



微拟球藻中的限速酶DGAT的探究

Bioinformatic Analysis of DGAT in *Nannochloropsis oceanica*

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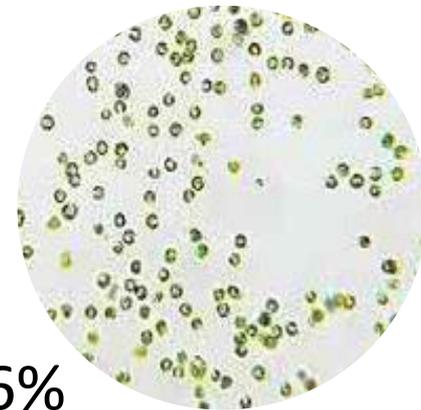
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研究背景

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微拟球藻 (Nannochloropsis) 属于单细胞光合微藻中的眼点藻纲，细胞直径2-5 μm ，生长快，可在盐碱、荒地等非耕地使用海水进行培养。



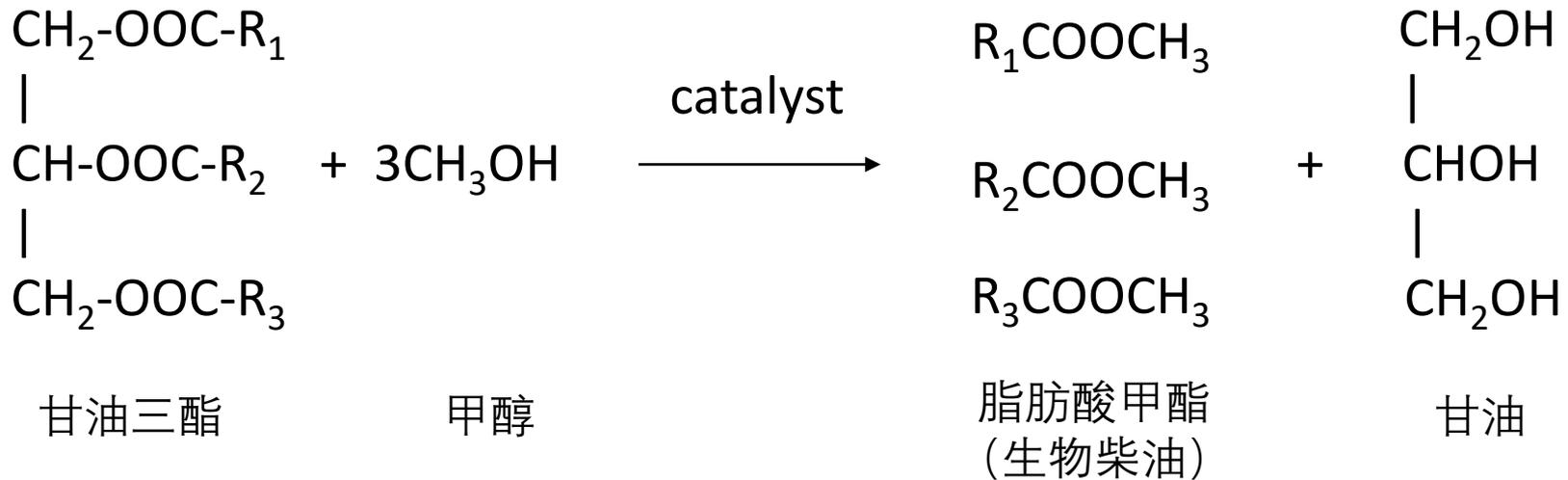
优势：

1. 生长快，易培养
2. 甘油三脂(TAG)产量在 10%-36%
3. 细胞壁较薄易破碎
4. 20世纪80年代就已有较好的规模化生产方法和技术基础
5. 有较为成熟的分子工程平台



研究背景

生产生物柴油的转酯化反应



研究背景

DGAT

DGAT是Kennedy途径合成TAG的唯一限速酶，能催化DAG和acyl-CoA，将acyl-CoA的酰基基团转移到DAG的sn-3位置合成TAG。



Kennedy 途径

DGAT被认为在TAG合成及积累方面具有重要的作用，相关研究表明DAGT与植物种子发育、萌发，动物饮食性肥胖有关。



研究背景

DGAT分类及特点

	分布	类型	跨膜结构域	分子量 kDa	等电点	氨基酸数	基因定位	cDNA长度 bp	外显子/内含子数
DGAT1	动物、植物	跨膜蛋白	9-10	约59	8.71-9.63	约500	染色体II (拟南芥)	1998	16/15 (拟南芥)
DGAT2	动物、植物、酵母	跨膜蛋白	2-3	约39	8.86-9.70	约340	染色体III (拟南芥)	1330	8/7 (拟南芥)
DGAT3	植物 (花生)	胞质溶解蛋白	0	约41	8.17-9.47	约350	*	1637	*

Cases,1998; Cao,2011; Saha,2006; Hobbs,1999; Bouvier,2000



DGAT基因及氨基酸序列

- 微拟球藻 *Nannochloropsis oceanica*
基因序列(CDs) (Genbank:KY073295.1)
氨基酸序列(Genbank:ASL69957.1)
- 衣藻 *Chlamydomonas reinhardtii*
基因序列(CDs) NCBI Reference Sequence: XM_001693137.1
Cre01.g045903.t1.1 CDS (phytozome数据库)
氨基酸序列 NCBI Reference Sequence: XP_001693189.1
Cre01.g045903.t1.1 (phytozome数据库)
- 小球藻 *Chromochloris zofingiensis*
基因序列(CDs) Cz03g14080.t1 CDS (phytozome数据库)
Cz06g04190.t1 CDS (phytozome数据库)
Cz09g23020.t1 CDS (phytozome数据库)
氨基酸序列 Cz03g14080.t1 (phytozome数据库)
Cz06g04190.t1 (phytozome数据库)
Cz09g23020.t1 (phytozome数据库)



DGAT基因及氨基酸序列

- 三角褐指藻 *Phaeodactylum tricornutum*
基因序列(CDs) (Genbank: >JF828904.1)
氨基酸序列 (Genbank: >AEG79729.1)
- 拟南芥 *Arabidopsis thaliana*
基因序列(CDs) AT1G48300.1 CDS
AT3G51520.1 CDS
氨基酸序列 AT1G48300.1
AT3G51520.1
- 花生 *Arachis hypogaea*
基因序列(CDs) GenBank: KC736067.1
GenBank: EU183333.1
氨基酸序列 GenBank: AGT57760.1
GenBank: ABW34442.1



DGAT基因及氨基酸序列

- 小鼠 *Mus musculus*
基因序列(CDs) GenBank: AF078752.1
氨基酸序列 GenBank: AAC72917.1
- 大鼠 *Rattus norvegicus*
基因序列(CDs) GenBank: AB062763.1
氨基酸序列 GenBank: BAC43743.1
- 人 *Homo sapiens*
基因序列(CDs) NCBI Reference Sequence: NM_012079.5
氨基酸序列 NP_036211.2



多序列比对



利用Muscle进行mRNA多序列比对

Species/Abbrv	Group Name	
1. Nannochloropsis_oceanica_DGAT1		-- GGGGCGTCTA AAAATGACGGCCAAACGTCCCGTTTTTCGCTGTCTCGGCCTTCTTCCACGAGCTGCTG -- ATCTCGATAACCTGCG --
2. Chlamydomonas_reinhardtii_DGAT1_1		--- GCGCCGGCGCCGTA ---
3. Chlamydomonas_reinhardtii_DGAT1_2		-- GGCAGCAGCAGGTTCAATGCCATCCTGCTCACGTTCTTCTGTCTCGGCCTGTGTTCCACGAGCTGCTA --- CTGGGGGTGCCGTG ---
4. Chromochloris_zofingiensis_DGAT1_1		--- CTGGGCTTCCCCTTC ---
5. Chromochloris_zofingiensis_DGAT1_2		-- GGTATCAACAGGTGGTGGGCAATGCTGTTAGTGTTCCTTGTGAGTGGAGTGTTCATGAGTTGGCA --- GTGGGTGTTCATTAG ---
6. Chromochloris_zofingiensis_DGAT1_3		--- CTGGGCTTCCCCTTC ---
7. Phaeodactylum_tricornutum_DGAT1		-- AAGATGCCGAAAGTCCGCTGCAACCTTTGTCGTTTTTTTCTCTCCGCGGTTATGCAAGAGGTGCTT --- GTCAAGCTACCTTT ---
8. Arabidopsis_thaliana_DGAT3		--- ATGACGGGTTTCGAA ---
9. Arabidopsis_thaliana_DGAT2		---
10. Arachis_hypogaea_DGAT3_3		--- GGTGCTGAA ---
11. Arachis_hypogaea_DGAT3_1		--- GGTGCT ---
12. Mus_musculus_DGAT1		TTGGTTTTTCCACTCCGTCTCAATGCTGTGGCAGAGCTTCTGCAAGTTTGGAGAACCGCGAGTTCTACAGAGATTGGTGGAAATGCTG
13. Rattus_norvegicus_DGAT1		TTGGCTTTTCCACTCAATGCTCAATGCTGTGGCAGAGCTCCTGCAAGTTTGGAGAACCGCGAGTTCTACAGGGACTGCTGGAAATGCTG
14. Homo_sapiens_DGAT1		TGCAGACAGCGAGCCCTGTCAGCCTTCAAGAACCTTGTTTCCAGGCCCTGCAATCCACCAAGCTGGATAGATGGGCCCCCTTGA

Species/Abbrv	Group Name	
1. Nannochloropsis_oceanica_DGAT1		--- T T C G G G G A T C G A T T G T T T T C A A G G A T T G G --- T G G A A C A G C A C C A C G T T C T C C C G C T A T T G G C G A A C T ---
2. Chlamydomonas_reinhardtii_DGAT1_1		--- T T C G G C A G A C G C G C G T A C A G C T G G T T C G G C C G --- T G G A A T G C G G C T A C G G T G G G C G A G T A C T G G A A G C T T ---
3. Chlamydomonas_reinhardtii_DGAT1_2		--- T T C G G C A C C G C G A G T T C T A C A A G G A C T G G --- T G G A A T G C G G C T A C G G T G G G C G A G T A C T G G A A G C T A ---
4. Chromochloris_zofingiensis_DGAT1_1		--- T T G G G G A G A C G C T A C A G C T G A G G A A T A T C --- T G G A A T G C A G C T A C T G T G G G T G A C T A T T G G A A G C T A ---
5. Chromochloris_zofingiensis_DGAT1_2		--- T T T G G G A C C G G G A G T T T T A C A A G G A T T G G --- T G G A A T G C A G C T A C T G T G G G T G A C T A T T G G A A G C T A ---
6. Chromochloris_zofingiensis_DGAT1_3		--- T T G G G G A G A C G C T A C A G C T G A G G A A T A T C --- T G G A A T T C G T C G G A A G T A T C T G C A T A T T G G A G G C T T ---
7. Phaeodactylum_tricornutum_DGAT1		--- T T T G G A G A T C G T G T G T T C T A C A A G A T T G G --- T G G A A T T C G T C G G A A G T A T C T G C A T A T T G G A G G C T T ---
8. Arabidopsis_thaliana_DGAT3		--- A T G G G A G G G A A G --- T G T A A G A G A T C A ---
9. Arabidopsis_thaliana_DGAT2		--- T T T G G T C A G G C A C G C G T G T A C A A A T G G T G G A A G ---
10. Arachis_hypogaea_DGAT3_3		--- A T G G G A A A C A A G --- T G C A A G A A A T C A ---
11. Arachis_hypogaea_DGAT3_1		--- A T G G G A A A C A A G --- T G C A A G A A A T C A ---
12. Mus_musculus_DGAT1		--- T A C G A C G A G T T C T T G A G A T G C T C T T T T T A --- C C C A G C T T C A A G T G G G G C T G A T C C A A C A G T G G A T G G T C C C T A C ---
13. Rattus_norvegicus_DGAT1		--- T A C G G C G G G T T C T T G A G A T G C T C T T T T T C A --- C C C A G C T T C A A G T G G G G C T G A T C C A G C A G T G G A T G G T C C C T A C ---
14. Homo_sapiens_DGAT1		A G G G G C T A C G G T A C A C A A T G G C T A T G T C C A T G G C T A --- T T C G C C T T G G G G C A G G G C T C G G T T ---



利用ClustalOmega进行蛋白多序列比对

Arabidopsis_thaliana_DGAT3	-----	360
Arachis_hypogaea_DGAT3_3	-----	340
Arachis_hypogaea_DGAT3_1	-----	340
Homo_sapiens_DGAT1	GDREFYRDWWNSESVTYFWQNWNI PVHKWCIRHFYKPMLRRGSSKWMARTGVFLASAFFH	415
Mus_musculus_DGAT1	GDREFYRDWWNAESVTYFWQNWNI PVHKWCIRHFYKPMLRHGSSKQVARTGVFLTSAFFH	426
Rattus_norvegicus_DGAT1	GDREFYRDWWNAESVTYFWQNWNI PVHKWCIRHFYKPMLRLGSKWMARTGVFLASAFFH	428
Nannochloropsis_oceanica_DGAT1	GDRLFFKDWWNSTSFSRYWRTWNL PVHQFVVRHVYFPLL RAGASKMTANVAVFAVSAFFH	360
Phaeodactylum_tricornutum_DGAT1	GDRVIFYKDWWNSSEVSAYWRLWNMPVHYWLI RHVYFPCVRLKMPKVAATFVVFLLSVMH	433
Chlamydomonas_reinhardtii_DGAT1_2	GDREFYKDWWNAATVGEYWKLWNMPVHKWLLRHVYFPAIRAGSSRFNAILLTFFVSAVFH	248
Chromochloris_zofingiensis_DGAT1_2	GDREFYKDWWNAATVGDYWKLWNMPVHKWLLRTIYFPALRNGINRWAMLLVFFVSGVFH	79
Chromochloris_zofingiensis_DGAT1_1	GETLQLRNIFDVPTLQ-QL-----TYKRLGFPFPYMLVGRWG-C--SPFPKKVPL	206
Chromochloris_zofingiensis_DGAT1_3	GETLQLRNIFDVPTLQ-QL-----TYKRLGFPFPYMLVGRWG-C--SPFPKKVPL	206
Chlamydomonas_reinhardtii_DGAT1_1	GQTRAYSWFRPGPLVPTW-----LVERISRAAGAVPIGMFG-QYGTMPHREPL	271
Arabidopsis_thaliana_DGAT2	GQARVYKWKPKDCDLY-----LKL SRAIRFTPICFWG-VFGSPLPCRQPM	262
Arabidopsis_thaliana_DGAT3	-----GSVSY*-----	360
Arachis_hypogaea_DGAT3_3	-----	340
Arachis_hypogaea_DGAT3_1	-----	340
Homo_sapiens_DGAT1	-----KMDYSRIIERLLKLA---VPNHLIWLI---FFYWLFHSCLNVAEELMQF	355
Mus_musculus_DGAT1	-----KMDYSRIIERLLKLA---VPNHLIWLI---FFYWFFHSCLNVAEELLQF	366
Rattus_norvegicus_DGAT1	-----KMDYSRIIERLLKLA---VPNHLIWLI---FFYWLFHSCLNVAEELLQF	368
Nannochloropsis_oceanica_DGAT1	--V---D--SLDLLKALGELLRLA---IPSTFVWLI---FFYVYFHCTLNLF AELTRF	300
Phaeodactylum_tricornutum_DGAT1	SDLDETNGS--YTAIFA EYWLKLS---IANTYLWLL---MFYTYFHL YLNLFAELLRF	373
Chlamydomonas_reinhardtii_DGAT1_2	FSVFDSSGGSLLL DLPRVAERVLKLA---LPSTYAWLL---GFYCLFHLWLNVLAE LTRF	188
Chromochloris_zofingiensis_DGAT1_2	-----MFYCLFHLWLNILAE L TCF	19
Chromochloris_zofingiensis_DGAT1_1	VRALKKEGAVMLC-PGGQAELLHCYRAF KQREFVLHIGHKGFC LAIEHGAAVVPV LAL	160
Chromochloris_zofingiensis_DGAT1_3	VRALKKEGAVMLC-PGGQAELLHCYRAF KQREFVLHIGHKGFC LAIEHGAAVVPV LAL	160
Chlamydomonas_reinhardtii_DGAT1_1	SGLLRARKVAVLV-PGGVQEV L NME---HGKEVAYLSSRTGFVRLAVQHGA PLVPVWAF	222
Arabidopsis_thaliana_DGAT2	TSLLDSGYSCVLV-PGGVQET FHMQ---HDAENVFLSRRRGFVRIAMEQGSPLVPVFCF	218



藻类之间的蛋白多序列比对

Nannochloropsis_oceanica_DGAT1	--SLDLLKALGELLRLAIPSTFVWLIFVYFHC TLNLF AELTRFGDRLFFKDWNSTSF	315
Phaeodactylum_tricornutum_DGAT1	S--YTA AIFA EYWLKLSIANTYLWLLMFYTYFHL YLNLFAELLRF GDRVFKDWNNSSEV	388
Chlamydomonas_reinhardtii_DGAT1_2	LLLLDLPRVAERVLK LALPSTYAWLLGFYCLFHLWLNVL AELTRFGDREFYKDWNAATV	203
Chromochloris_zofingiensis_DGAT1_2	-----MFYCLFHLWLNILAELTCFGDREFYKDWNAATV	34
	** ** **::*** **** *:*****:: .	
Nannochloropsis_oceanica_DGAT1	SRYWRTWNL PVHQFVVRHVYFPLL RAGASKMTANVAVFAVS AFFHELLISIPCHV VRLWA	375
Phaeodactylum_tricornutum_DGAT1	SAYWRLWNMPVHYWLIRHVYFPCVRLKMPKVAATFVVFVLSAVMHEVLVSVPFHIIRPWS	448
Chlamydomonas_reinhardtii_DGAT1_2	GEYWKLWNMPVHKWLLRHVYFPAIRAGSSRFNAILLTFFVSAVFHELLLGVPLHMVRLWA	263
Chromochloris_zofingiensis_DGAT1_2	GDYWKLWNMPVHKWLLRTIYFPALRNGINRWWAMLLVFFVSGVFHELAVGVPLHMVQYSA	94
	. **:: **::*** :::* :*** :* : * .* :* *..**:: ::* *::: :	
Nannochloropsis_oceanica_DGAT1	FLAMMGQIPLIYLT DQLEKTLFKETQAGNYTFWLIFC IFGQPMVLLYADFSARTTSES	435
Phaeodactylum_tricornutum_DGAT1	FIGMMQIPLVAF TKYLYRK-FPGGSFGNVLFWMTFCVIGQPMAILLYTVDYQYKHHST	507
Chlamydomonas_reinhardtii_DGAT1_2	FAGIMFQVPLIMVTEMLRKK-LNRDELGN YIFWIAFCVVGQPVCVLLYHDYVVGIRPAL	322
Chromochloris_zofingiensis_DGAT1_2	FFGIMLQVPLISL TEALKKH-LKSDTWGN YIFWITFCIVGQPVSLMLYHDWVIAHRQTI	153
	* .:* *::**:: .* * : : ** **:: **::***::** *:	



系统发育树构建



利用ClustalW进行多序列比对

M7: Alignment Explorer (New Text Document_new.FASTA)

Data Edit Search Alignment Web Sequencer Display Help

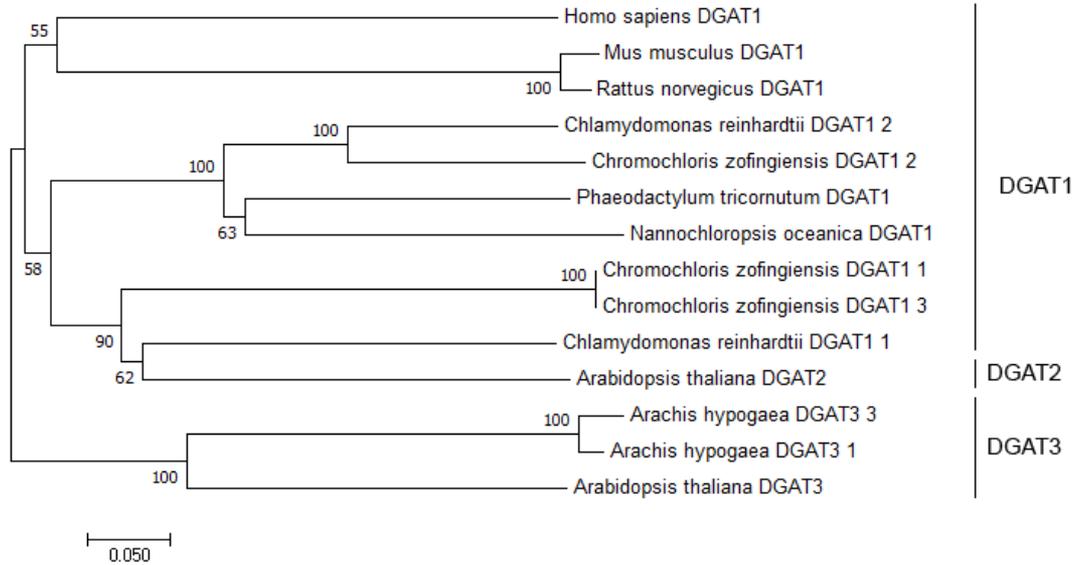
Protein Sequences

Species/Abbrv	Group Name	
1. Nannochloropsis_oceanica_DGAT1		VAVFAVS A F F H E L L I S I P C H - - - V V R L W A F L A M M G Q I P L I Y L T D Q L E K T L F K E T Q A G N Y T F W L I F C I F G Q P M A V L L Y Y A C
2. Chlamydomonas_reinhardtii_DGAT1_1		L V P T W L V E R I S R A A G A V P I G - - - M F G Q Y G - T P M P H R E P L T I V V G R P I P V P E L A P G Q L E P E P E V L A A L L K R F T D D L Q A L Y C
3. Chlamydomonas_reinhardtii_DGAT1_2		L L T F F V S A V F H E L L L G V P L H - - - M V R L W A F A G I M F Q V P L I M V T E M L R K - K L N R D E L G N Y I F W I A F C V V G Q P V C V L L Y Y H C
4. Chromochloris_zofingiensis_DGAT1_1		- V P T L Q Q L T Y K R L G F P F P Y M - - - L V G R W G C S P F P K K V P L V Y V V G K P L R A P E Y P E G - S P V S Q E L V D N L H Q Q Y V D S M V A M F H
5. Chromochloris_zofingiensis_DGAT1_2		L L V F F V S G V F H E L A V G V P L H - - - M V Q Y S A F F G I M L Q V P L I S L T E A L K K - H L K S D T W G N Y I F W I T F C I V G Q P V S L M L Y Y H C
6. Chromochloris_zofingiensis_DGAT1_3		- V P T L Q Q L T Y K R L G F P F P Y M - - - L V G R W G C S P F P K K V P L V Y V V G K P L R A P E Y P E G - S P V S Q E L V D N L H Q Q Y V D S M V A M F H
7. Phaeodactylum_tricornutum_DGAT1		F V V F F L S A V M H E V L V S V P F H - - - I I R P W S F I G M M M Q I P L V A F T K Y L Y R - K F P G G S F G N V L F W M T F C V I G P M A I L L Y T V C
8. Arabidopsis_thaliana_DGAT3		S G C A L L L D E F Q R A M T G F E G S - - - - A V A C K C M G K C R D G P N V R V V K E T D A V M T D - - - - S V R T P S K T L C V G V G L Q D V E T I V T S
9. Arabidopsis_thaliana_DGAT2		- - D C D L Y L K L S R A I R F T P I C - - - F W G V F G - S P L P C R Q P M H V V V G K P I E V T K T - - - - L K P T D E E I A K F H G Q Y V E A L R D L F E
10. Arachis_hypogaea_DGAT3_3		S G S I A L L Q E F E R V V G A E G G A D A A A V V G C K C M G K C K S A P N V R I Q N S T A D K I A E G F N D S V K V P A N P L F V G V A L E D V E T I V A F
11. Arachis_hypogaea_DGAT3_1		S G S I A L L Q E L E R V I G A E G G A - A A A V V G C K C M G K C K S A P N V R I Q N S T A D K I A E G F N D S V K V P A N P L C I G V A L E D V E T I V A F
12. Mus_musculus_DGAT1		T G V F L T S A F F H E Y L V S V P L R - - - M F R L W A F T A M M A Q V P L A W I V G R F F Q - - - - G N Y G N A A V W V T - L I I G Q P V A V L M Y V H C
13. Rattus_norvegicus_DGAT1		T G V F L A S A F F H E Y L V S I P L R - - - M F R L W A F T A M M A Q V P L A W I V N R F F Q - - - - G N Y G N A A V W V T - L I I G Q P V A V L M Y V H C
14. Homo_sapiens_DGAT1		T C V F L A S A F F H E Y L V S V P L R - - - M F R L W A F T G M M A Q I P L A W F V G R F F Q - - - - G N Y G N A A V W L S - L I I G Q P I A V L M Y V H C

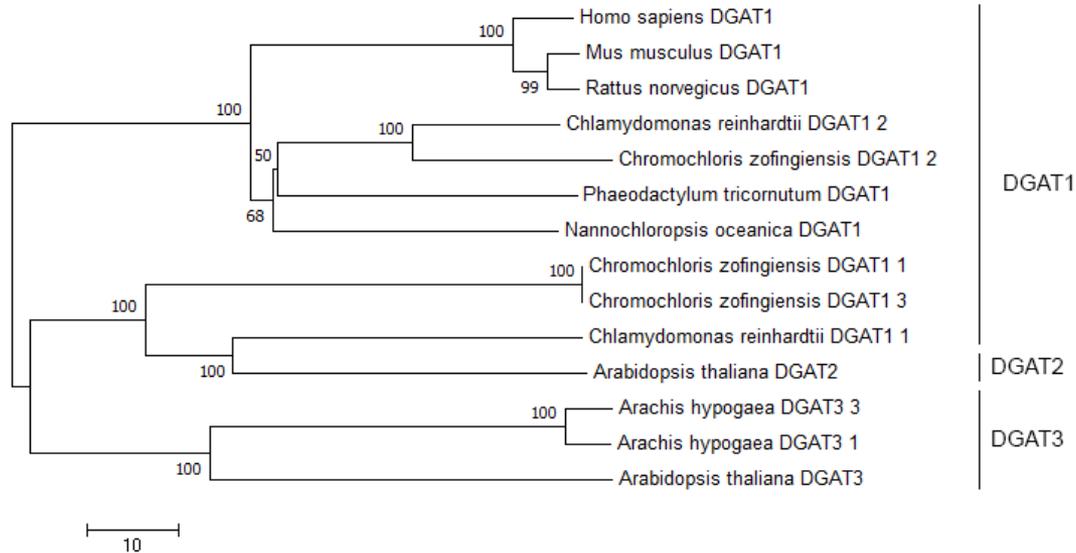
DGAT家族系统发育树构建



mRNA



Protein





结构分析



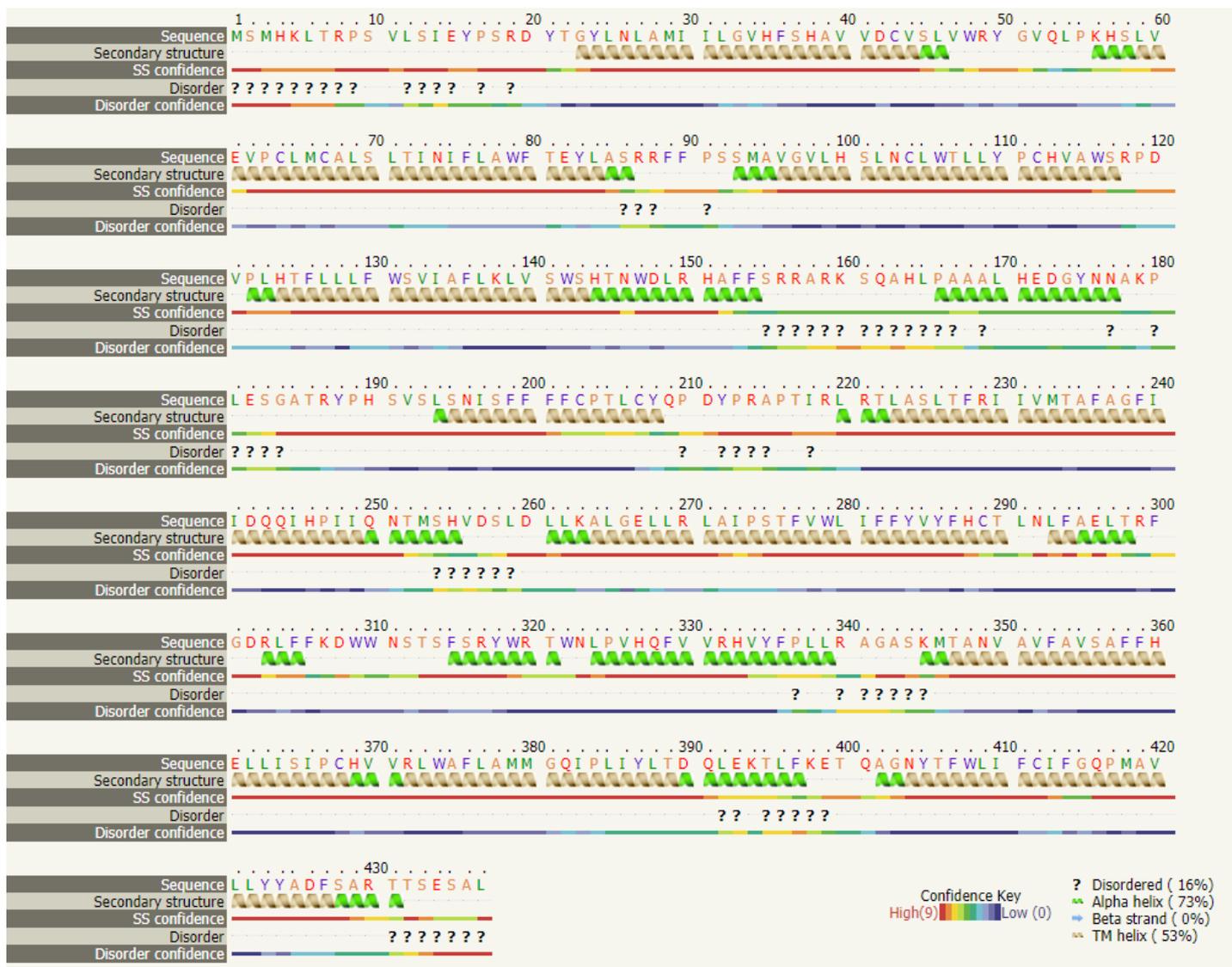
氨基酸组成

Amino acid composition:

Ala (A)	36	8.2%
Arg (R)	24	5.5%
Asn (N)	13	3.0%
Asp (D)	13	3.0%
Cys (C)	10	2.3%
Gln (Q)	11	2.5%
Glu (E)	11	2.5%
Gly (G)	13	3.0%
His (H)	19	4.3%
Ile (I)	23	5.3%
Leu (L)	60	13.7%
Lys (K)	10	2.3%
Met (M)	11	2.5%
Phe (F)	37	8.5%
Pro (P)	22	5.0%
Ser (S)	36	8.2%
Thr (T)	26	5.9%
Trp (W)	14	3.2%
Tyr (Y)	18	4.1%
Val (V)	30	6.9%
Pyl (O)	0	0.0%
Sec (U)	0	0.0%
(B)	0	0.0%
(Z)	0	0.0%
(X)	0	0.0%

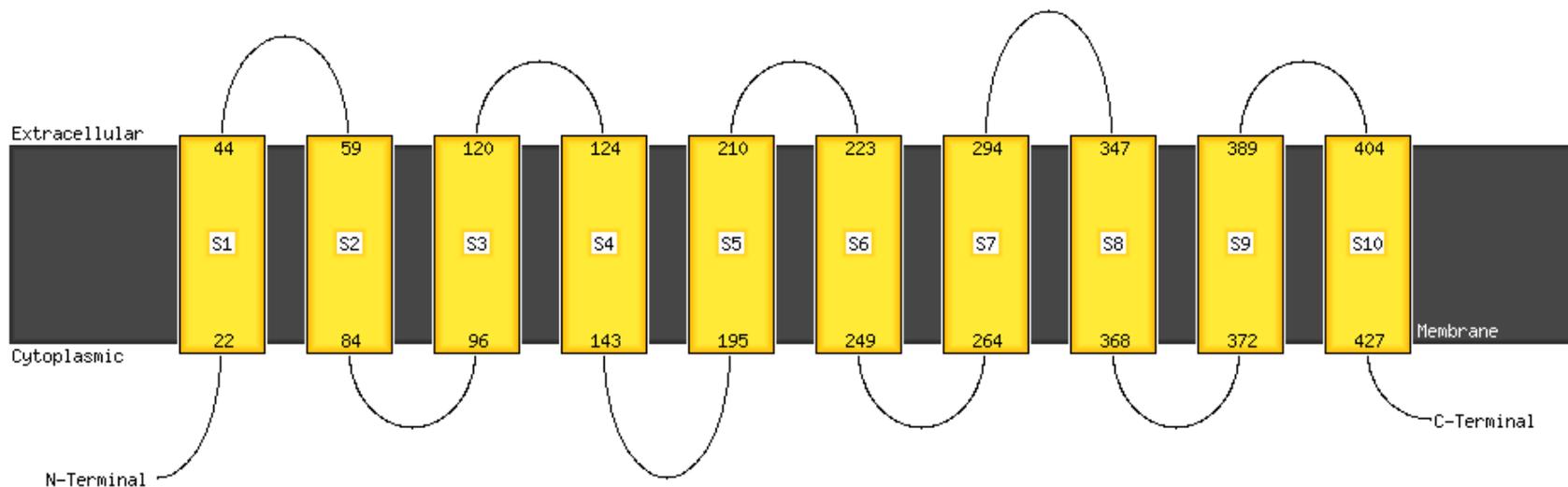


微拟球藻DGAT的二级结构预测





微拟球藻DGAT的跨膜螺旋预测





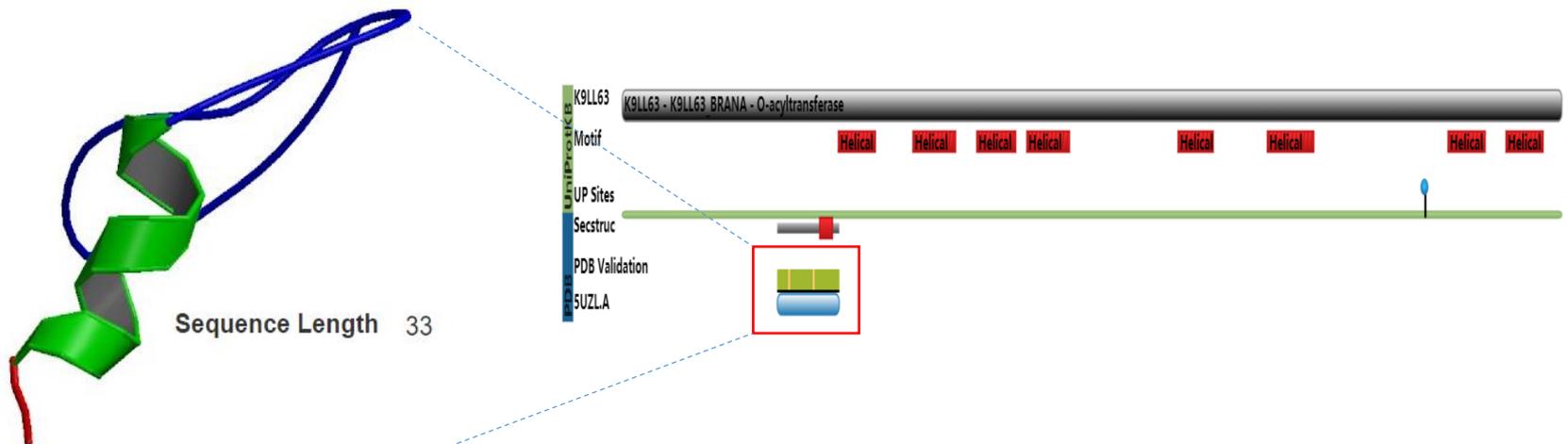
微拟球藻中的保守结构域

含有的结构域MBOAT, membrane-bound O-acyltransferase family

已经测定的结构：油菜O-乙酰基转移酶(NMR)

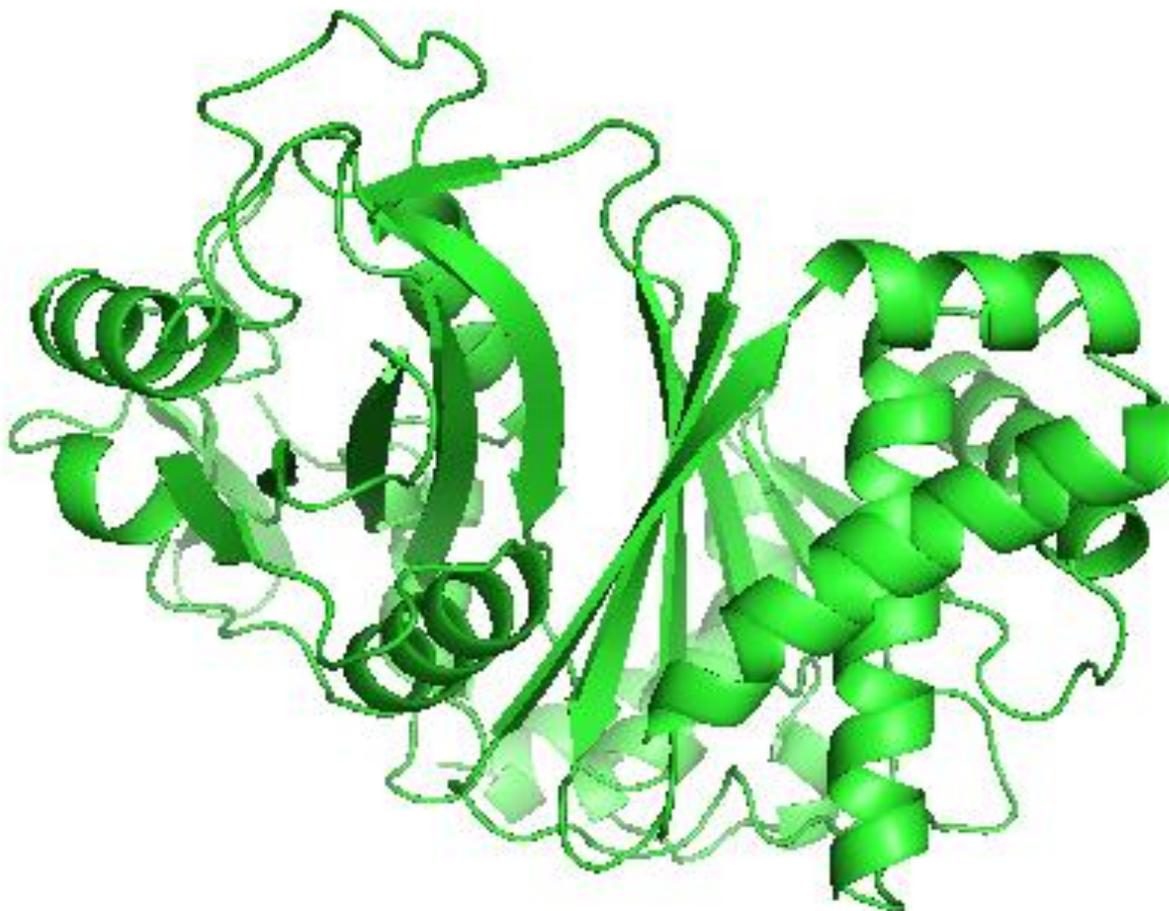


Brassica napus DGAT1 exosite

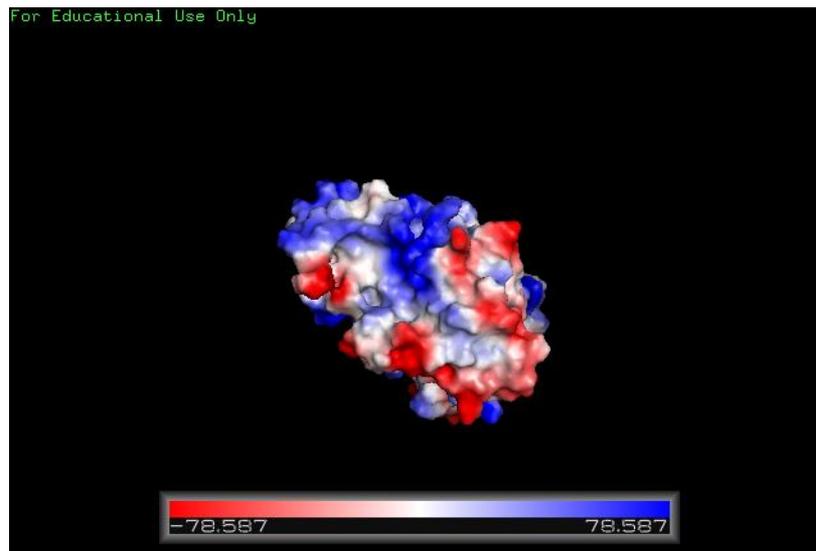
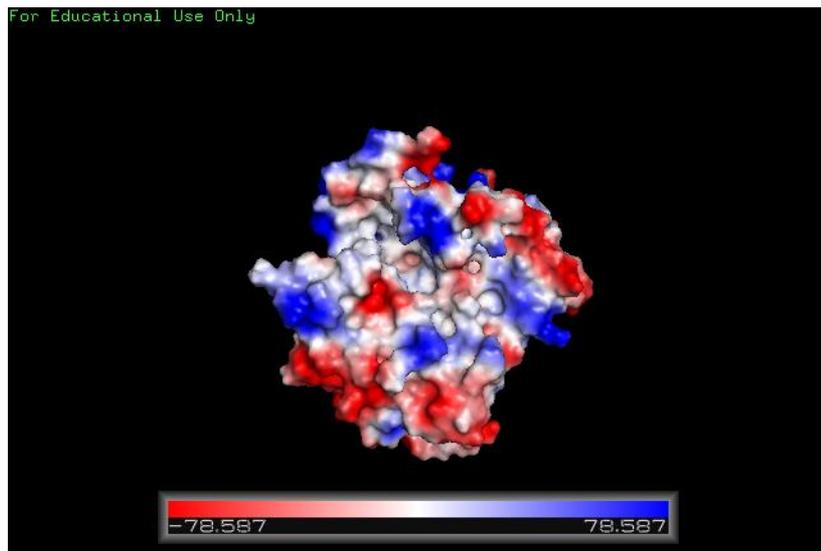


<https://www.rcsb.org/structure/5UZL>

来自海洋细菌的二酰基甘油酰基转移酶



蛋白活性口袋

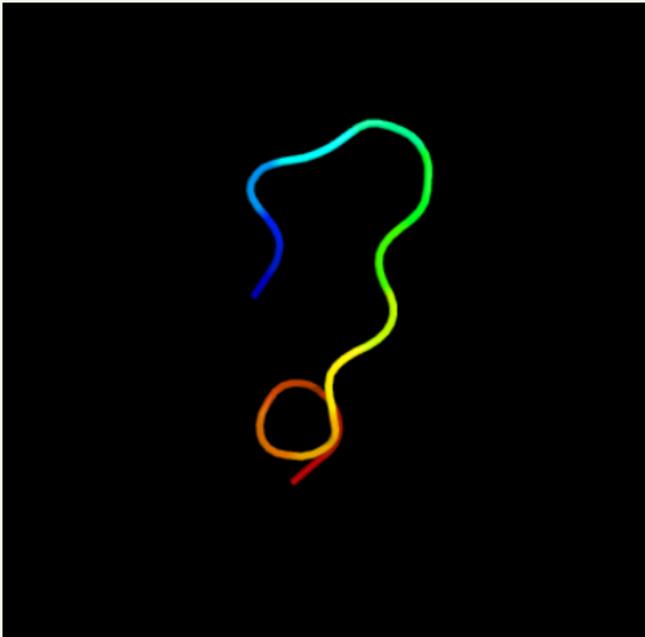




Phyre2 预测：微拟球藻DGAT

三级结构预测 normal:

Top model



Model (left) based on template [c5uzlA_](#)

Top template information

PDB header:transferase
Chain: A: **PDB Molecule:**o-acyltransferase;
PDBTitle: brassica napus dgat1 exosite

Confidence and coverage

Confidence: **62.2%** Coverage: **3%**

14 residues (3% of your sequence) have been modelled with 62.2% confidence by the single highest scoring template.

Confidence in the model: 62.2%

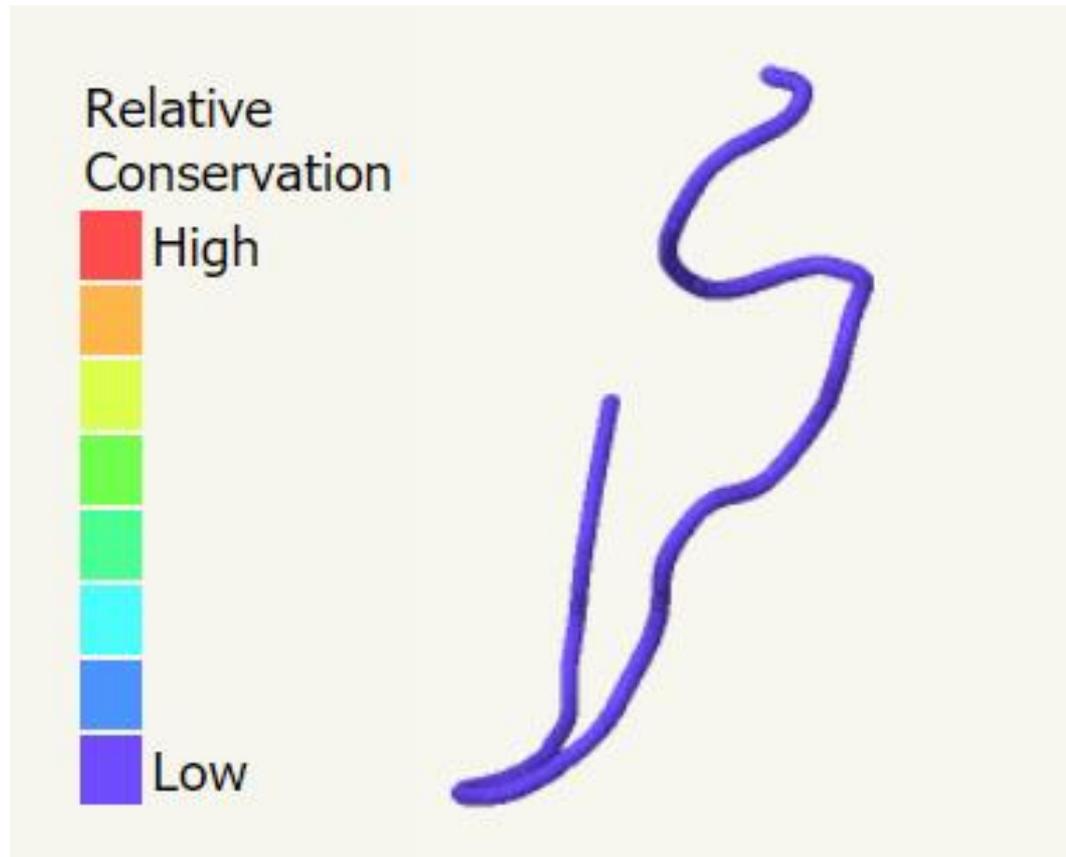
WARNING: Low confidence model. Please use with caution.

Image coloured by rainbow N → C terminus

Model dimensions (Å): **X:**26.997 **Y:**16.331 **Z:**18.285

Phyre2 预测：微拟球藻DGAT

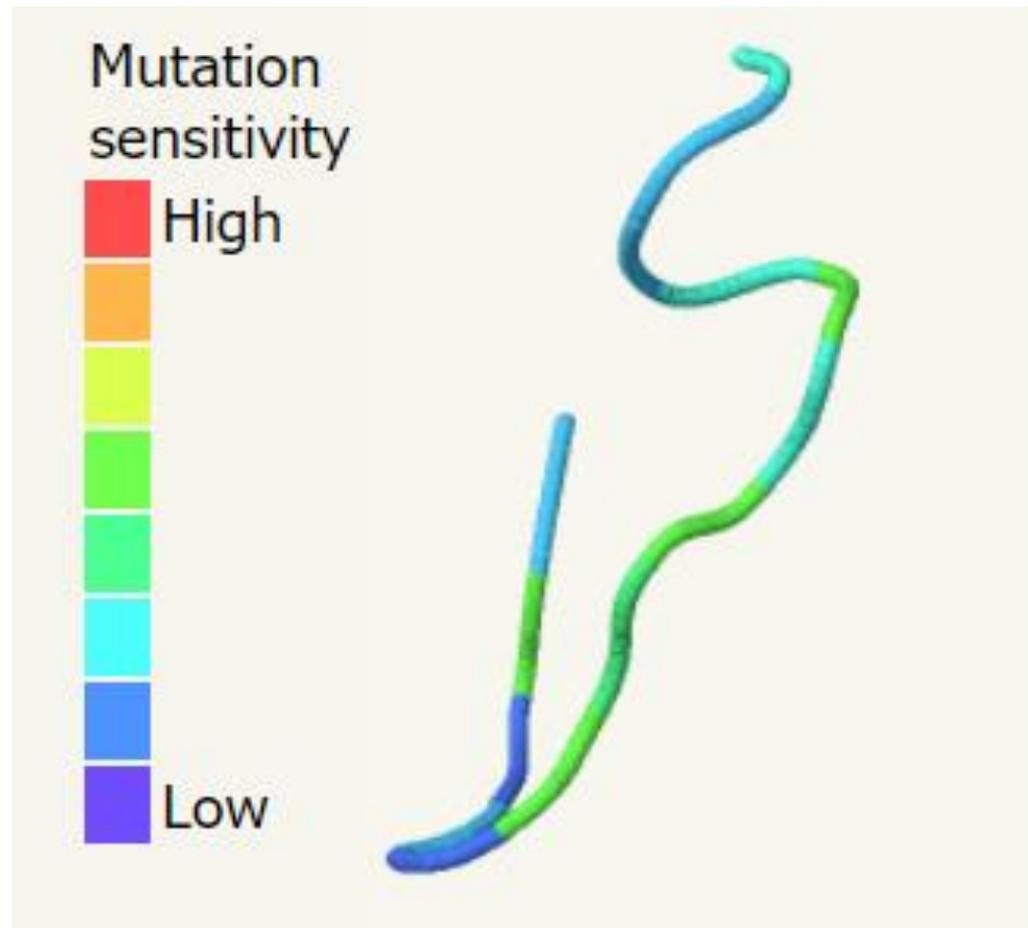
保守性

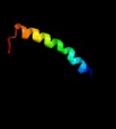




Phyre2 预测：微拟球藻DGAT

对突变的敏感性



3D Model	Confidence	% I.d.	3D Model	Confidence	% I.d.	3D Model	Confidence	% I.d.	3D Model	Confidence	% I.d.
	62.2	36		29.9	19		11.6	20		9.6	26
	32.7	16		16.7	50		11.2	27		9.5	25
	32.1	27		14.0	25		10.9	56		9.5	44
	32.1	27		12.3	17		10.9	22		9.5	44
	32.1	27		11.7	60		10.5	20		9.5	44

Domain analysis

Rank	Aligned region
1	c5uziA
2	c6bpzC_
3	
4	c2na6C_
4	c2na6A_
5	c2na6B_
6	c2i5yA_
7	c5n6yC_
8	c2k60A_
9	d1v54b2_
10	c1rpqY_
11	c2ounA_
12	c6ercA_
13	c5kevA_
14	c5djeB_
15	c3qi4A_
16	c1d2vD_
17	c3a16C_
18	c1kcoA_
19	c1rpqW_
20	c1rpqZ_
21	
22	
23	
24	

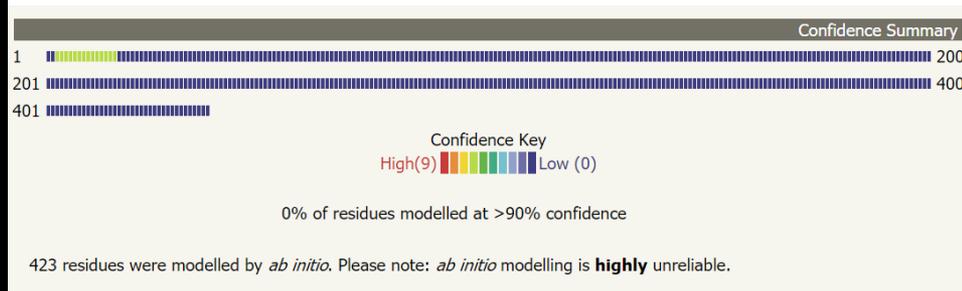
Phyre2 预测：微拟球藻DGAT

三级结构预测 intensive:



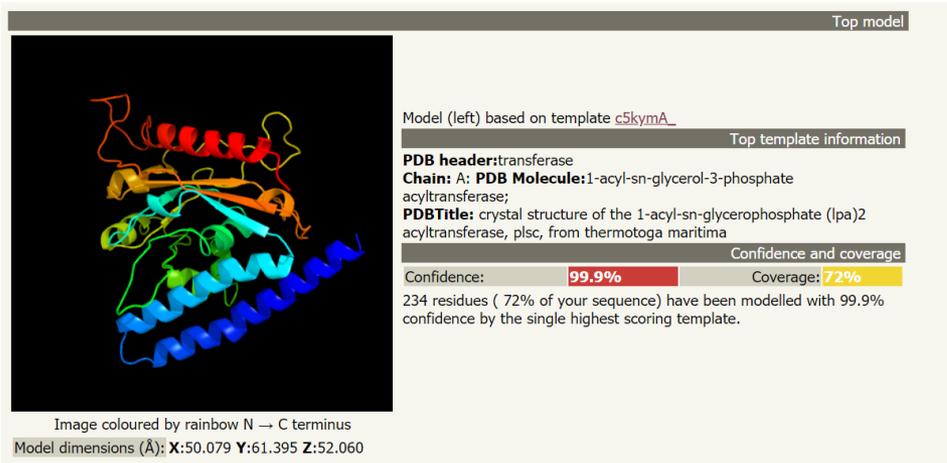
Image coloured by rainbow N → C terminus

Model dimensions (Å): **X**:64.261 **Y**:69.368 **Z**:78.838

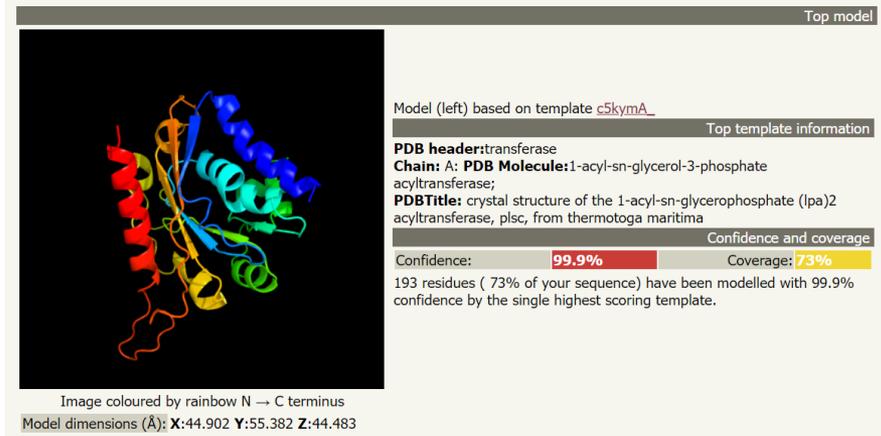


Phyre2 预测：其他物种

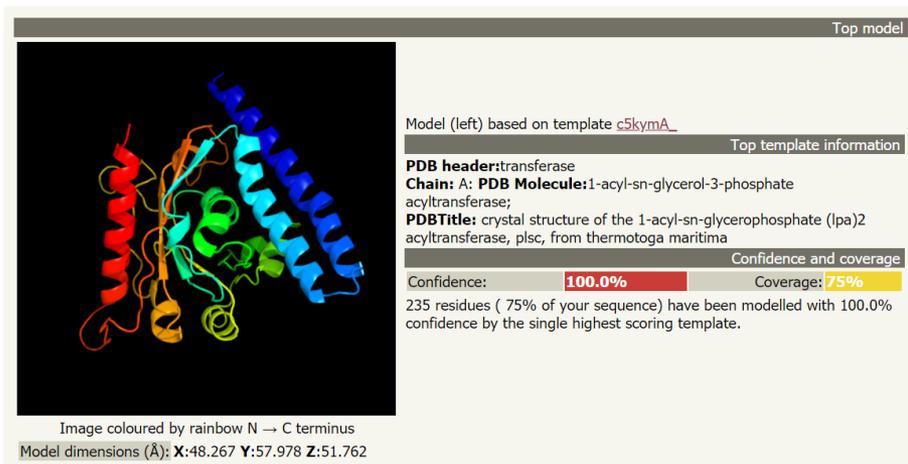
衣藻 *Chlamydomonas reinhardtii*



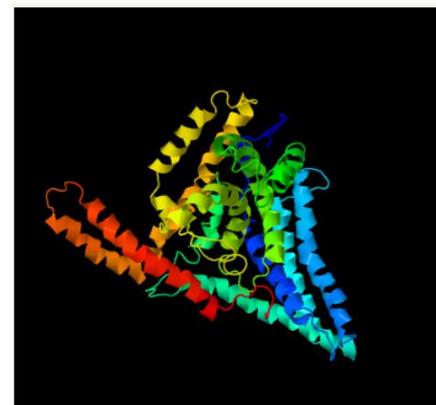
小球藻 *Chromochloris zofingiensis*



拟南芥 *Arabidopsis thaliana*



微拟球藻 *Nannochloropsis oceanica*





总结

- 多序列比对表明微拟球藻的DGAT序列与藻类相似度更高，今后对微拟球藻的DGAT酶的研究中可以参考其他研究成熟的藻类
- 系统发育树表明DGAT1、DGAT2和DGAT3均具有跨物种的保守性，说明这三个蛋白在物种演化之前就已经存在
- 从结构上对微拟球藻进行了预测，可以为下一步对酶活性的优化提供一些建议



展望

随着环保挑战日益严峻，开发环保可再生的替代能源成为了当下的迫切任务。本次讨论报告通过与其他物种比对，并对DGAT结构进行预测，为后续研究提供两条思路：1.通过湿实验研究活性位点的突变是否会改变底物作用位点或增加酶活，从而提高TAG产量；2.基因共定位，解析DGAT作用机制，明确代谢途径，通过代谢工程手段提高油脂产量。



谢谢！