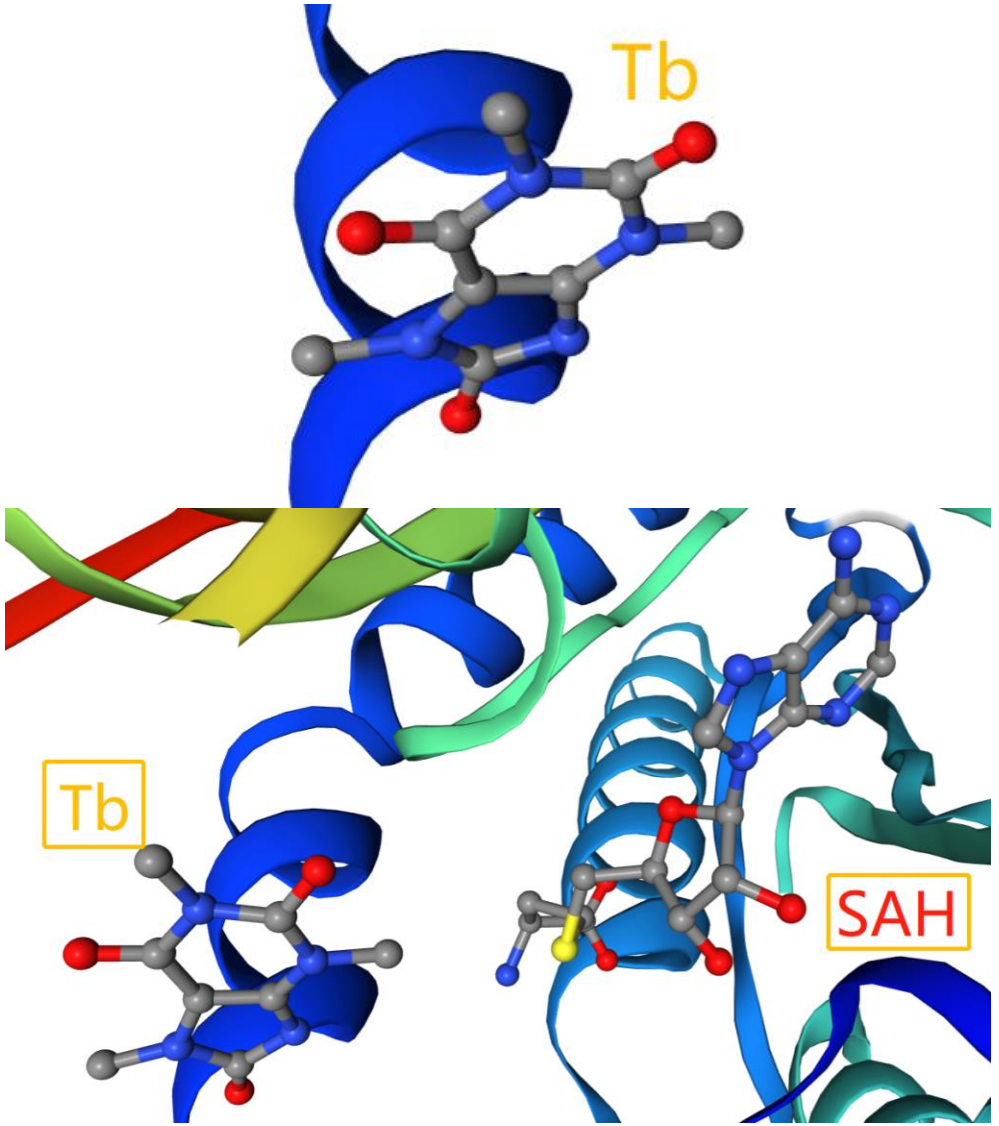




SAH

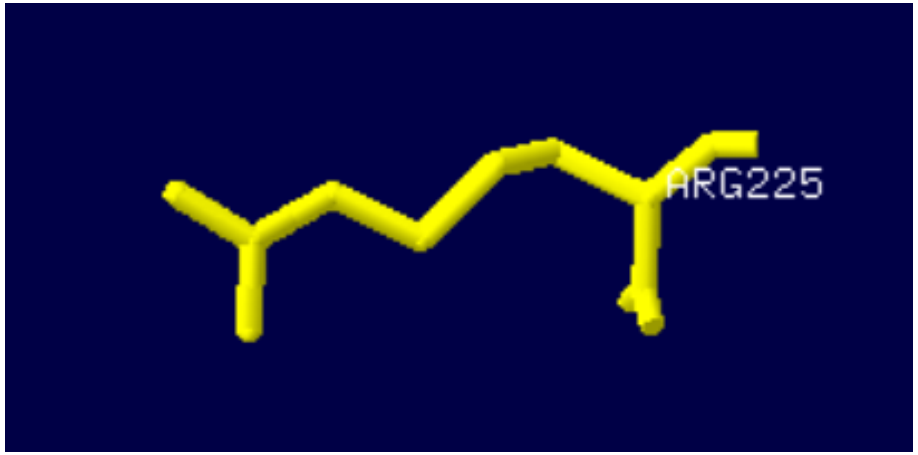
底物结构与底物结合位点

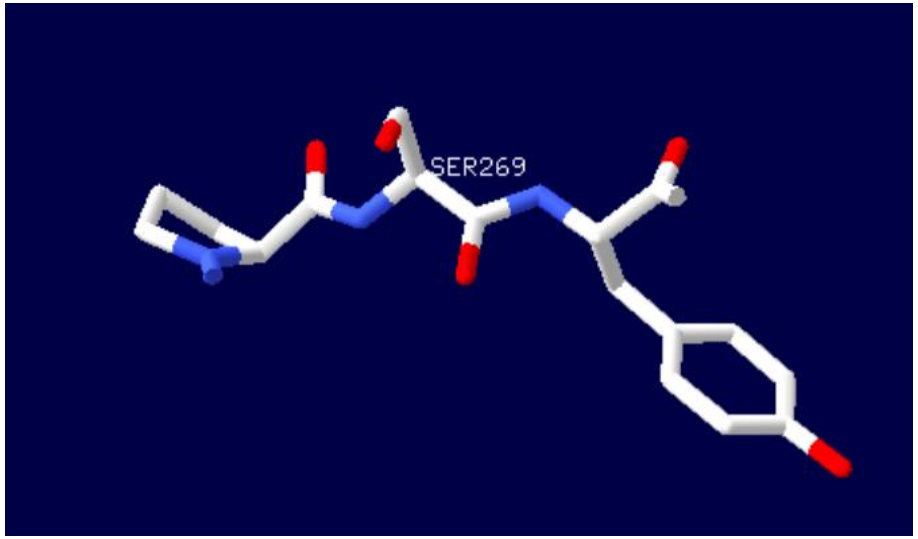
茶树咖啡碱合成酶1（TCS1）具有催化N-3和N-1甲基化的活性，能够催化7-甲基黄嘌呤（7mX）与S-腺苷-L-蛋氨酸（SAM）生成可可碱（theobromine），进而生成咖啡碱。



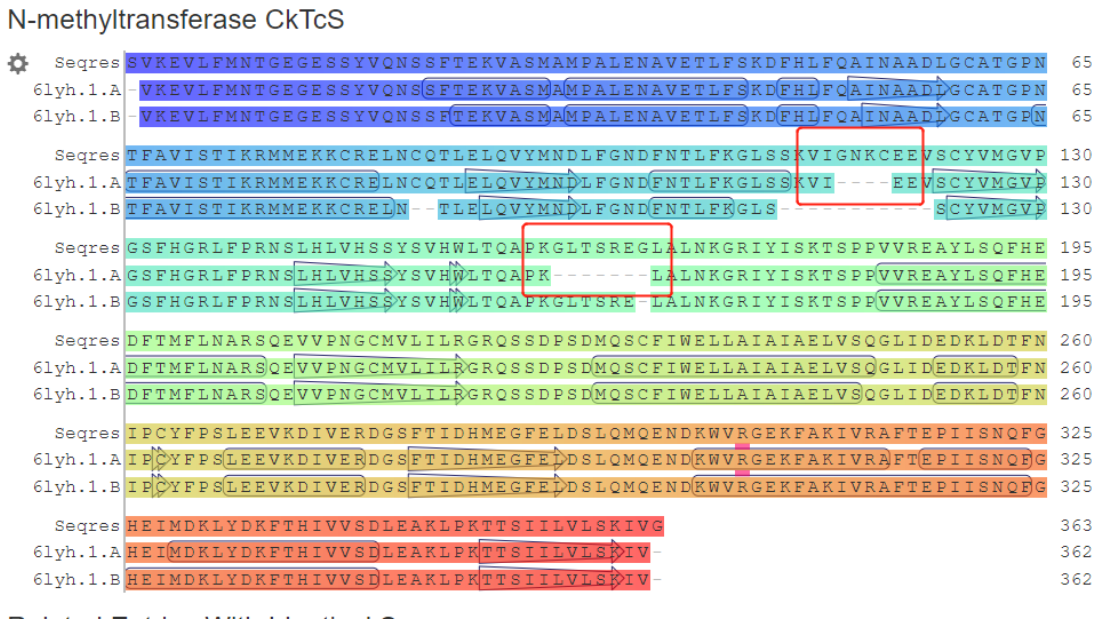
TCS1关键氨基酸位点

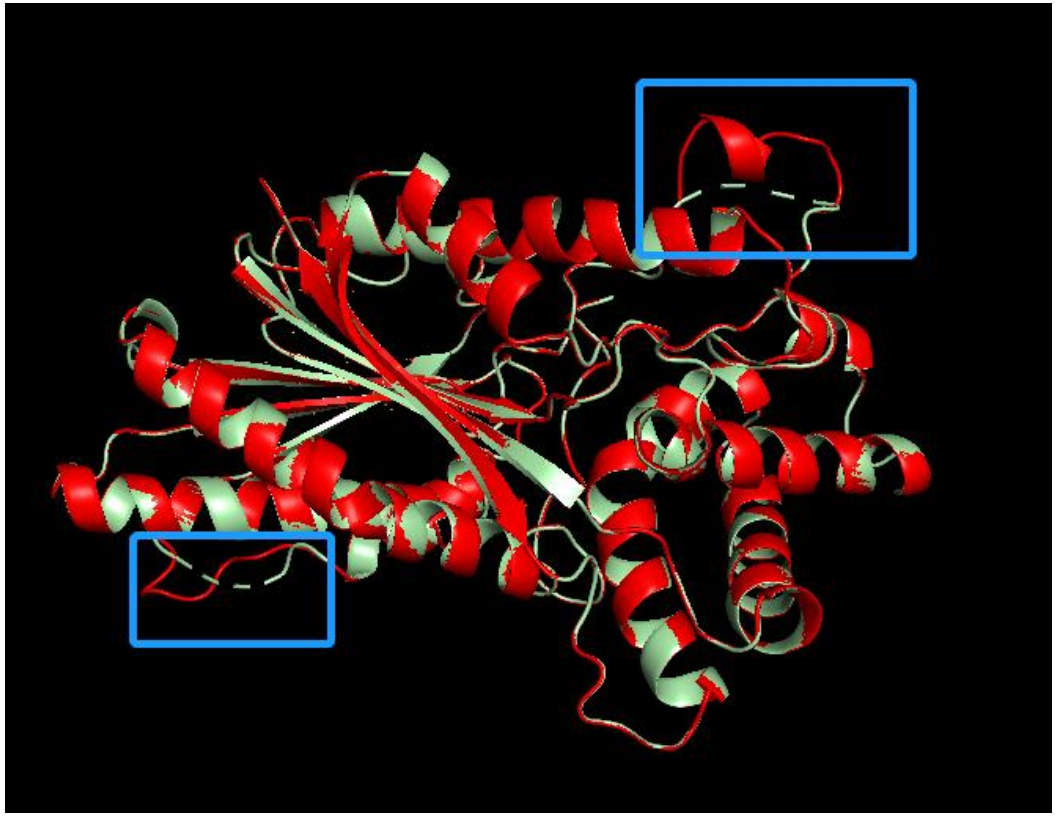
与*TCS1a-c*相比，*TCS1d-f* 具有Ser269Cys变化，*TCS1a*和*TCS1d*中269位氨基酸残基分别为丝氨酸和半胱氨酸，进行定点诱变实验证明TCS1中的第225 、 269位氨基酸残基在TCS（咖啡碱合成酶）活性和底物识别中起重要作用。





TCS1与CkTcS三级结构比较





注：红色为TCS1a
绿色为CkTcS

总结

a) 将新鉴定的N-甲基转移酶与已知的N-甲基转移酶进行系统发生分析建树，确定它们的进化与分枝情况，根据结果分析N-甲基转移酶是否通过趋同进化从两个独立的起源进化而来， 从而初步推断酶功能。

b) 对酶的蛋白质一级结构进行理化分析，为我们提供酶的基础信息用于鉴定、设计底物活性实验及酶的基础分离实验。

c) 蛋白质三级结构预测可以为我们提供酶与底物及辅因子的结合情况，判断其存在形式、核心区域、氢键作用、保守结构域、活性结构等基础信息，对于酶的功能活性研究，及后续的可变位点 或 关键位点的定点突变实验的设计具有重要意义。

总结

d) 在蛋白质的结构分析中找到主要作用残基位点，底物结合特异性，重要的相互作用位点，设计实验判断定向底物的位点,综上对一个未知蛋白进行系统的挖掘验证。

e) 茶树相关数据库如TAIP，拟南芥相关数据库TAIR和转录因子数据库Plant TFDB，为进一步研究调控咖啡碱合成酶基因的启动子及相关转录因子提供基础信息。根据已知元件进行转录水平研究，从转录水平分析影响茶树咖啡碱代谢合成的调控机制。

参考文献

[1]Jin, Ji-Qiang, Ma, et al. Natural allelic variations of TCS1 play a crucial role in caffeine biosynthesis of tea plant and its related species[J]. Plant Physiology & Biochemistry, 2016.

[2] Zhang YH, Li YF, Wang Y , et al. Identification and characterization of N9-methyltransferase involved in converting caffeine into non-stimulatory theacrine in tea. Nat Commun. 2020 Mar 19;11(1):1473. doi: 10.1038/s41467-020-15324-7.

[3] Jin JQ, Chai YF, Liu YF , et al. Hongyacha, a Naturally Caffeine-Free Tea Plant from Fujian, China. J Agric Food Chem. 2018 Oct 31;66(43):11311-11319. doi: 10.1021/acs.jafc.8b03433.

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