鸟嘌呤核苷酸结合蛋白(NOG2)的分析
Analysis of Nucleolar GTP-binding protein 2

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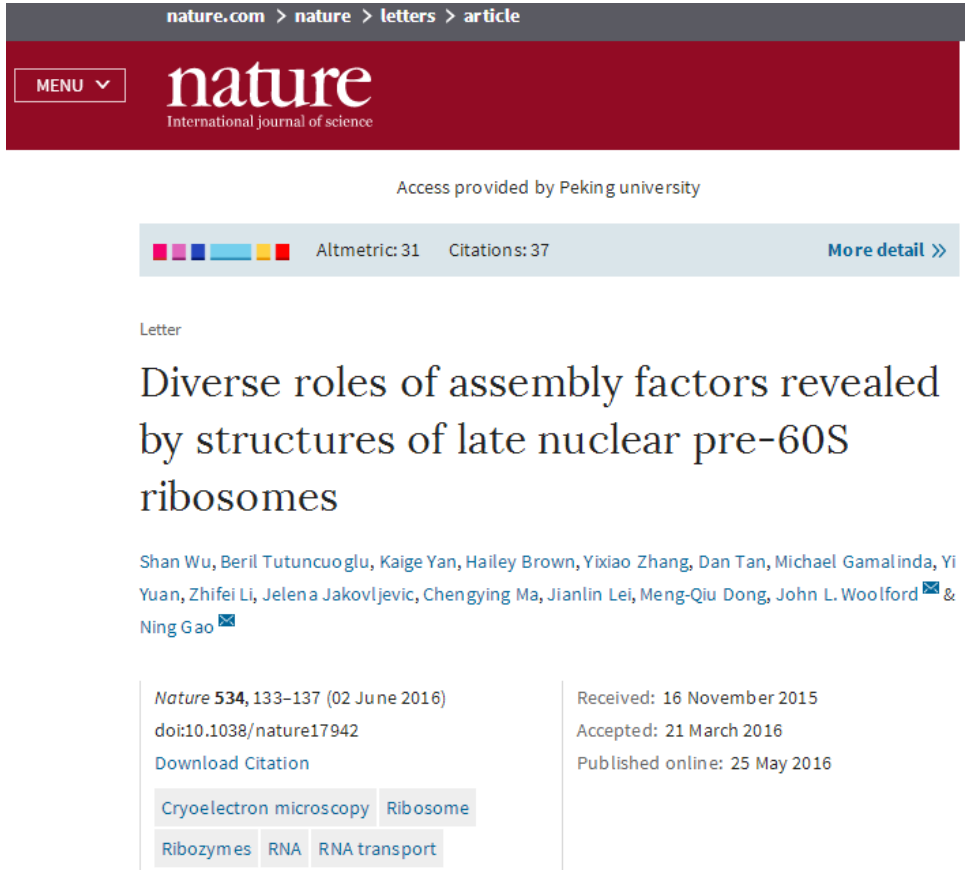
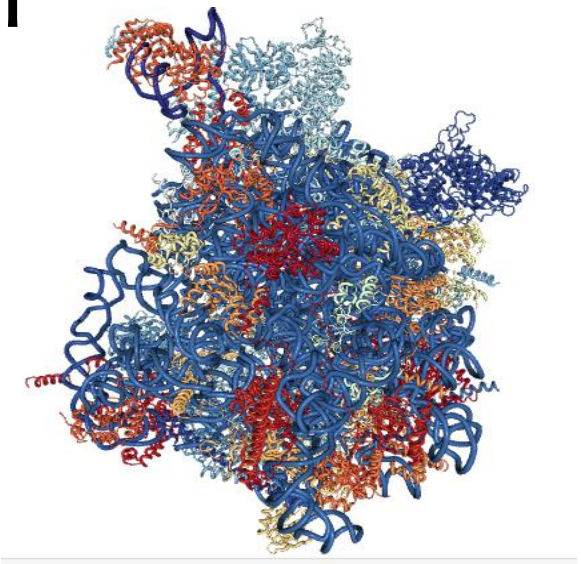
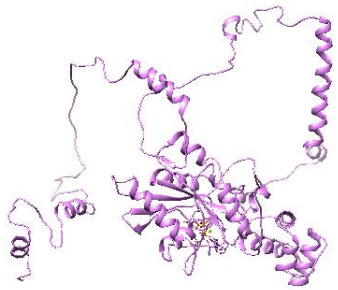
故事起源-NOG2蛋白结构的解析





60S ribosomes



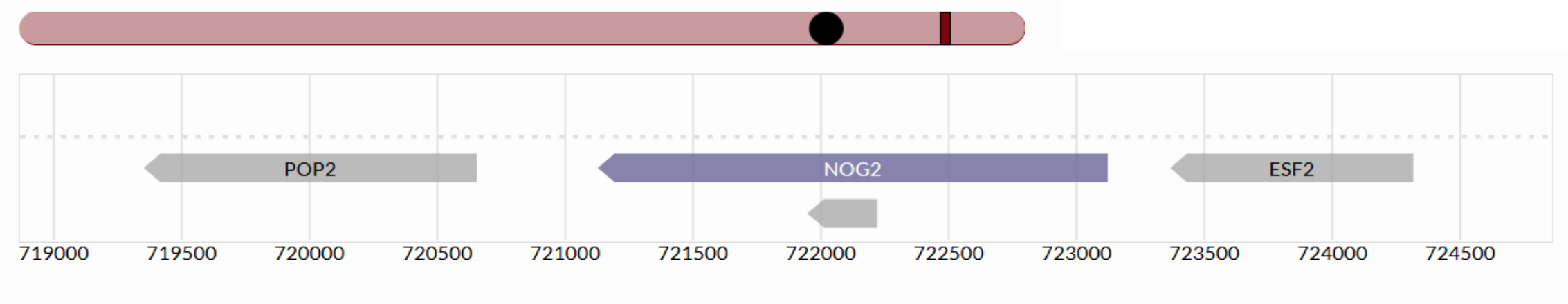
NOG2

- 高宁实验室在2016年解析出酵母核糖体蛋白NOG2结构
- 人源NOG2结构还未解出
- 通过使用生物信息课学到的相关知识初步解读NOG2蛋白的相关信息

Shan Wu，et al .Nature.2016 Jun 2;534(7605):133-7.

NOG2 基因信息以及氨基酸序列

NOG2 定位在14号染色体的 721120..723112



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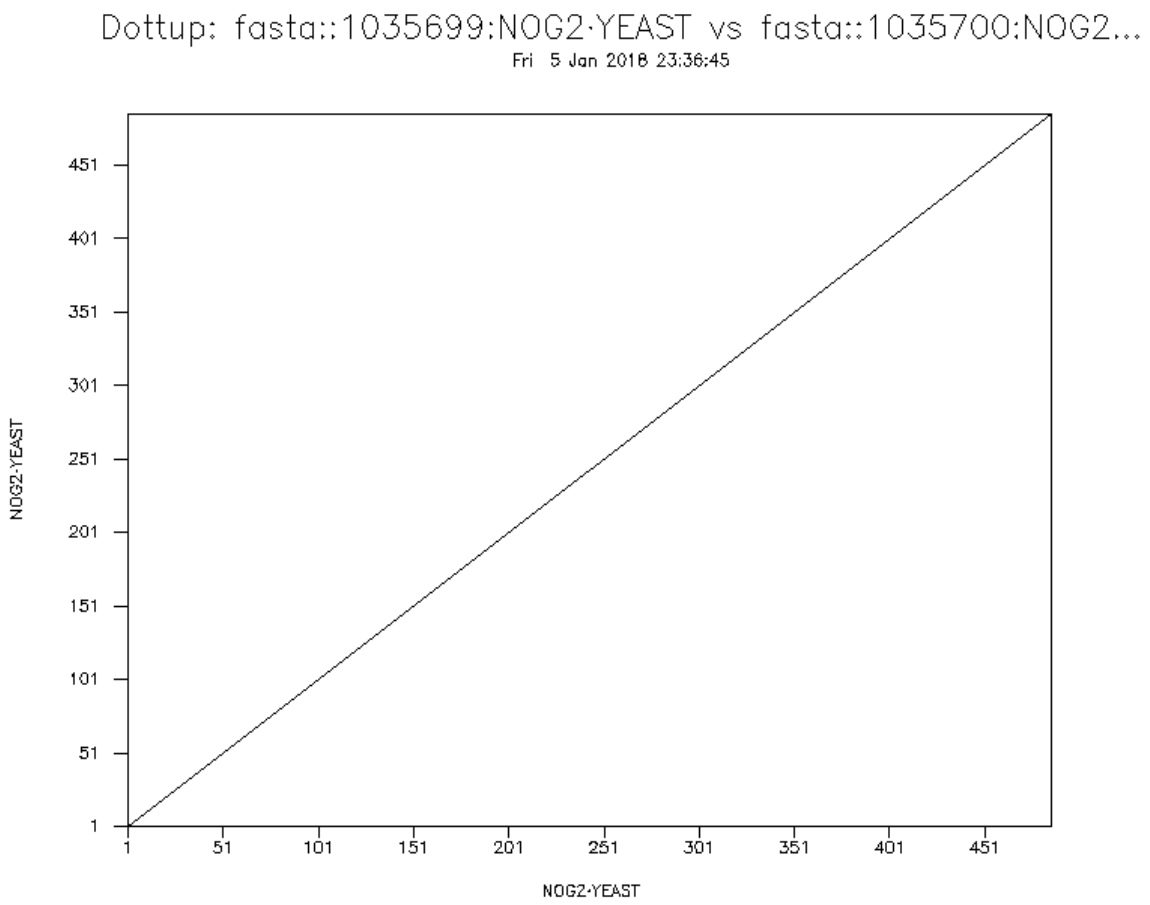
LGLMGNQEDKENGWTSAAKEAIFSKGQSKRIWNELYKVIDSSDVVIHVLDARDPLGTRCK
SVEEYMKKETPHKHLIYVLNKCDLVPTWVAAAWVKHLSKERPTLAFHASITNSFGKGSLI
QLLRQFSQLHTDRKQISVGFIGYPNTGKSSIINTLRKKKVCQVAPIPGETKVWQYITLMK
RIFLIDCPGIVPPSSKDSEEDILFRGVVRVEHVTHPEQYIPGVLKRCQVKHLERTYEISG
WKDATEFIEILARKQGRLLKGGEPDESGVSKQILNDFNRGKIPWFVLPPEKEGEEKPKKK
EVEKTA

共由486个氨基酸组成，
55.49KD大小

NOG2p定位在生物体内的核仁和核质上

运用WebLab进行重复序列分析



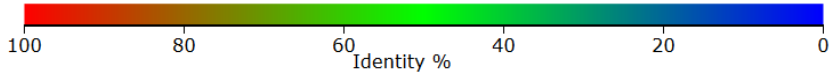
- 【Tool】WebLab-Dottup
- 【Parameter】default

- Dottup 是精确匹配，两个序列比对中，word size 内精确匹配时以图上的点表示，匹配程度较高时适用，重复匹配
- DotMatcher 是近似匹配，用给定的计分矩阵对windowsize 内的序列进行打分，高于threshold 的在图上以点显示，可用于匹配程度不高的两个序列，重复匹配
- DotPath 与Dottup 相似，是word size 内的精确匹配，但不进行重复匹配。

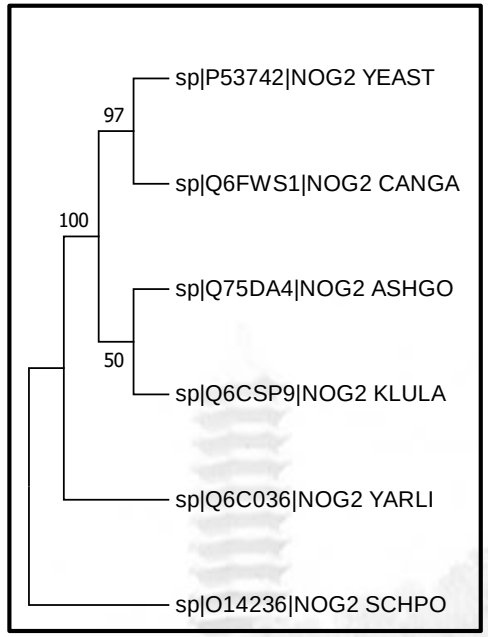
✓该蛋白不含重复序列

Blast 酵母NOG2蛋白序列

- 运用UniProt数据库对酵母NOG2进行blast，得到250条相似序列，reviewed6条如下；
- 其中100%一条是自己，其他均为其他物种的NOG2，说明高度保守；



Entry	Alignment overview	Info	Status
Query: sp|P53742|NOG2_YEAST B20180109ACFE4208EAFA842A78A1B3BA7138A93D996171Q			
P53742	NOG2_YEAST - Nucleolar GTP-binding protein 2 - Saccharomyces ce... - View alignment	E-value: 0.0 Score: 2,533 Ident.: 100.0%	
Q6FWS1	NOG2_CANGA - Nucleolar GTP-binding protein 2 - Candida glabrata... - View alignment	E-value: 0.0 Score: 2,268 Ident.: 88.6%	
Q75DA4	NOG2_ASHGO - Nucleolar GTP-binding protein 2 - Ashbya gossypii ... - View alignment	E-value: 0.0 Score: 2,118 Ident.: 82.4%	
Q6CSP9	NOG2_KLULA - Nucleolar GTP-binding protein 2 - Kluyveromyces la... - View alignment	E-value: 0.0 Score: 2,112 Ident.: 82.0%	
Q6C036	NOG2_YARLI - Nucleolar GTP-binding protein 2 - Yarrowia lipolyt... - View alignment	E-value: 0.0 Score: 1,824 Ident.: 71.9%	
O14236	NOG2_SCHPO - Nucleolar GTP-binding protein 2 - Schizosaccharomy... - View alignment	E-value: 0.0 Score: 1,607 Ident.: 66.4%	

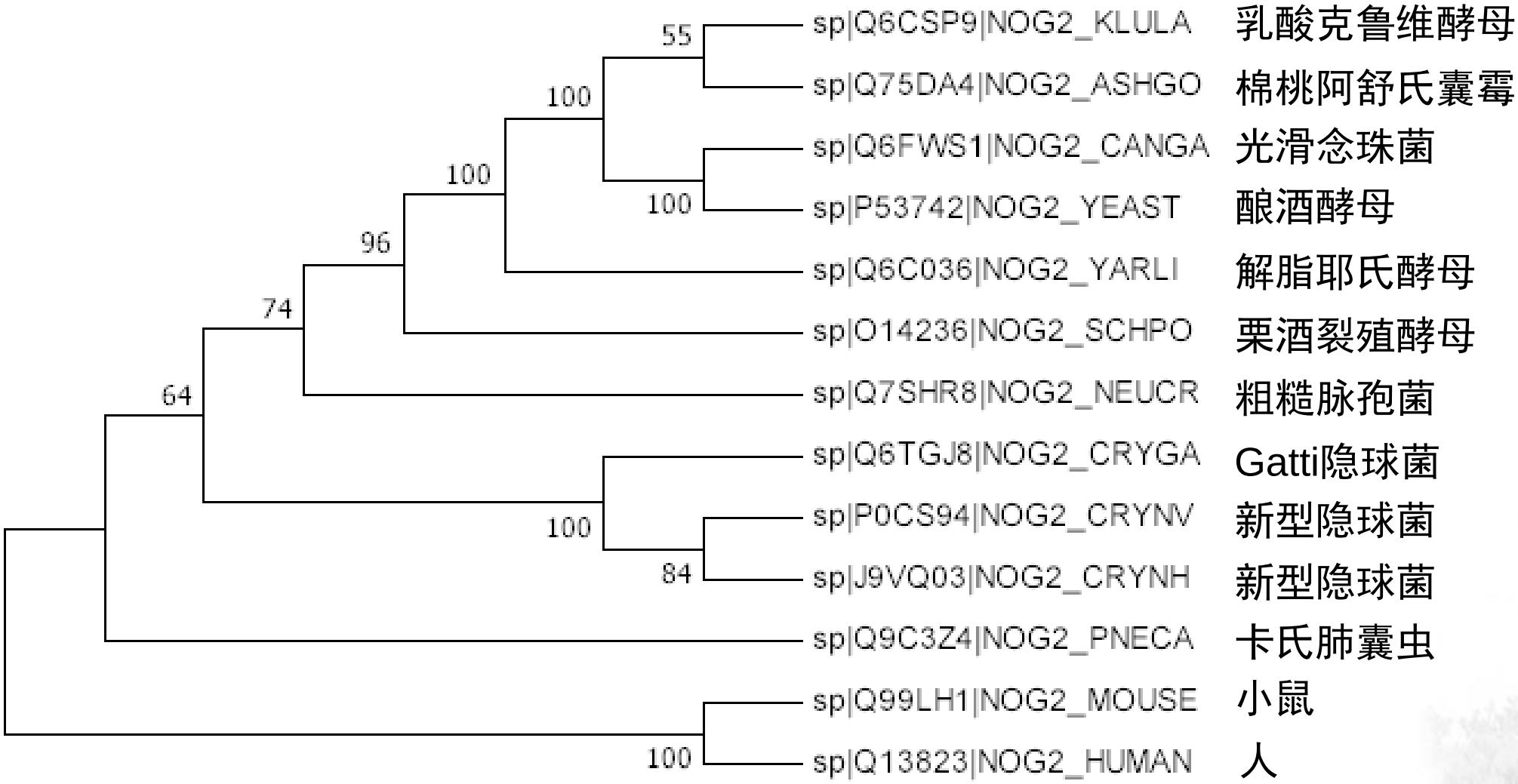


NOG2家族

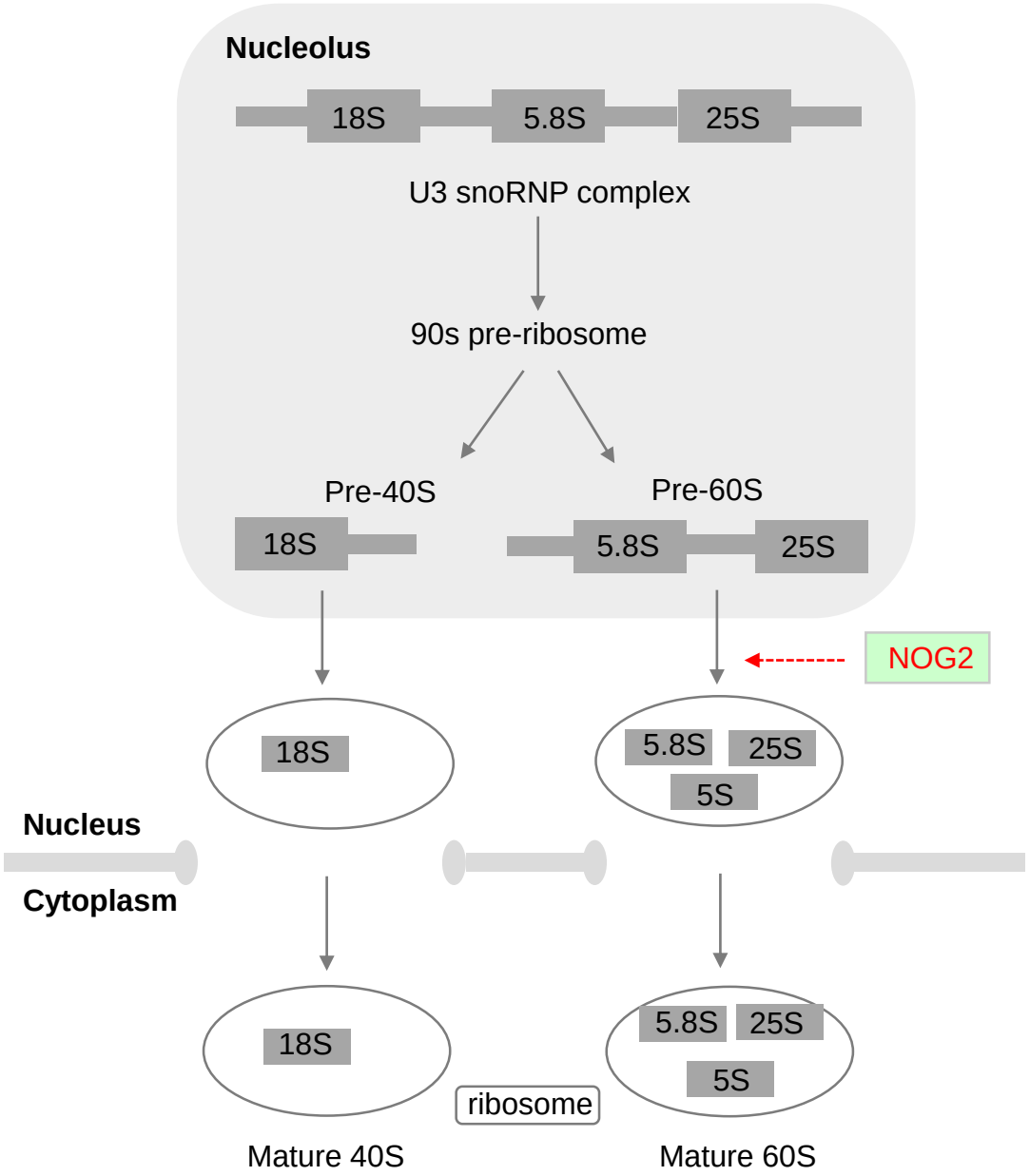
- 酵母NOG2属于TRAFAC class YlqF/YawG GTPase 大家族和NOG2 亚家族
- NOG2亚家族在UniProt数据库里reviewed共有13个成员

编号	物种	uniprot登录号	蛋白长度	Domain	Nucleotide binding位点
NOG2_CRYNV	*Cryptococcus neoformans var. grubil* 新型隐球菌	P0CS94	693	198-359	308-315；352-356
NOG2_PNECA	*Pneumocystis carinli* 卡氏肺囊虫	Q9C3Z4	483	189-350	299-306；343-347
NOG2_YARLI	*Yarrowia lipolytica*解脂耶氏酵母	Q6C036	509	204-365	314-321；358-362
NOG2_KLULA	*Kluyveromyces*乳酸克鲁维酵母	Q6CSP9	513	211-372	321-328；365-369
NOG2_MOUSE	*Mus musculus 鼠*	Q99LH1	728	207-368	317-324；361-365
NOG2_ASHGO	*Ashbya gossypii* 棉桃阿舒氏囊霉	Q75DA4	502	211-372	321-328；365-369
NOG2_CRYGA	*Cryptococcus gatti* Gatti隐球菌	Q6TGJ8	731	223-396	345-352；389-393
NOG2_CANGA	*Candida glabrata* 光滑念珠菌	Q6FWS1	494	212-373	322-329；366-370
NOG2_CRYNH	*Cryptococcus neoformans var. grubii serotype A*新型隐球菌	J9VQ03	720	225-386	335-342；379-383
NOG2_HUMAN	*Homo sapiens 人*	Q13823	731	207-368	317-324；361-365
NOG2_NEUCR	*Neurospora crassa* 粗糙脉孢菌	Q7SHR8	619	222-383	332-339；376-380
NOG2_SCHPO	*Schizosaccharomyces pombe* 栗酒裂殖酵母	O14236	537	207-368	317-324；361-365
NOG2_YEAST	*Saccaromyces cerevisiae 酿酒酵母*	P53742	486	212-373	322-329；366-370

最优化后的NOG2建树



KEGG 通路—核糖体生物机理



Yeast NOG2

90S pre-ribosome components

UTP-C complex

CK2B
CK2A
UTP22 Rrp7

t-UTP complex

UTP5 UTP8 UTP15 UTP9 UTP4 UTP10 NAN1

UTP-B complex

UTP18 UTP13 UTP21 Dip2 UTP6 PWP2

MPP10 complex

MPP10
Imp3 Imp4

Human NOG2

90S pre-ribosome components

UTP-C complex

CK2B
CK2A
UTP22 Rrp7

t-UTP complex

UTP5 UTP8 UTP15 UTP9 UTP4 UTP10 NAN1

UTP-B complex

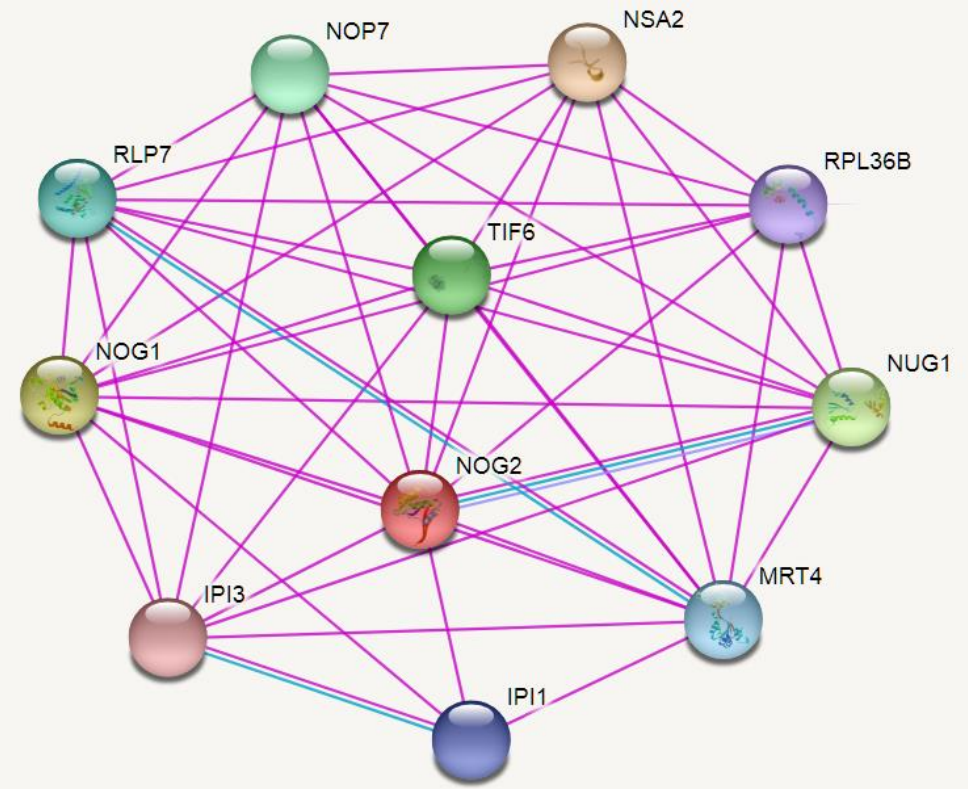
UTP18 UTP13 UTP21 Dip2 UTP6 PWP2

MPP10 complex

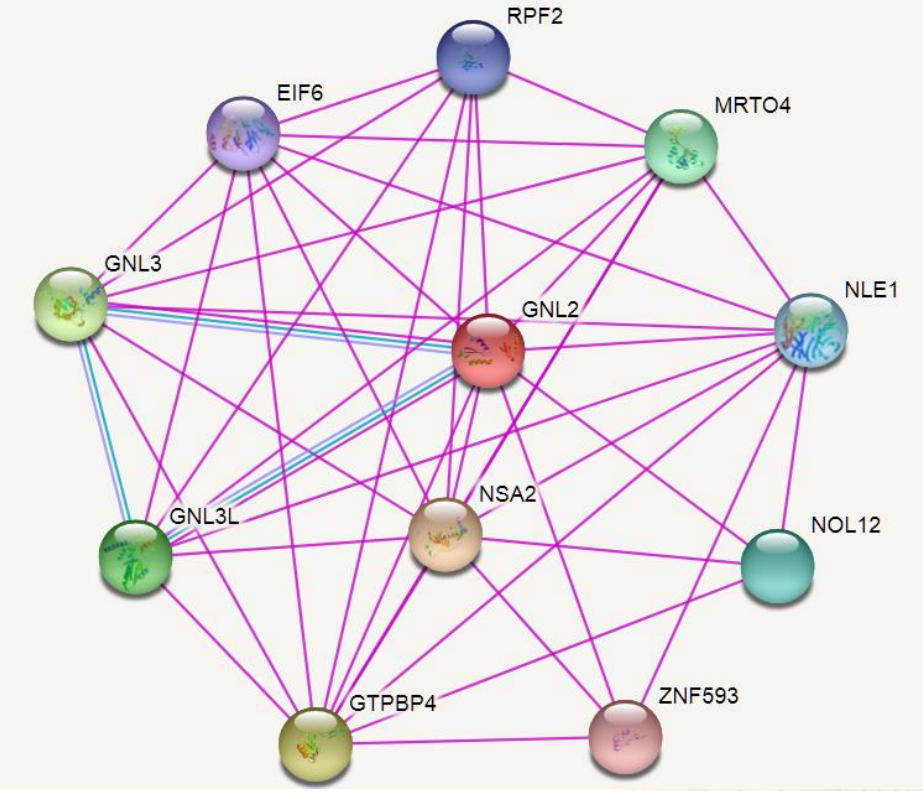
MPP10
Imp3 Imp4

蛋白相互作用信息

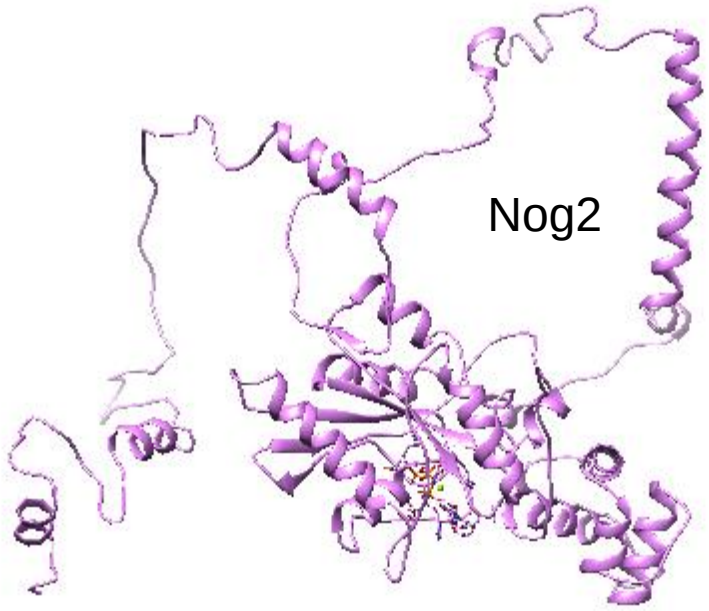
Yeast NOG2

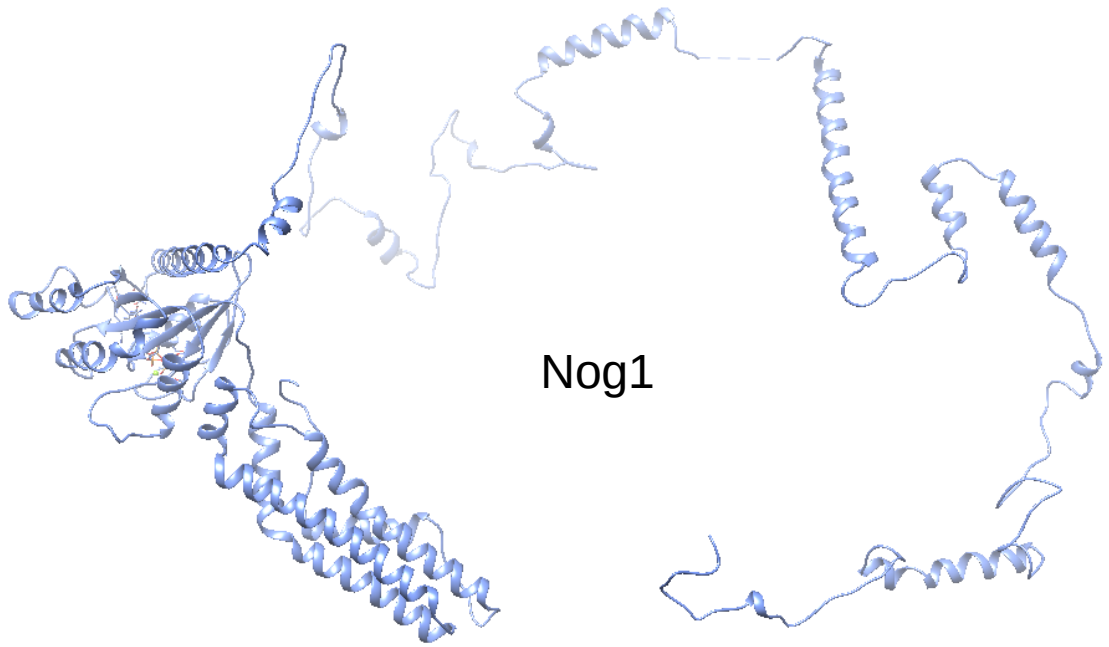


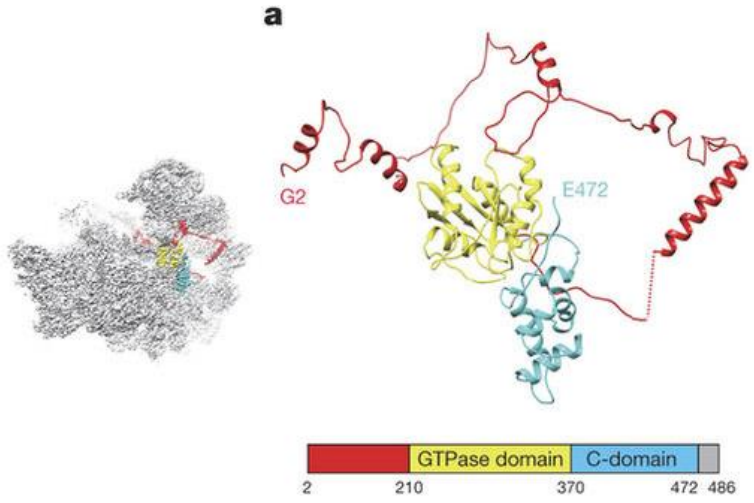
Human NOG2



原子模型组装结构-NOG2



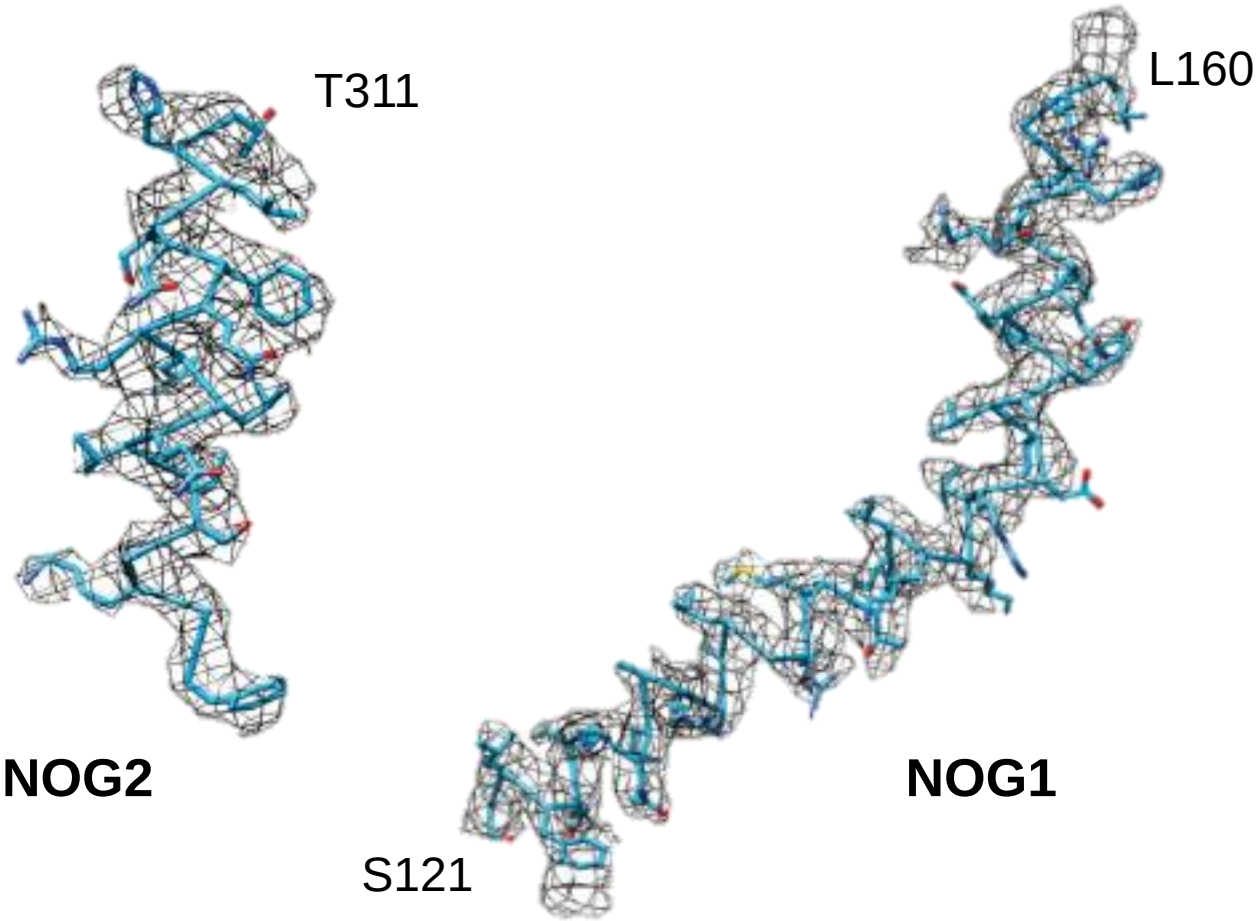


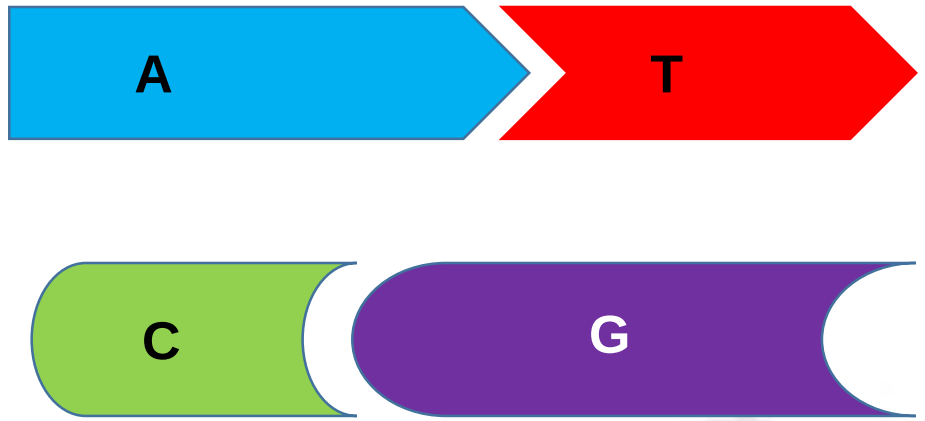


- NOG2主要结构域可分为三大部分，GTPase结构域，羧基（C）-末端结构域与多螺旋连接结构域。

Gao N *et al. Nature.* 534, 133–137 (2016)

酵母NOG2-冷冻电镜密度图





- 通过碱基互补配对原则搭建模型

- NOG2是一种重要的GTP酶，
- 与Nog1一同作为中枢蛋白与多个远处的装配因子和功能性核糖体RNA元件相互作用。

酵母NOG2和人NOG2序列比对

54.78%
57.81%

人的NOG2蛋白在C端多200bp的序列

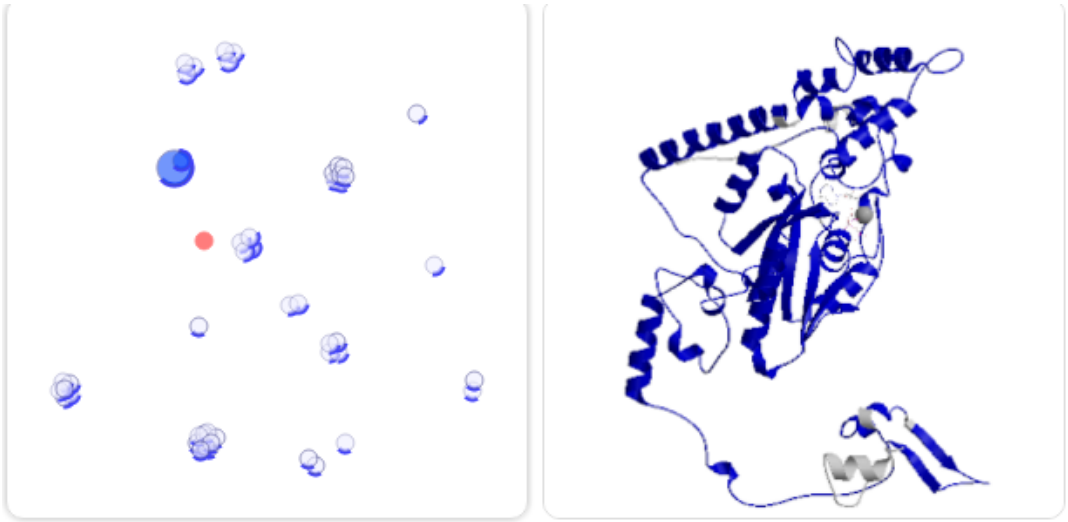
人源NOG2蛋白预测模板

Templates | Quaternary Structure | Sequence Similarity | Alignment of Selected Templates | More

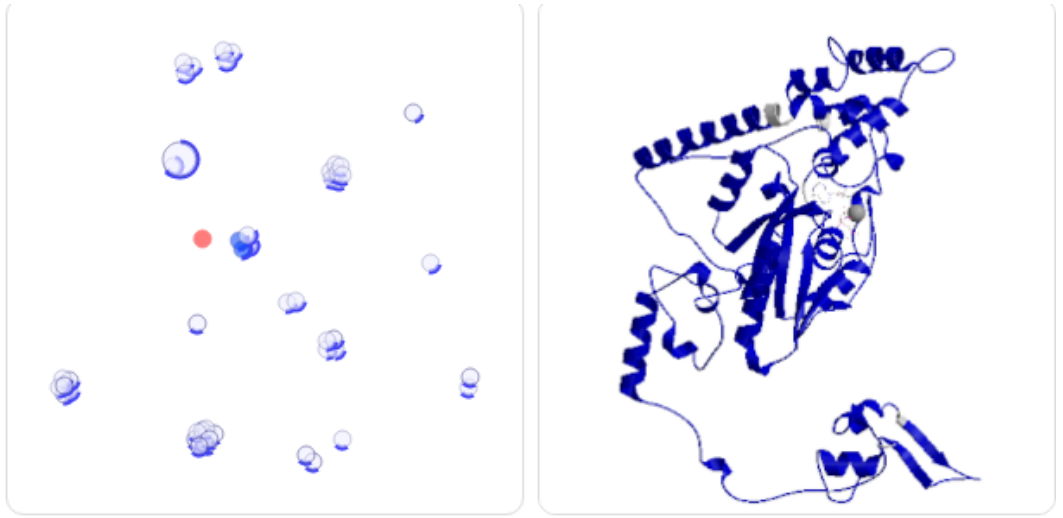
	Name	Title	Coverage	Identity	Method	Oligo State	Ligands
✓ ✓	3jct.1.c	Nucleolar GTP-binding protein 2		54.78	EM	hetero-54-mer ⚠	2 x GTP, 4 x ZN, 2 x MG
✓	3jct.1.c	Nucleolar GTP-binding protein 2		57.81	EM	hetero-54-mer ⚠	2 x GTP, 4 x ZN, 2 x MG
	3cnn.1.A	Putative uncharacterized protein		34.51	X-ray, 2.3Å	monomer ✓	1 x GTP
	3cno.1.A	Putative uncharacterized protein		34.51	X-ray, 2.3Å	monomer ✓	1 x GDP
	3cnl.1.A	Putative uncharacterized protein		34.51	X-ray, 2.0Å	monomer ✓	1 x GNP
	3cno.1.A	Putative uncharacterized protein		25.63	X-ray, 2.3Å	monomer ✓	1 x GDP
	3cnl.1.A	Putative uncharacterized protein		25.63	X-ray, 2.0Å	monomer ✓	1 x GNP
	3cnn.1.A	Putative uncharacterized protein		25.63	X-ray, 2.3Å	monomer ✓	1 x GTP
	2rcn.1.A	Probable GTPase engC		19.52	X-ray, 2.2Å	monomer ✓	1 x GDP, 1 x ZN, 1 x MG
	5uz4.1.U	Small ribosomal subunit biogenesis GTPase RsgA		20.00	EM	hetero-20-mer ⚠	1 x GGM, 1 x ZN
✓	5no2.1.S	Small ribosomal subunit biogenesis GTPase RsgA		20.00	EM	hetero-18-mer ⚠	6 x MG, 1 x ZN, 1 x GNP
	2ykr.1.V	PUTATIVE RIBOSOME BIOGENESIS GTPASE RSGA		19.05	EM, 9.8Å	hetero-21-mer ⚠	None

60S ribosomal protein L4-A

基于酵母NOG2预测人NOG2结构

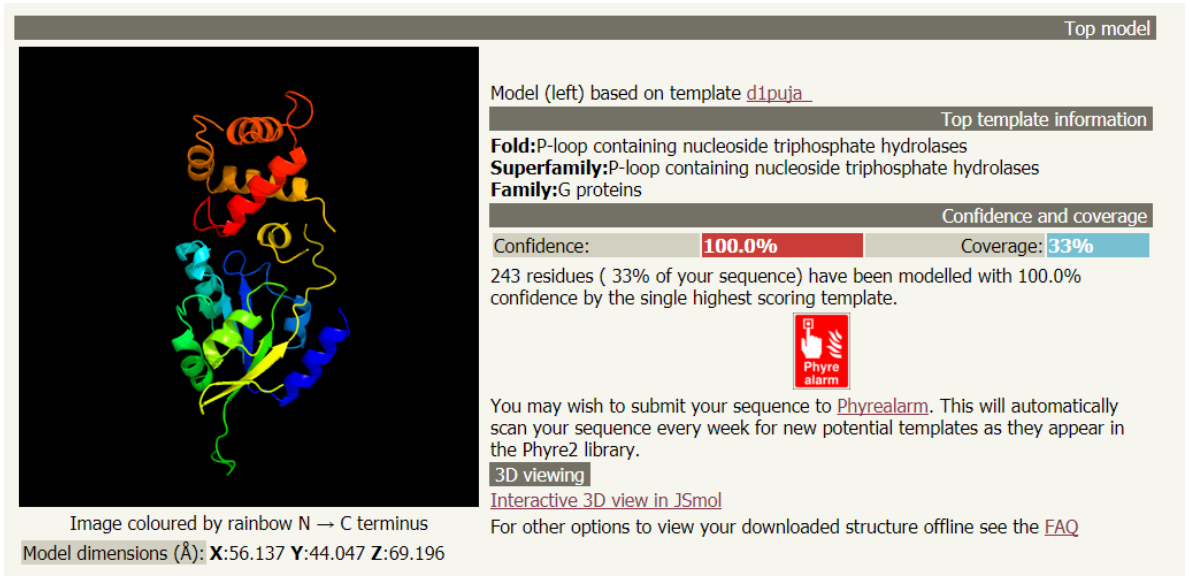


SMTL ID	3jct.1.c
Title	Nucleolar GTP-binding protein 2
Coverage	
Identity	57.81
Similarity	0.47
Ligands	2 x GTP4 x ZN2 x MG
Method	EM 0.00Å
Oligo State	monomer
Found By	BLAST



SMTL ID	3jct.1.c
Title	Nucleolar GTP-binding protein 2
Coverage	
Identity	54.78
Similarity	0.46
Ligands	2 x GTP4 x ZN2 x MG
Method	EM 0.00Å
Oligo State	monomer
Found By	HHblits

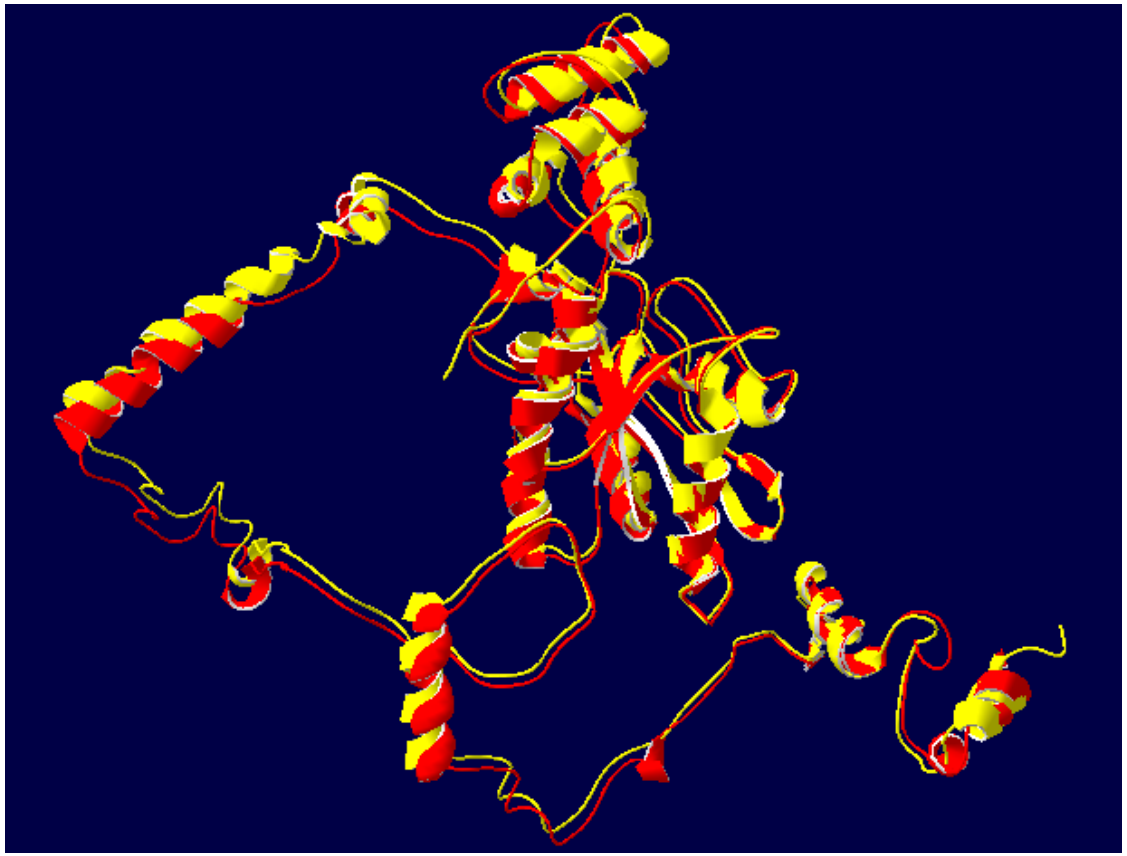
NOG2_Human结构预测

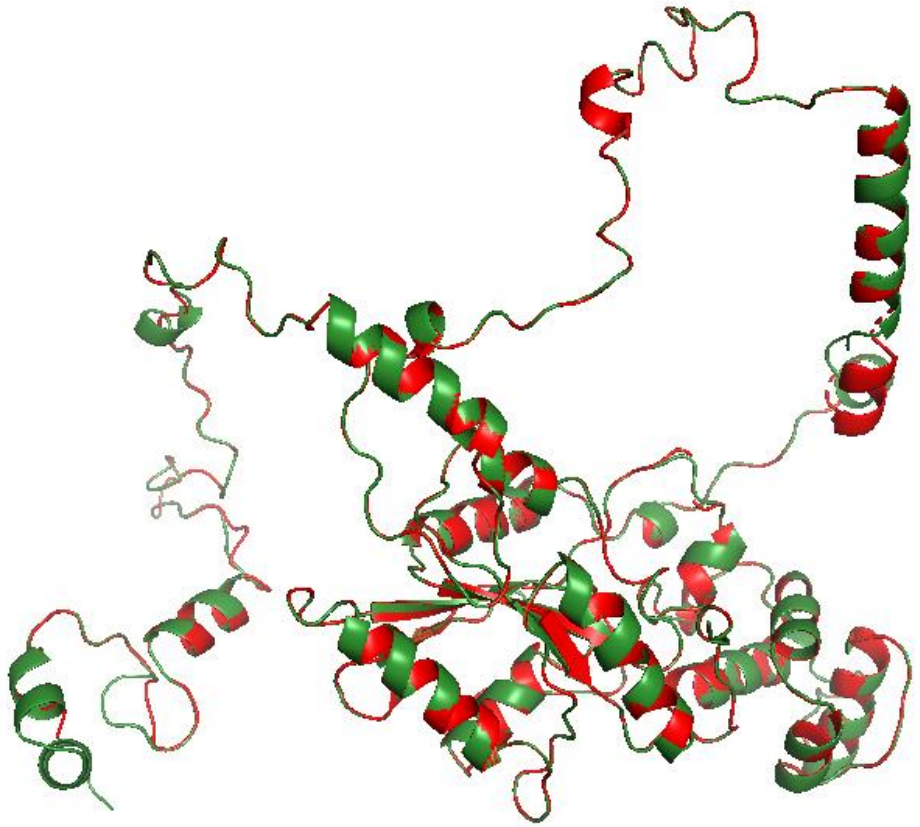


通过 Swiss-Model 对NOG2_Human蛋白进行结构模拟后，选取最优结果，与已测定结构的NOG2_Yeast蛋白进行结构比对。

通过 Phyre2 对NOG2_Human蛋白进行结构模拟后，选取最优结果，与已测定结构的NOG2_Yeast蛋白进行结构比对。

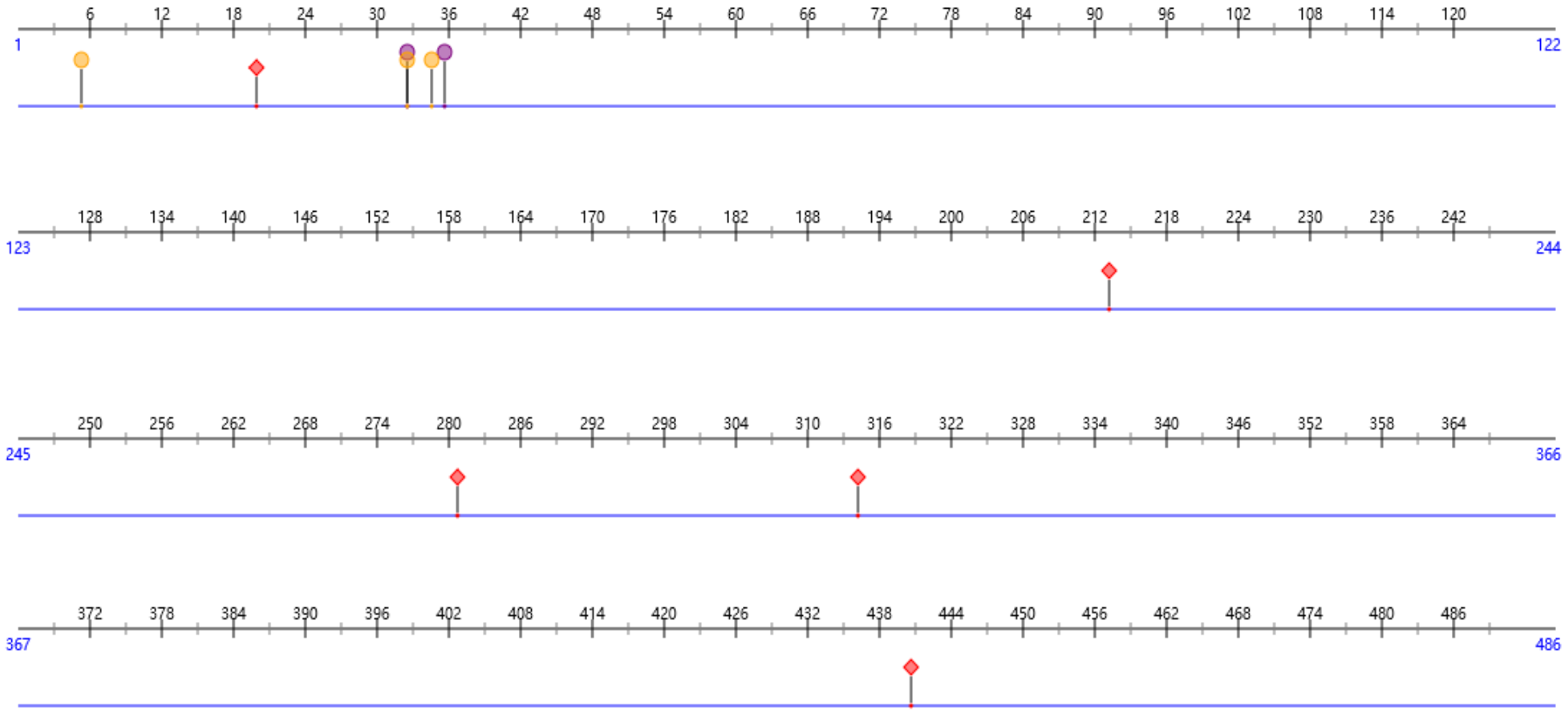
人和酵母NOG2结构比对





红色：预测的人NOG2结构
绿色、黄色：酵母的NOG2结构

酵母NOG2 蛋白-蛋白，蛋白-核酸作用位点

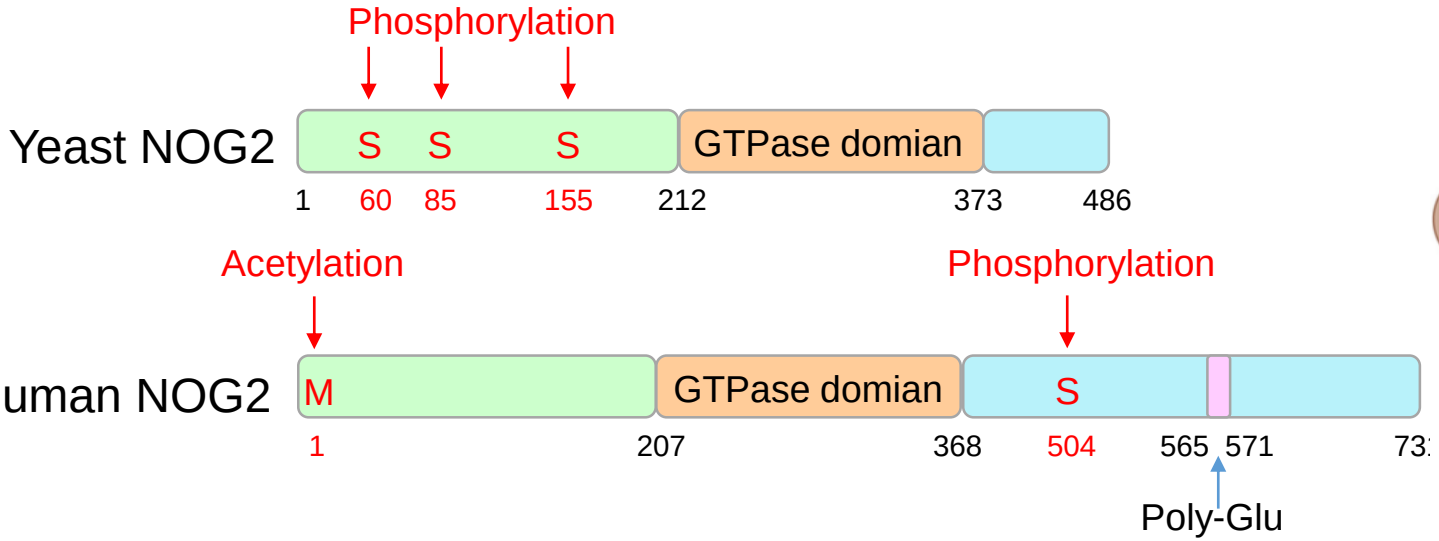


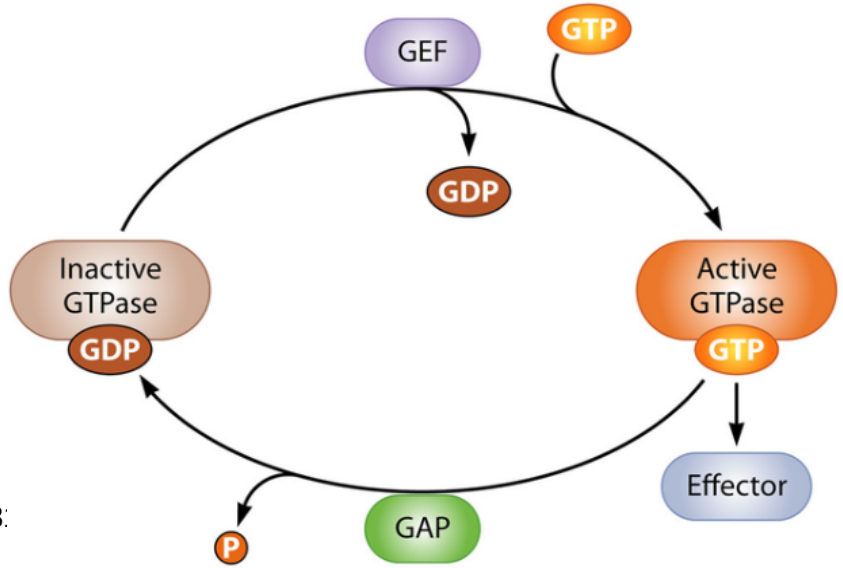
DNA-binding region

RNA-binding region

Protein binding region

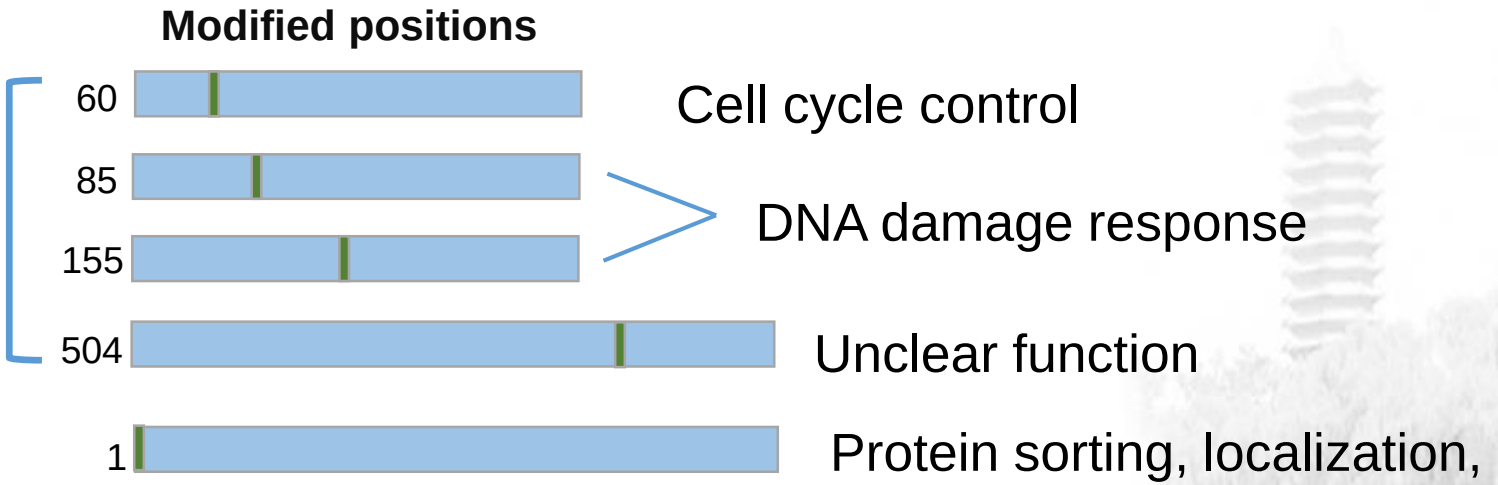
蛋白结构域和修饰位点



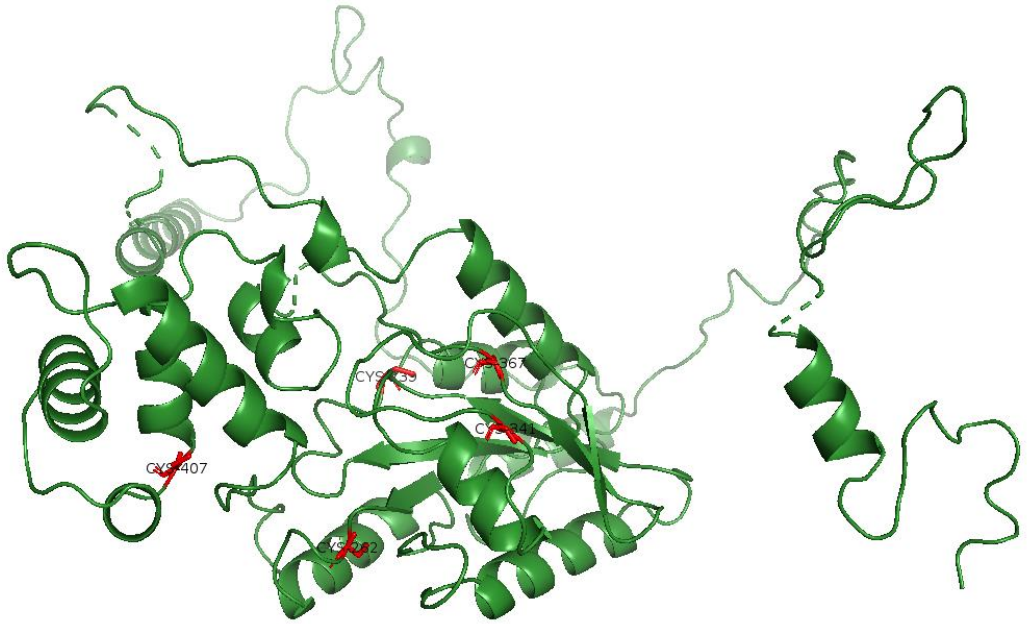


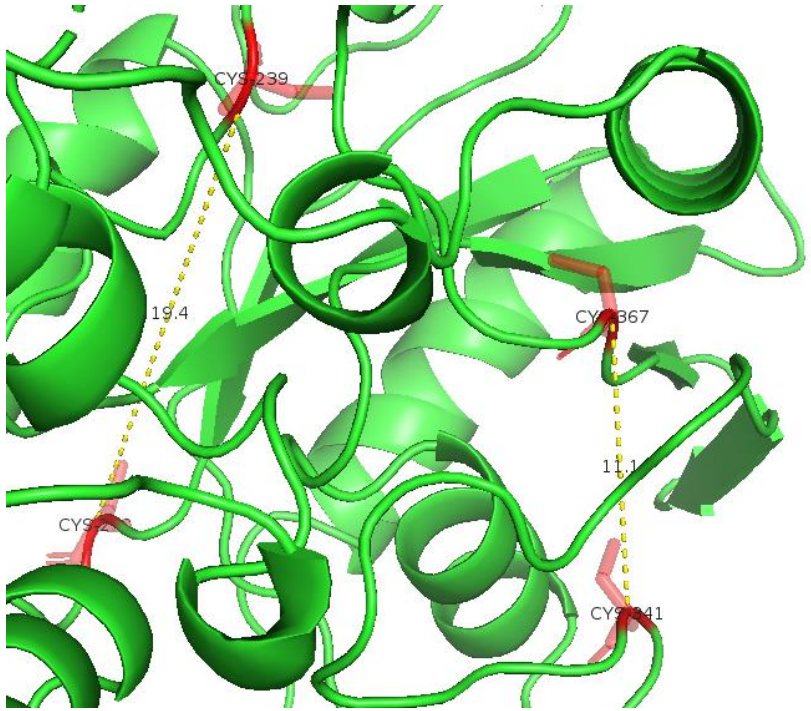
Phosphoproteome analysis

N-terminal acetylome analysis



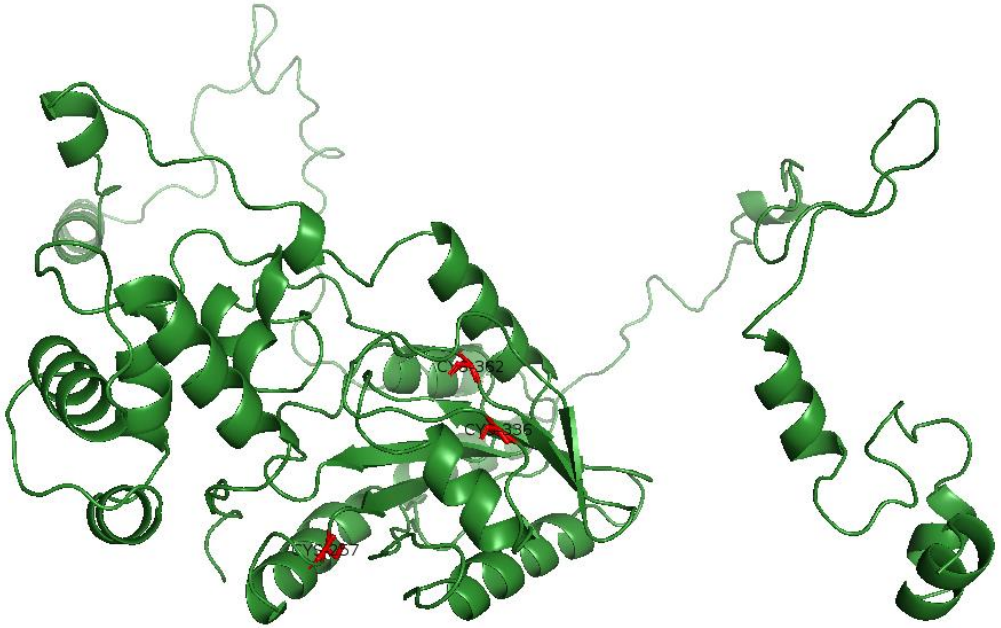
酵母NOG2二硫键分析

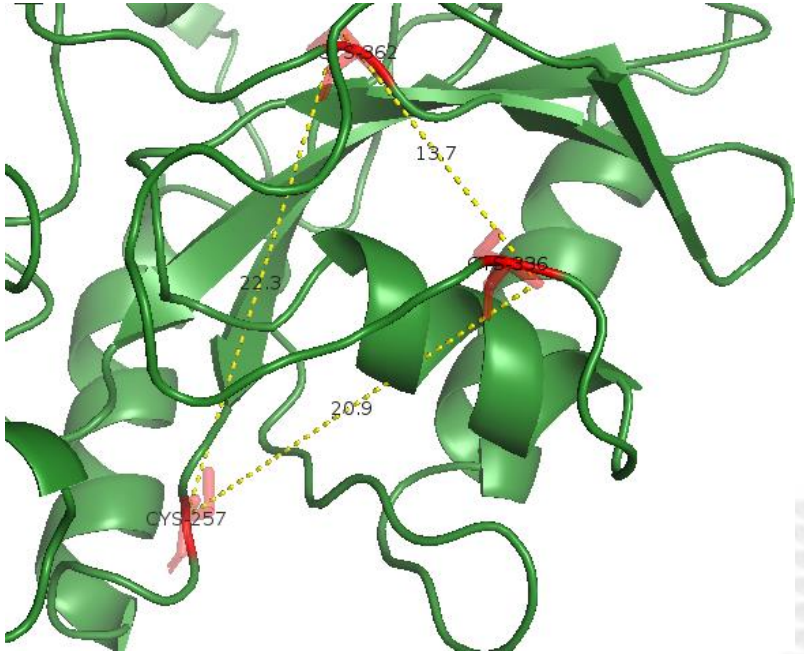




- NOG2_Yeast
- 有五个Cys残基，分别为Cys239，Cys262，Cys341，Cys367，Cys407

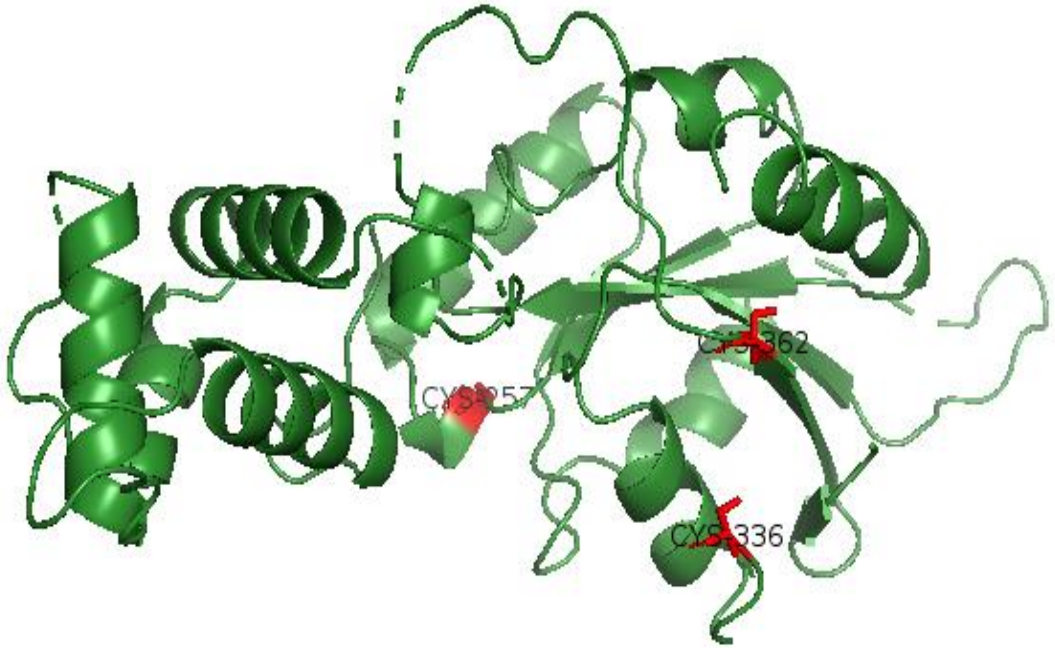
Swiss-Model预测的人NOG2二硫键分析

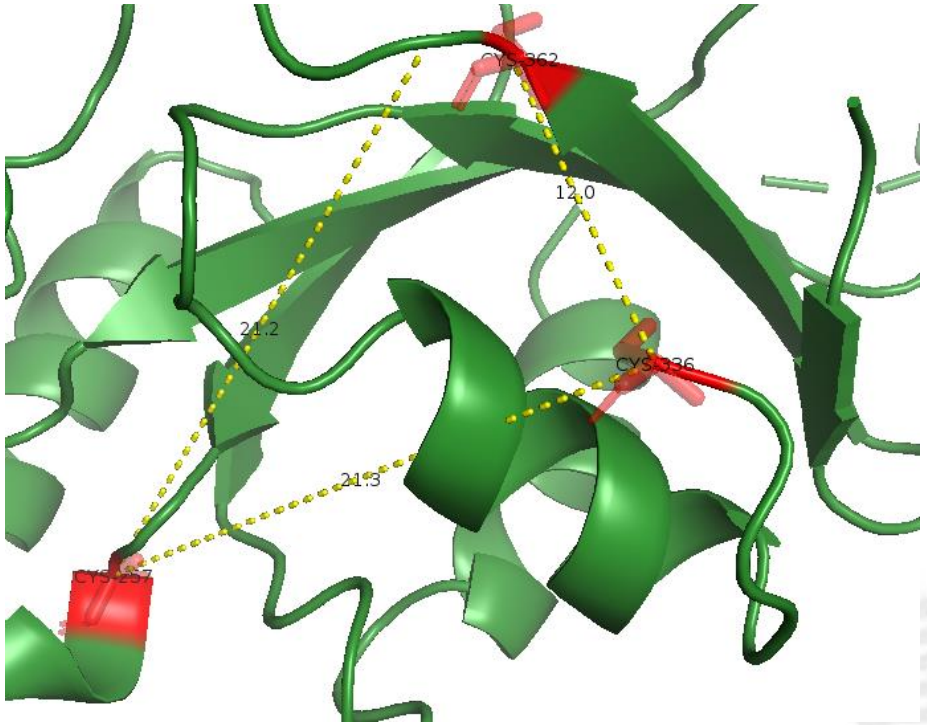




- NOG2_Human Swiss-Model
- 有三个Cys残基，分别为Cys257，Cys336，Cys362

Phyre2预测的人NOG2二硫键分析

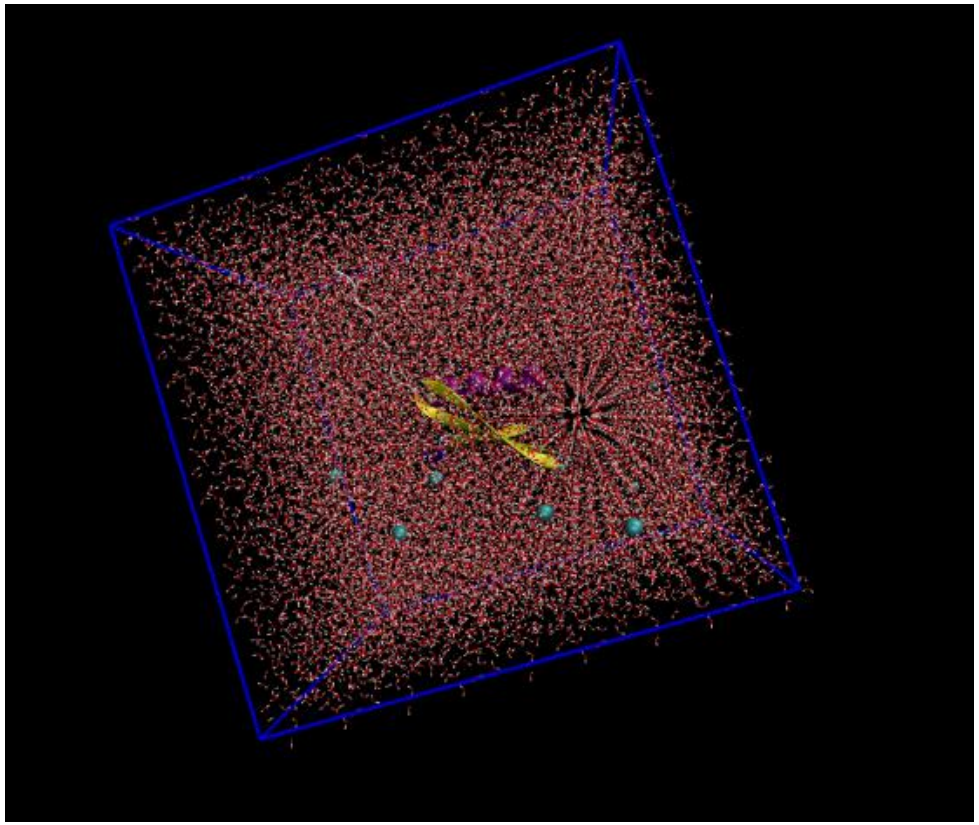


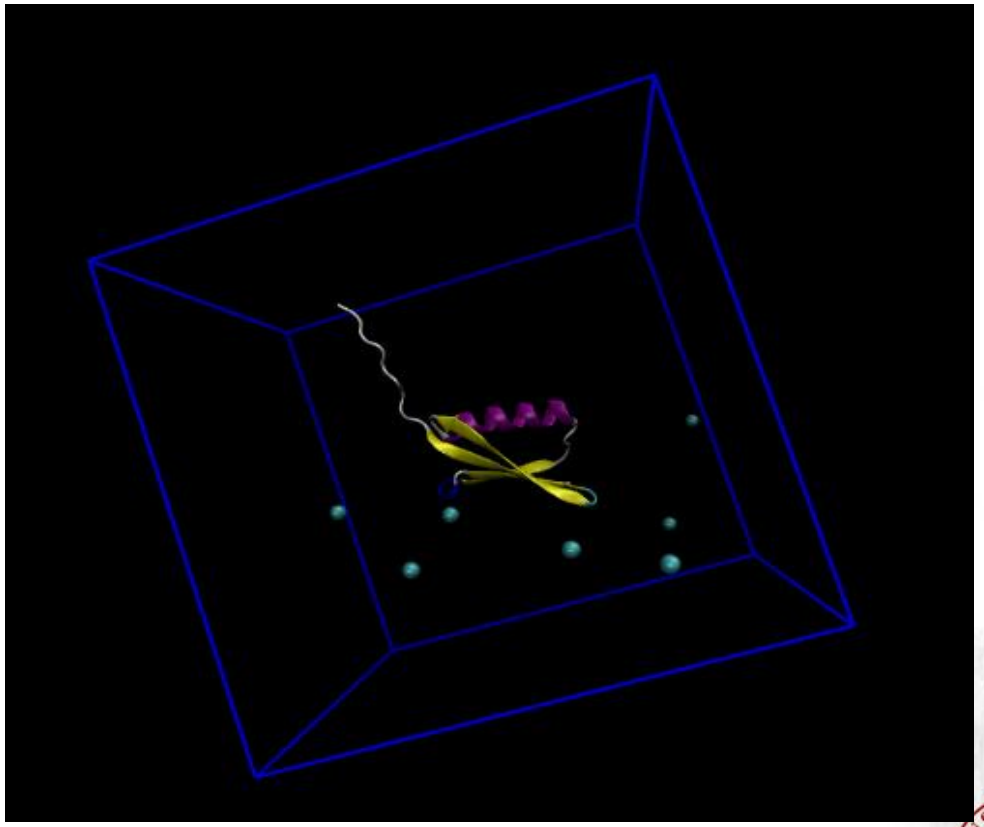


- NOG2_Human Phyre2
- 有三个Cys残基，分别为Cys257，Cys336，Cys362

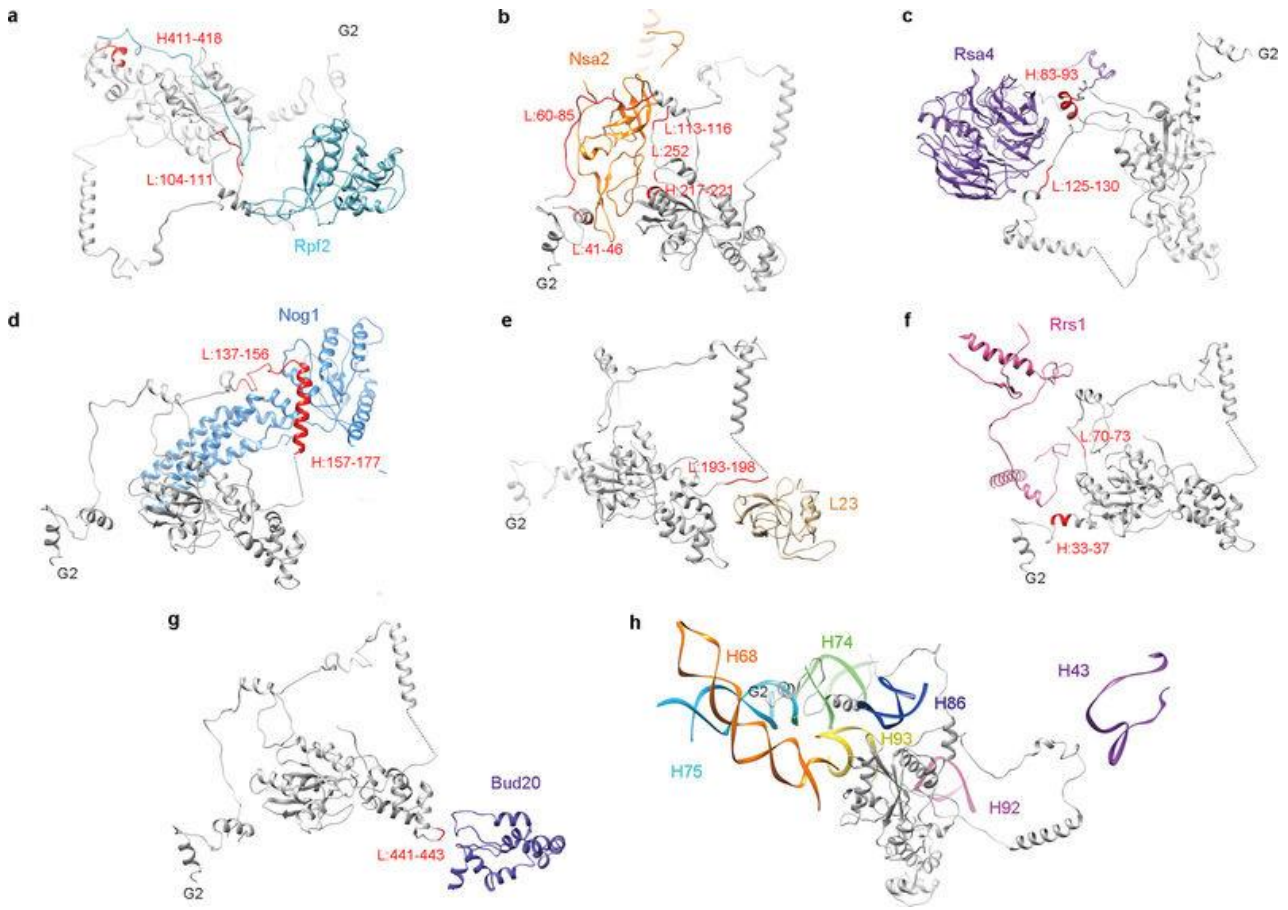
场环境下的NOG2

- 对NOG2部分结构域进行水环境模拟，由于生命体系中不存在净电荷, 所以必须往体系中添加了7个钠离子, 以保证总电荷为零。
- 左图水分子可视化，右图忽略水分子看到蛋白和加入的离子条件





谢谢聆听，欢迎提问！



Gao N *et al. Nature.* 534, 133–137 (2016)

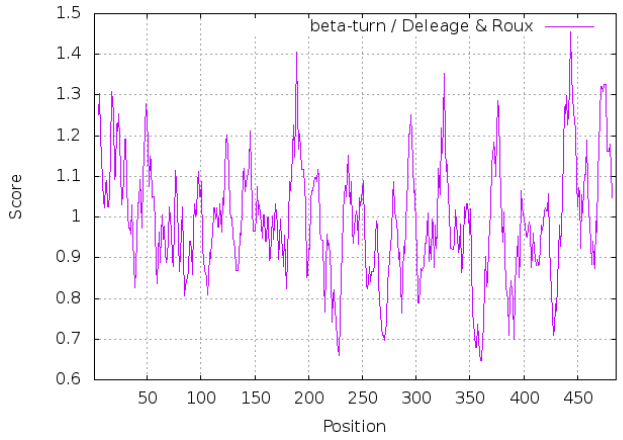
“Nog2 binds at the centre of the pre-60S particle, via interaction of its GTPase domain and carboxy (C)-terminal domain with a multi-helical junction , making extensive contacts with H93, H62, H64, H67, H69 and H71 of 25S ribosomal RNA (rRNA), and Bud20. This interaction stabilizes H69 and H71 in a nearly 180° -flipped position compared with their mature forms17. In addition, the C-terminal extended loop of Rpf2 (residues 275–300) is inserted into the interface of Nog2–GTPase domain–C-terminal domain and H69–H71 , also contributing to the displacement of H69–H71.”

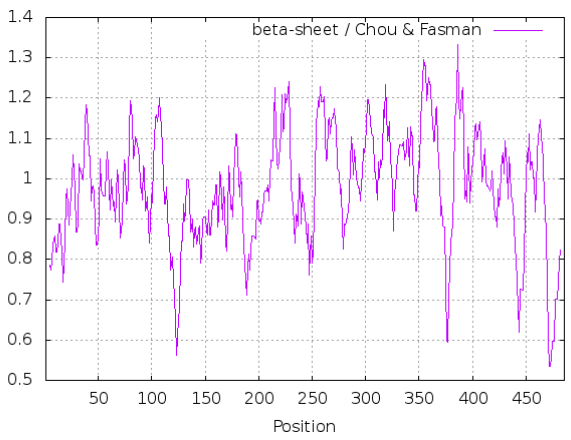
引物设计 (NM_001183230.1)

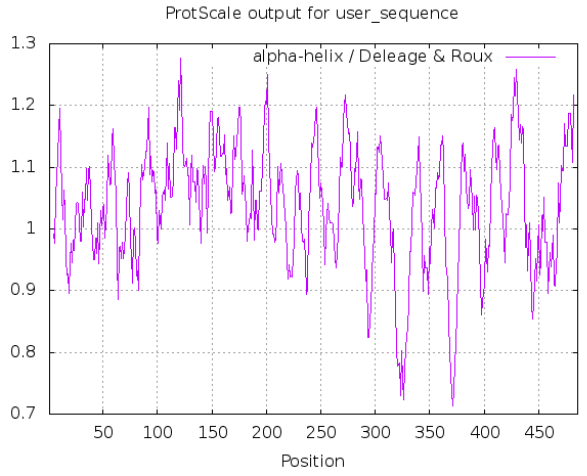
- 【Tool】WebLab-Eprimer32
- 【Database】default
- 【Product size range】1400-2000

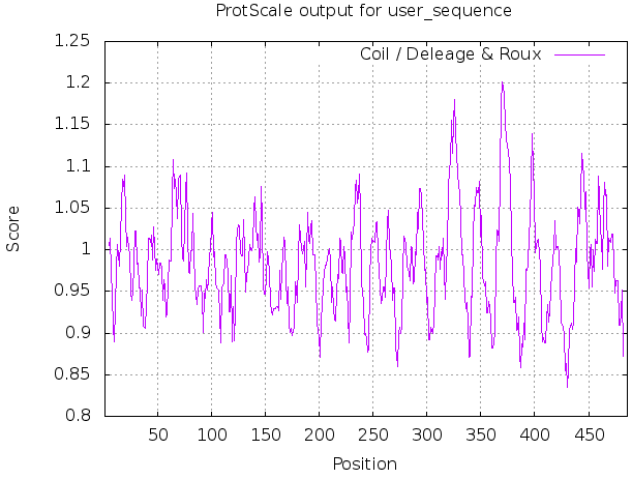
#		Start	Len	Tm	GC%	Sequence
1	PRODUCT SIZE: 1400					
	FORWARD PRIMER	28	26	58.27	46.15	AGACGTATTCGTGAAGGTGACACCAA
	REVERSE PRIMER	1402	26	57.17	46.15	TTTTCTTCCCCTTCCTTTTCAGGTGG
2	PRODUCT SIZE: 1400					
	FORWARD PRIMER	28	26	58.27	46.15	AGACGTATTCGTGAAGGTGACACCAA
	REVERSE PRIMER	1401	27	58.12	44.44	TTTTCTTCCCCTTCCTTTTCAGGTGGA
3	PRODUCT SIZE: 1400					
	FORWARD PRIMER	29	25	57.33	48.00	GACGTATTCGTGAAGGTGACACCAA
	REVERSE PRIMER	1402	27	57.50	44.44	TTTTTCTTCCCCTTCCTTTTCAGGTGG

NOG2蛋白的二维结构分析

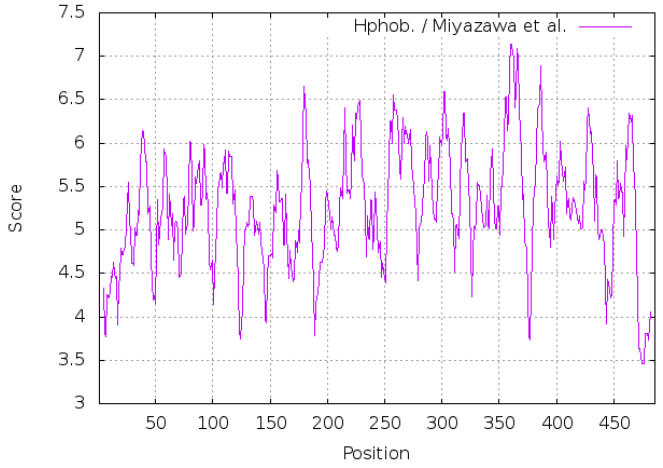


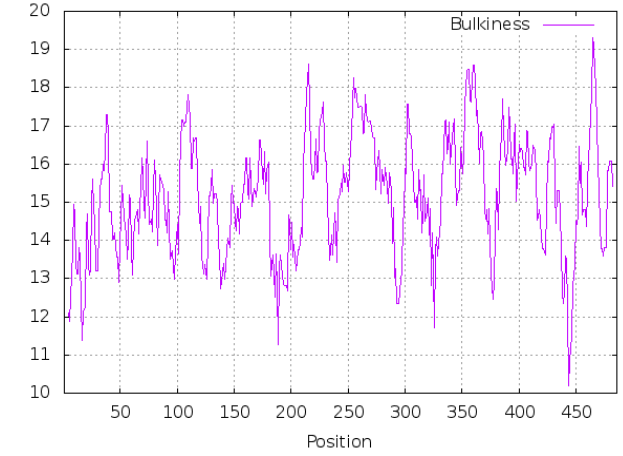


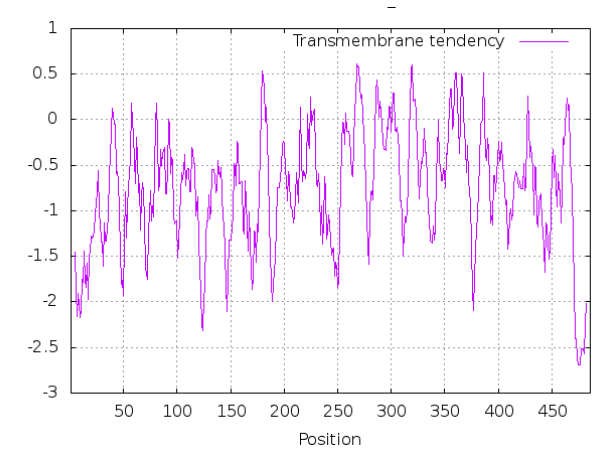


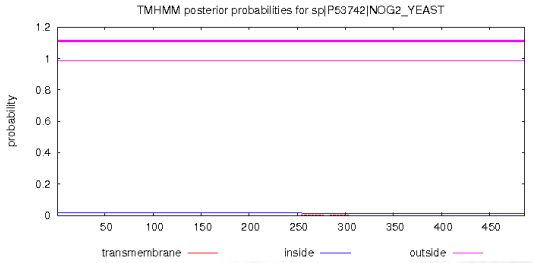


β-sheet/β-turn/α-helix/coil



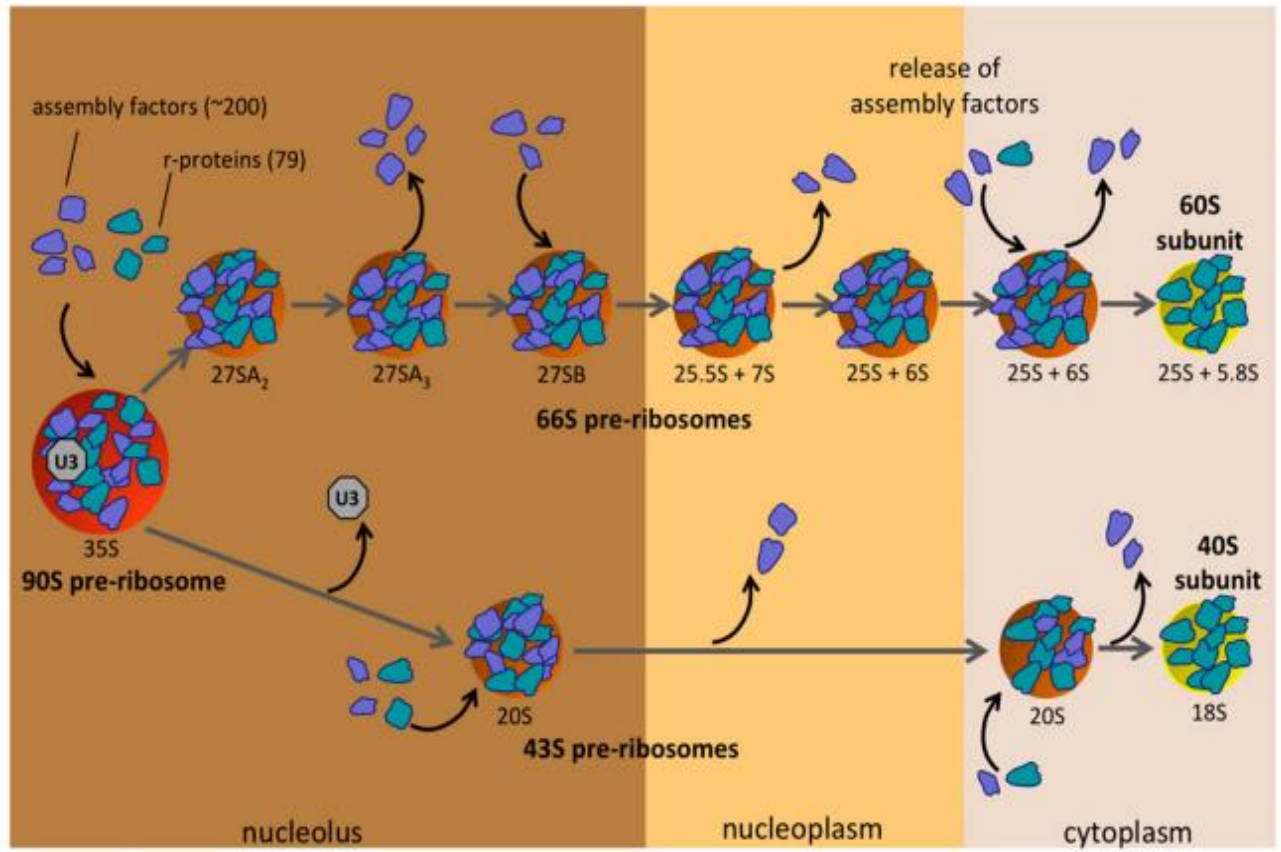


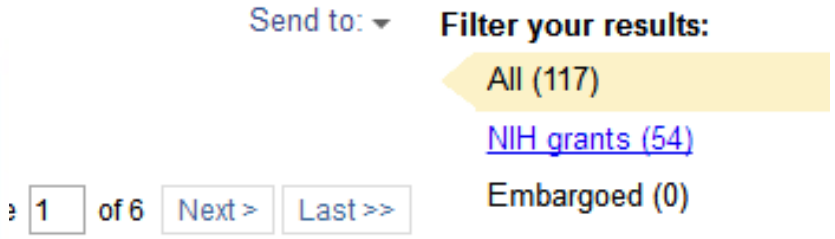




亲水/疏水，空间位阻和跨膜结构预测20

背景–40s/60s 核糖体小体的通路



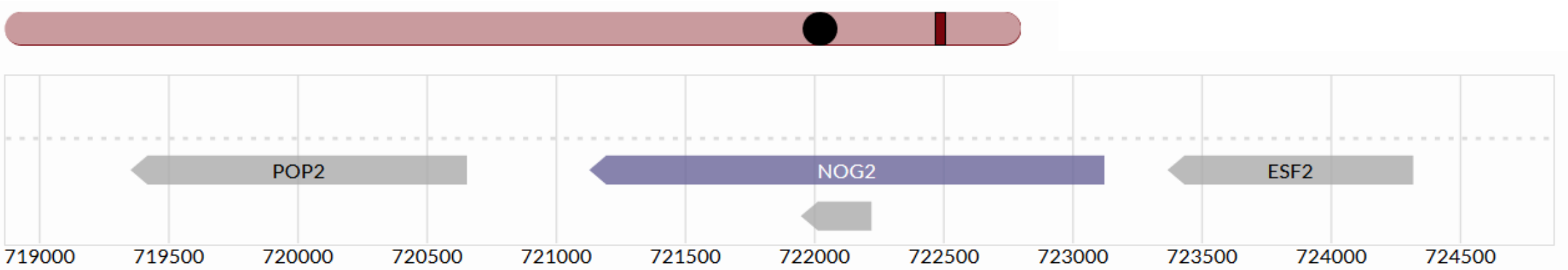


- PMC检索NOG2共有117篇相关文献
- 该蛋白与60S亚基装配过程息息相关
- 图示为生物体中核糖体前体的装配过程

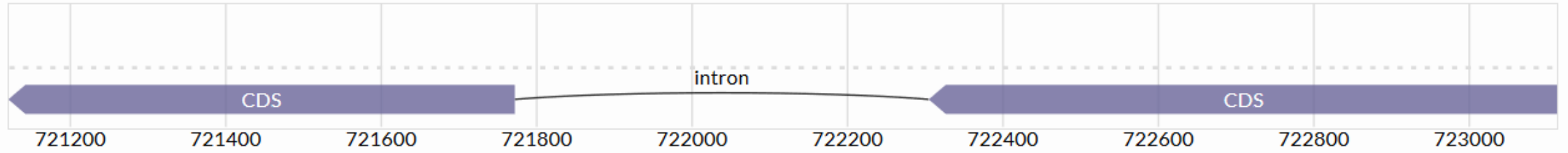
Matsuo Y, et al. Nature. 2014; 505:112–116.

NOG2 基因信息

NOG2 定位在14号染色体的 721120..723112



NOG2 基因信息:



Feature	Relative Coordinates	Coordinates	Coord. Version	Seq. Version
CDS	1..810	chrXIV:723112..722303	2011-02-03	1997-01-28
intron	811..1342	chrXIV:722302..721771	2011-02-03	2011-02-03
CDS	1343..1993	chrXIV:721770..721120	2011-02-03	1997-01-28

酵母NOG2氨基酸序列

MGTGKKEKSRRIREGDTKDGNLRVKGENFYRDSKRVKFLNMYTSGKEIRNKKGNLIRAAS
FQDSTIPDARVQPDRRWFGNTRVISQDALQHFRSALGETQKDTYQVLLRRNKLPMSLLEE
KDADESPKARILDTESYADAFGPKAQRKRPRLAASNLEDLVKATNEDITKYEEKQVLDAT
LGLMGNQEDKENGWTSAAKEAIFSKGQSKRIWNELYKVIDSSDVVIHVLDARDPLGTRCK
SVEEYMKKETPHKHLIYVLNKCDLVPTWVAAAWVKHLSKERPTLAFHASITNSFGKGSLI
QLLRQFSQLHTDRKQISVGFIGYPNTGKSSIINTLRKKKVCQVAPIPGETKVWQYITLMK
RIFLIDCPGIVPPSSKDSEEDILFRGVVRVEHVTHPEQYIPGVLKRCQVKHLERTYEISG
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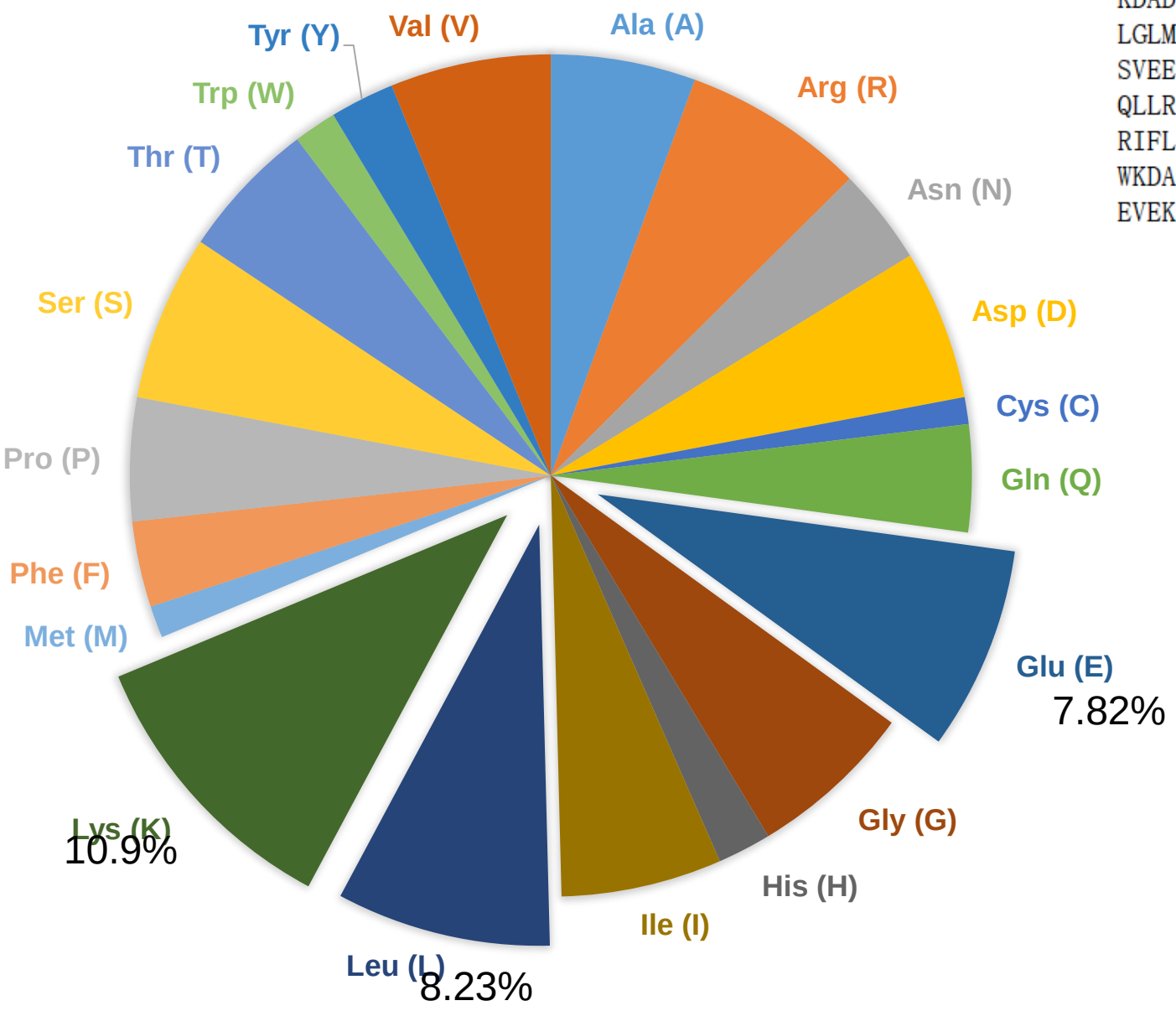
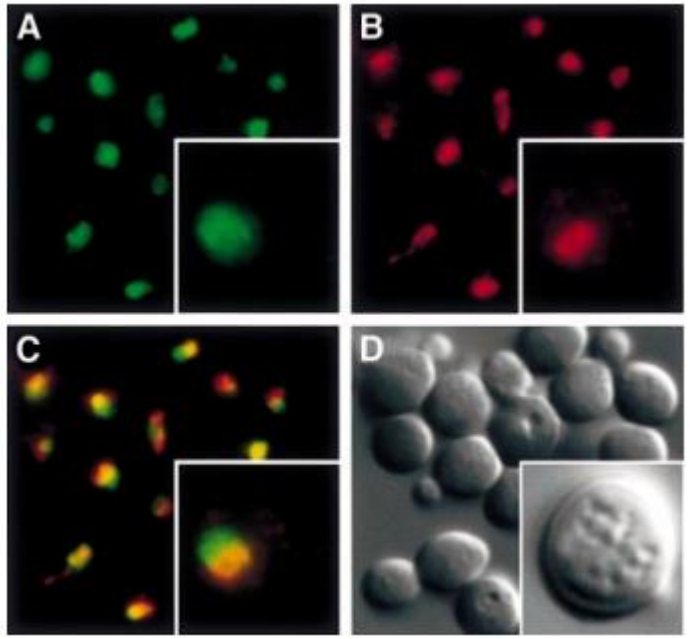
EVEKTA

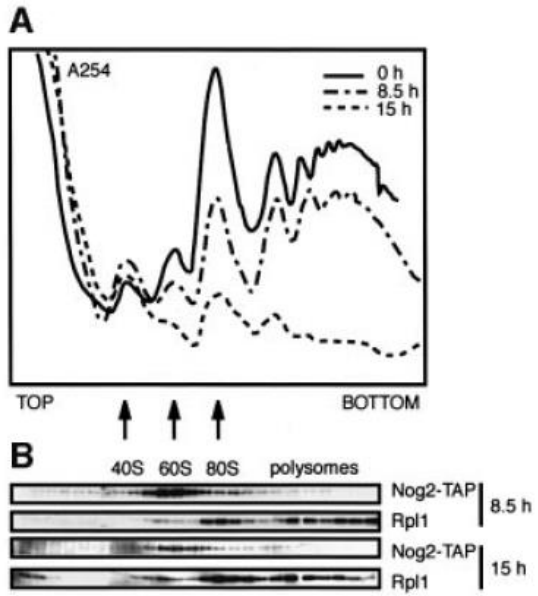


共由486个氨基酸组成，
55.49KD大小

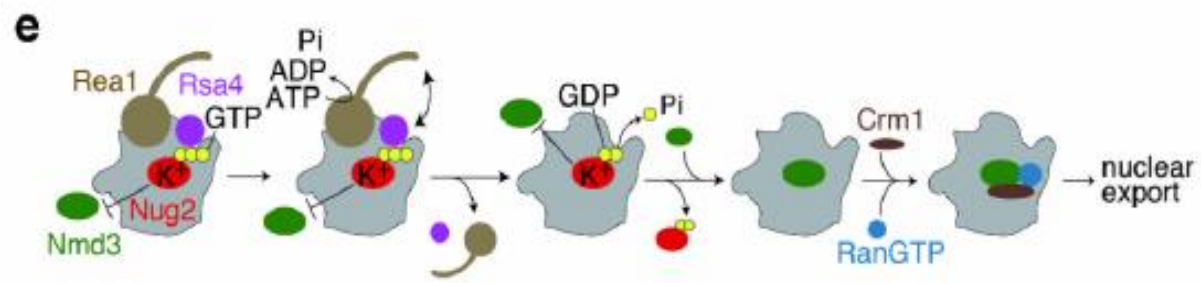
背景-NOG2，一个重要的GTP酶

- NOG2p定位在生物体内的核仁和核质上





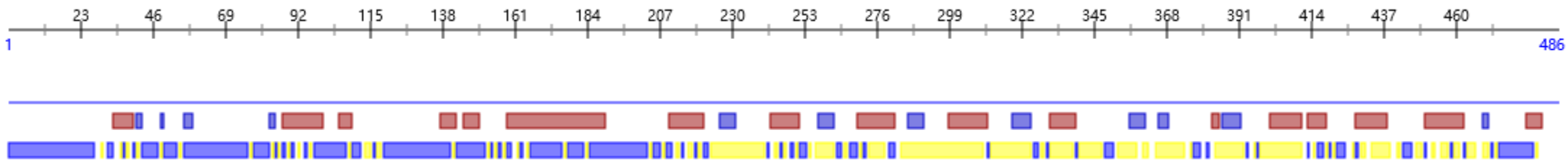
Saveanu C, et al. EMBO J. 2001; 20:6475–6484.

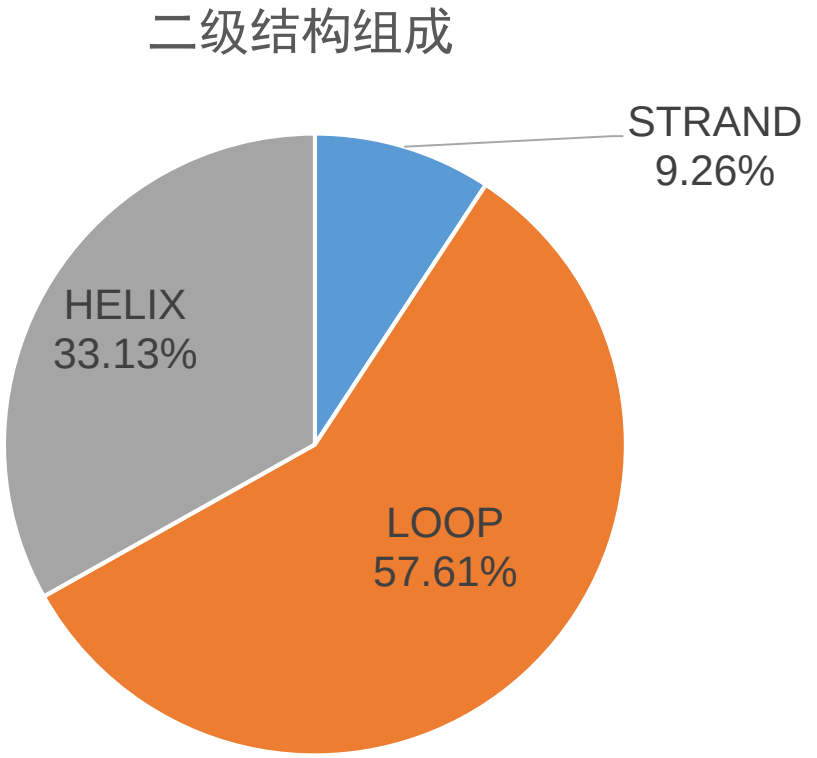


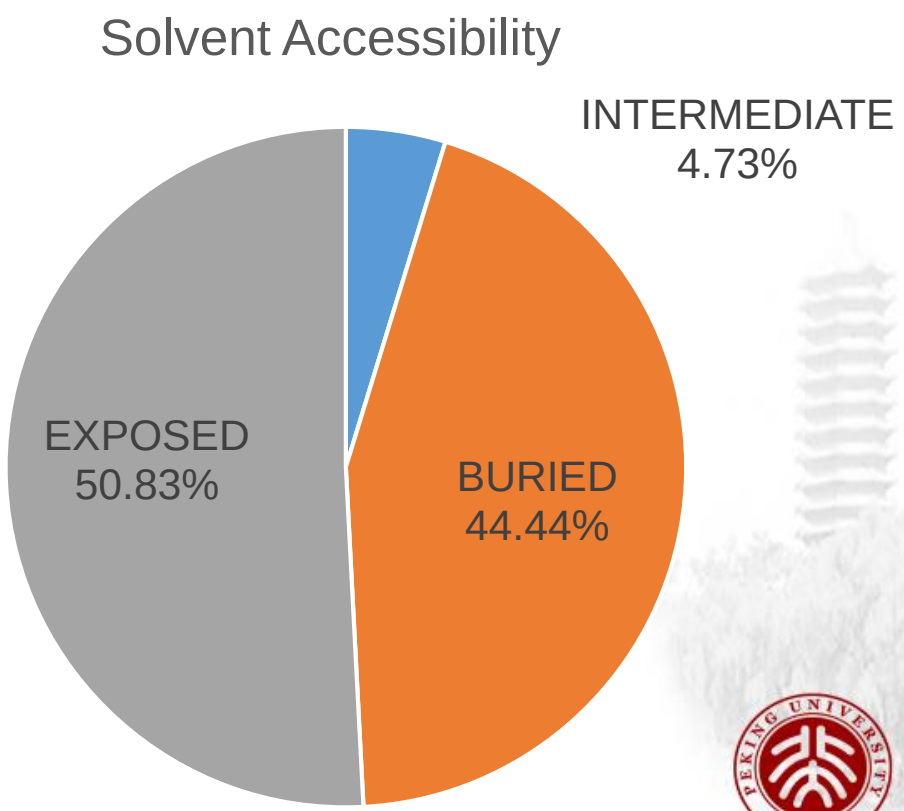
Model of pre-60S subunit maturation starting from the Rix1-particle with final Nmd3-Crm1-RanGTP recruitment

Matsuo Y, et al. Nature. 2014; 505:112–116.

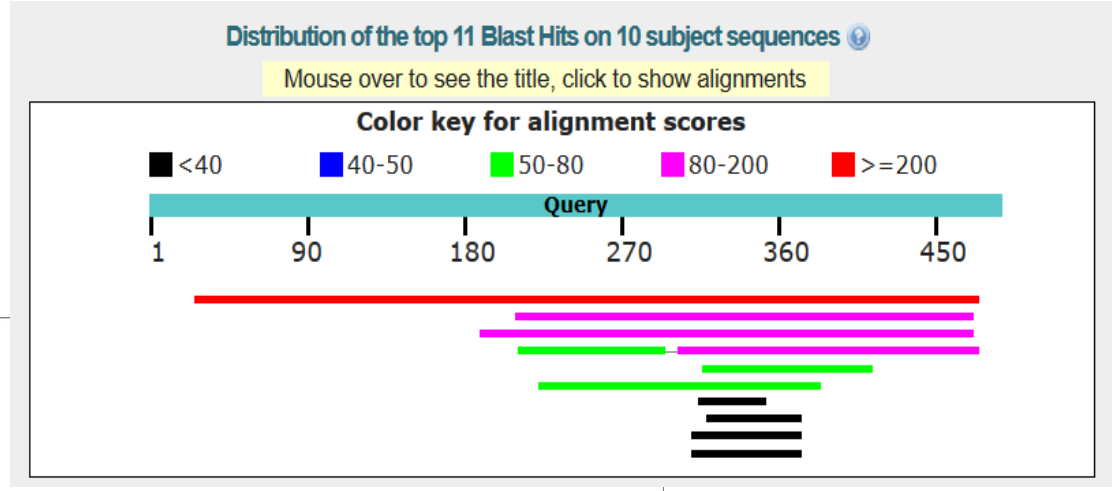
酵母NOG2 二级结构信息

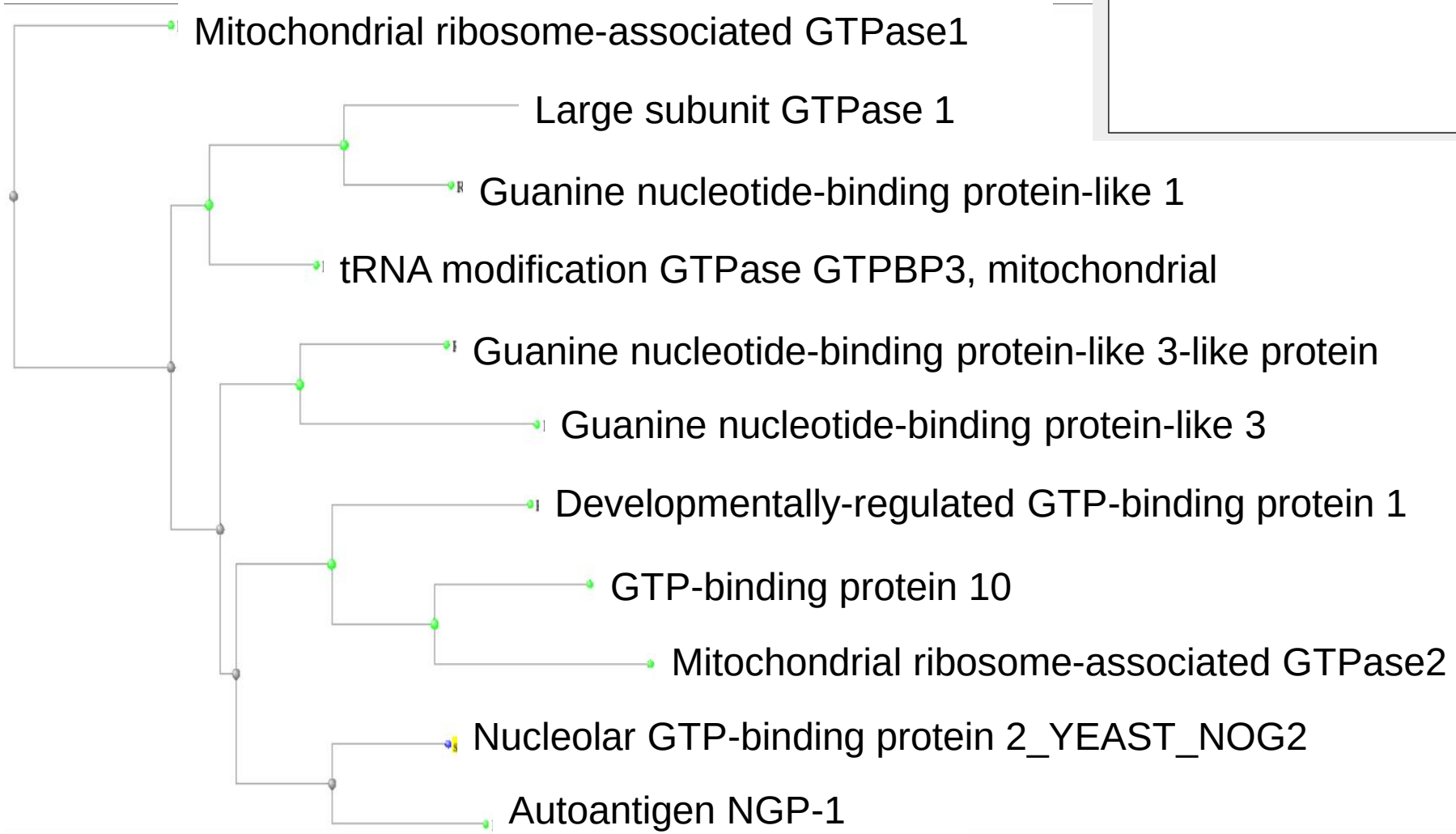




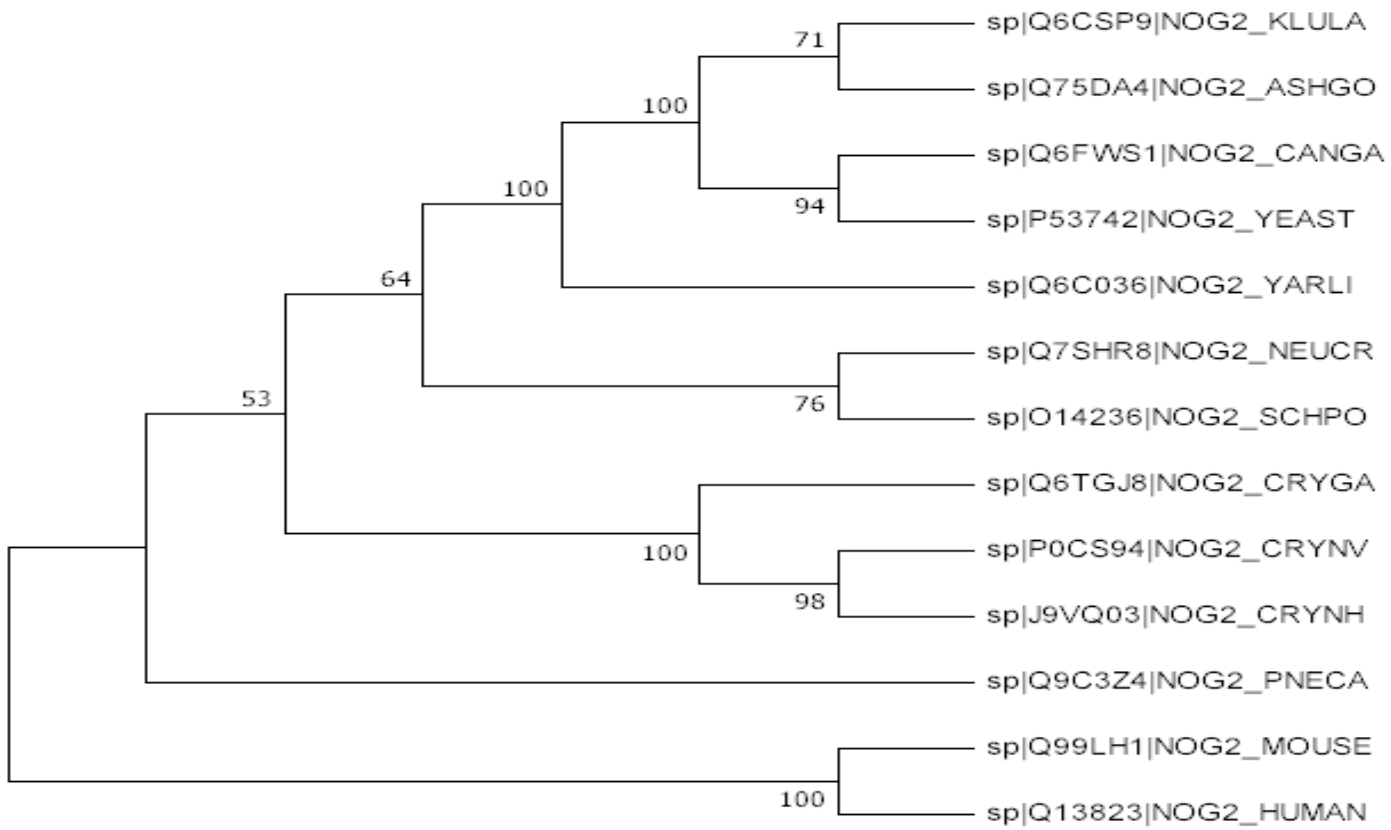


Blast 酵母NOG2蛋白序列-1



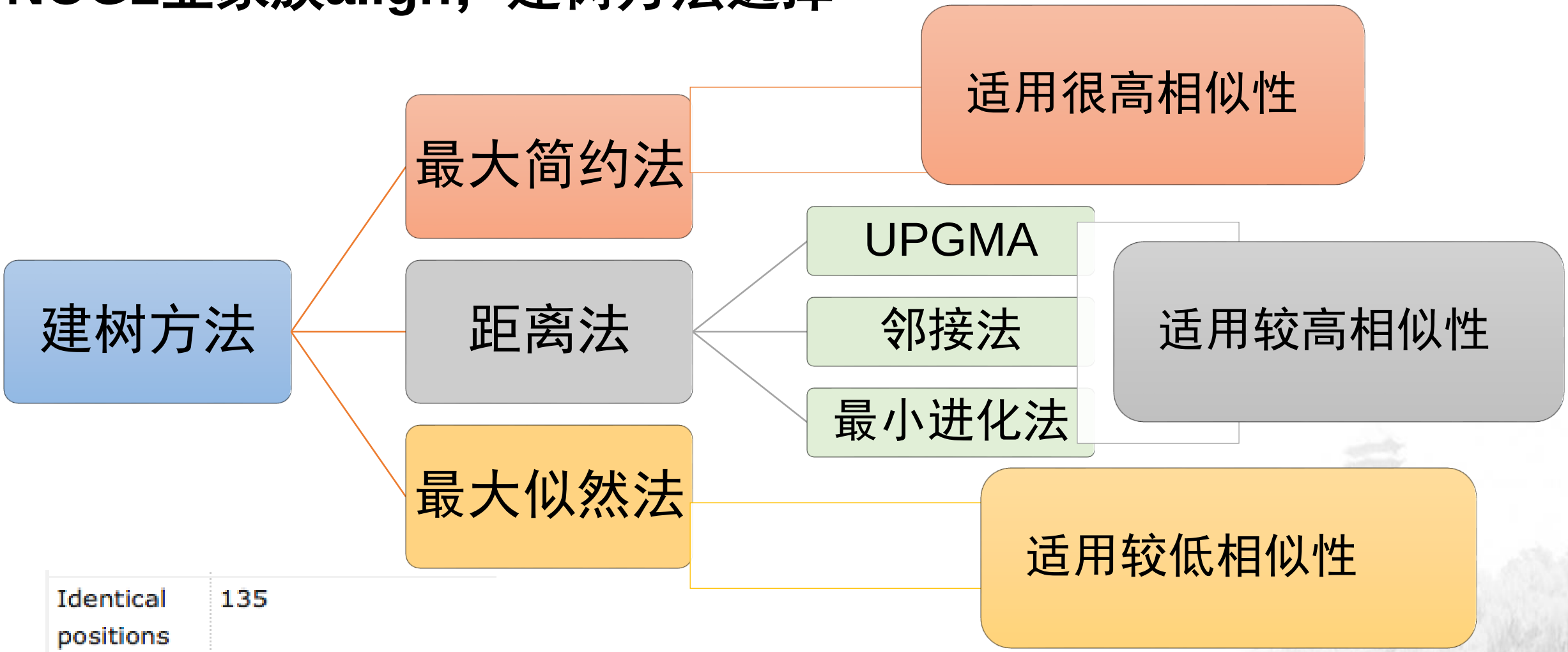


建树



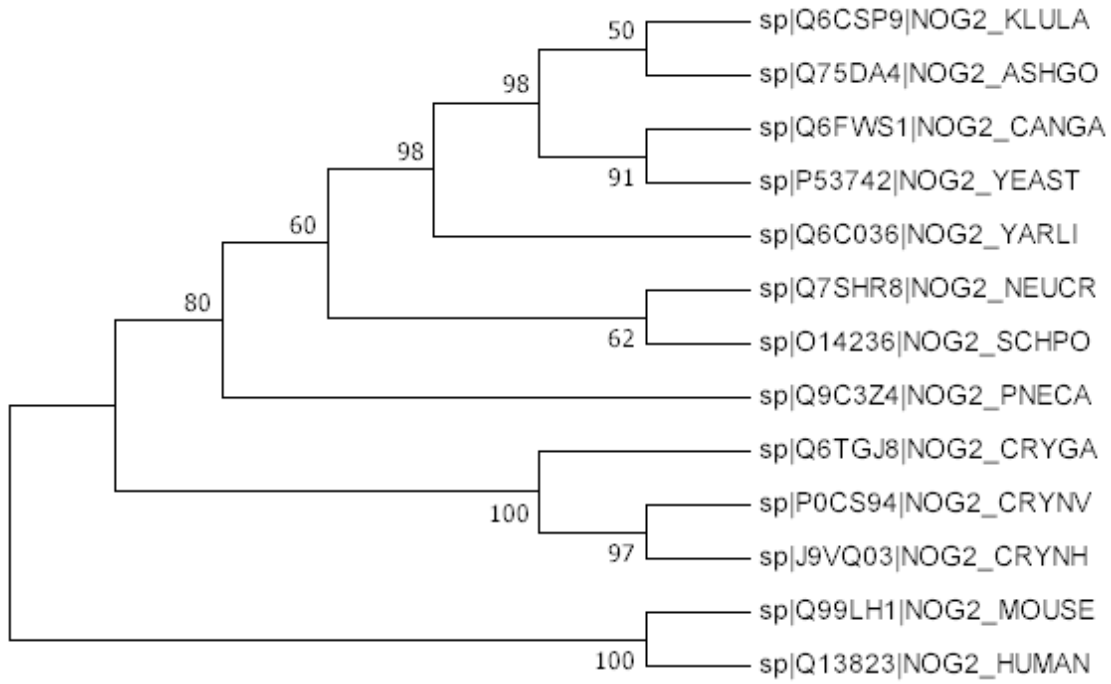
最小进化法
（Minimum Evolution Tree）

NOG2亚家族align，建树方法选择

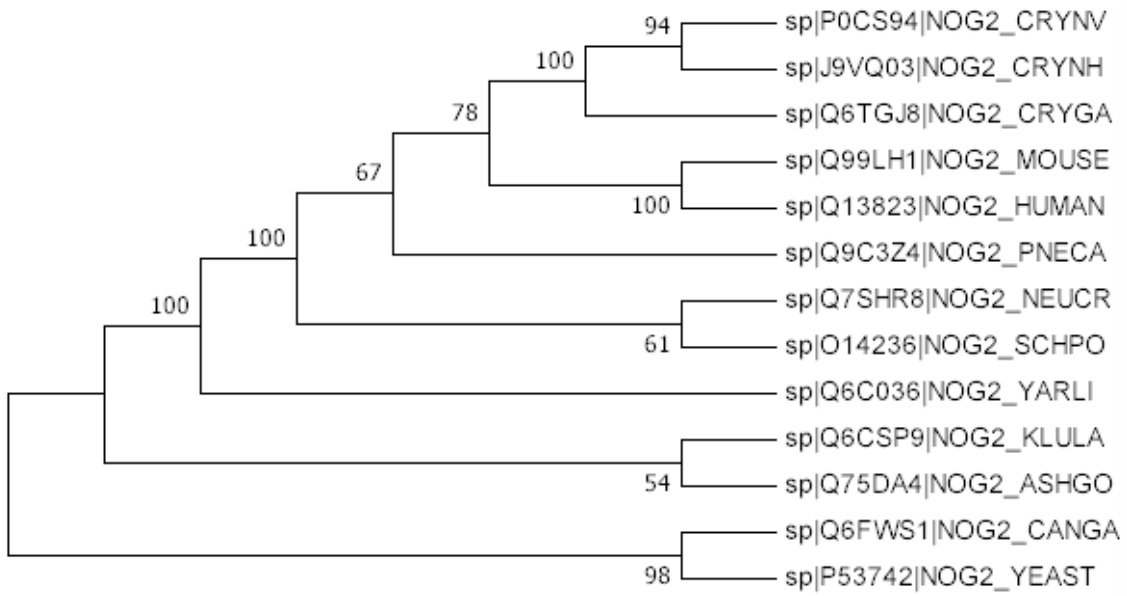


Identical positions	135
Identity	16.896%
Similar positions	124

建树

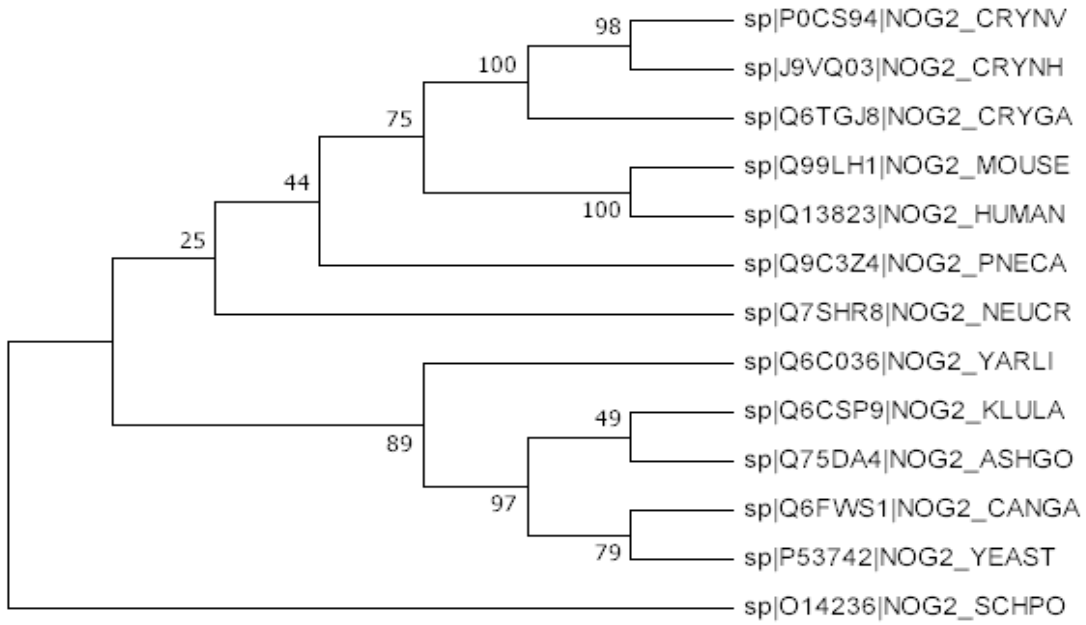


最大似然法
（Maximum Likelihood Tree)

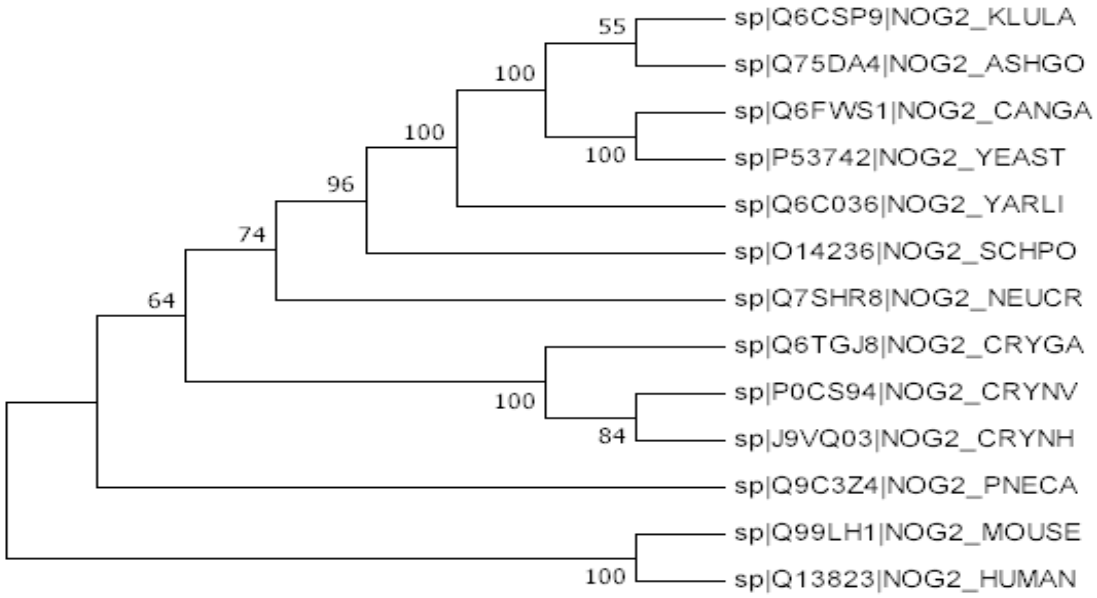


邻接法
（Neighbor-joining Tree)

建树

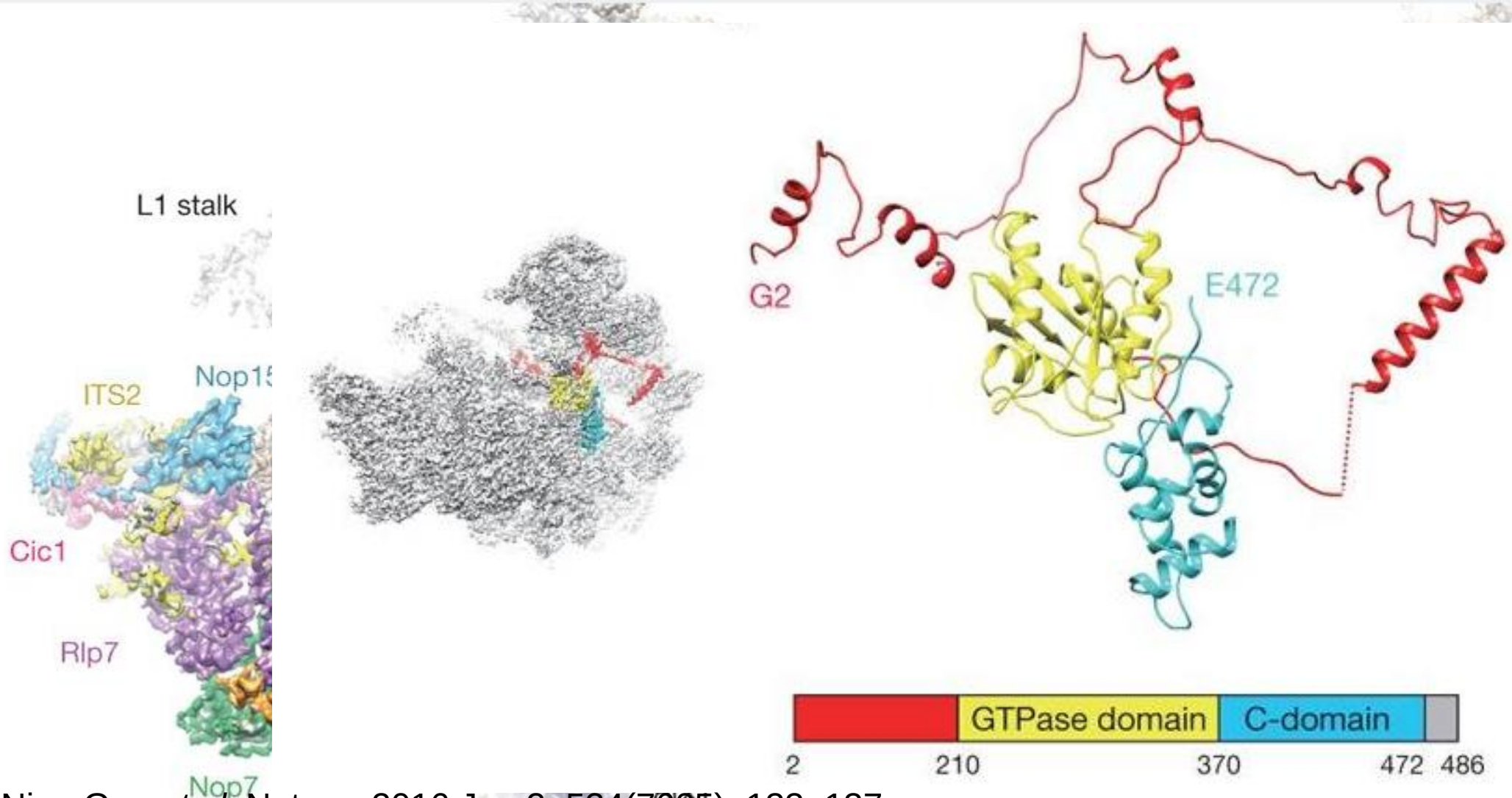


最大简约法
（Maximum Parsimony Tree)



UPGMA法

NOG2结构解析



Ning Gao *et al*. Nature. 2016 Jun 2; 534(7605): 133–137.

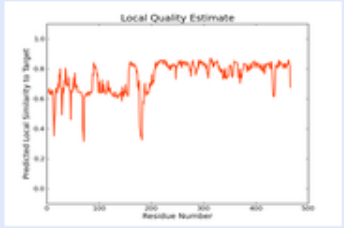
同源建模算法比较

Oligo-State	Ligands	GMQE	QMEAN
Monomer	None	0.51	-5.91

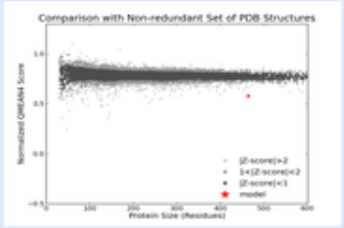
Global Quality Estimate

QMEAN	-5.91
Cβ	-0.62
All Atom	-1.03
Solvation	0.57
Torsion	-5.95

Local Quality Estimate



Comparison



Template	Seq Identity	Coverage	Description
3jct.1.c	54.78%		Nucleolar GTP-binding protein 2

Biounit Oligo State	Method	Seq Similarity	Range	Coverage
hetero-54-mer	EM	0.46	3 - 466	0.63

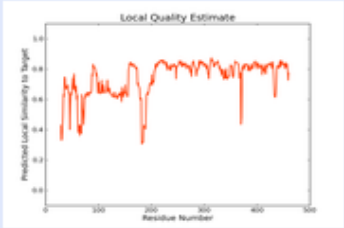
Ligand	Added to Model	Description
GTP	✕ - Binding site not conserved.	GUANOSINE-5'-TRIPHOSPHATE
GTP	✕ - Binding site not conserved.	GUANOSINE-5'-TRIPHOSPHATE
MG	✕ - Binding site not conserved.	MAGNESIUM ION
MG	✕ - Binding site not conserved.	MAGNESIUM ION
ZN	✕ - Binding site not conserved.	ZINC ION
ZN	✕ - Binding site not conserved.	ZINC ION
ZN	✕ - Binding site not conserved.	ZINC ION
ZN	✕ - Binding site not conserved.	ZINC ION

Oligo-State	Ligands	GMQE	QMEAN
Monomer	None	0.48	-5.20

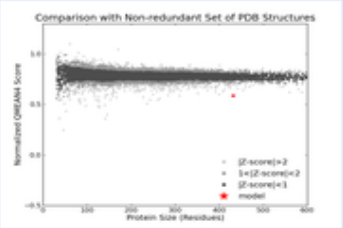
Global Quality Estimate

QMEAN	-5.20
Cβ	-0.58
All Atom	-0.97
Solvation	0.46
Torsion	-5.33

Local Quality Estimate



Comparison

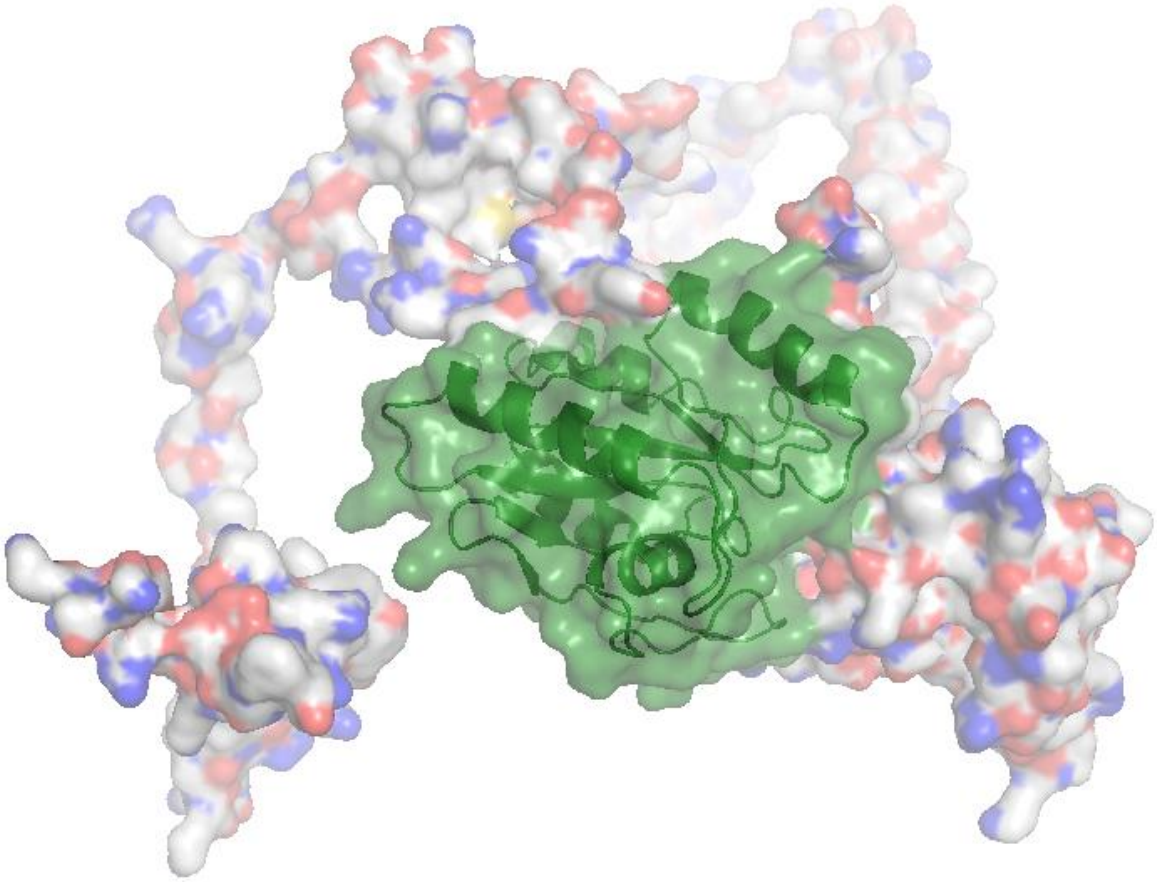


Template	Seq Identity	Coverage	Description
3jct.1.c	57.81%		Nucleolar GTP-binding protein 2

Biounit Oligo State	Method	Seq Similarity	Range	Coverage
hetero-54-mer	EM	0.47	29 - 460	0.59

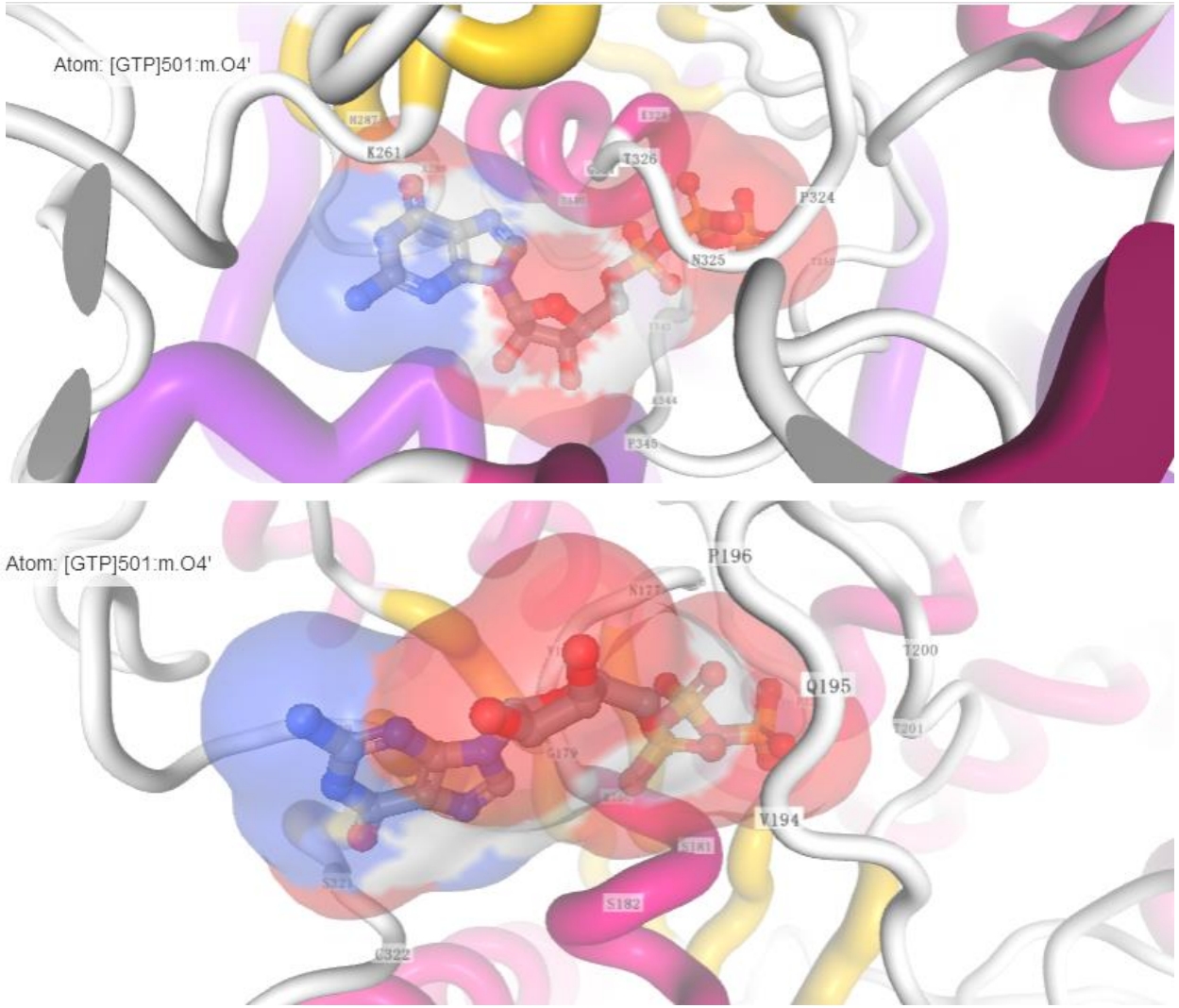
Ligand	Added to Model	Description
GTP	✕ - Binding site not conserved.	GUANOSINE-5'-TRIPHOSPHATE
GTP	✕ - Binding site not conserved.	GUANOSINE-5'-TRIPHOSPHATE
MG	✕ - Binding site not conserved.	MAGNESIUM ION
MG	✕ - Binding site not conserved.	MAGNESIUM ION
ZN	✕ - Binding site not conserved.	ZINC ION
ZN	✕ - Binding site not conserved.	ZINC ION
ZN	✕ - Binding site not conserved.	ZINC ION
ZN	✕ - Binding site not conserved.	ZINC ION

酵母NOG2的GTP结合位点（绿）



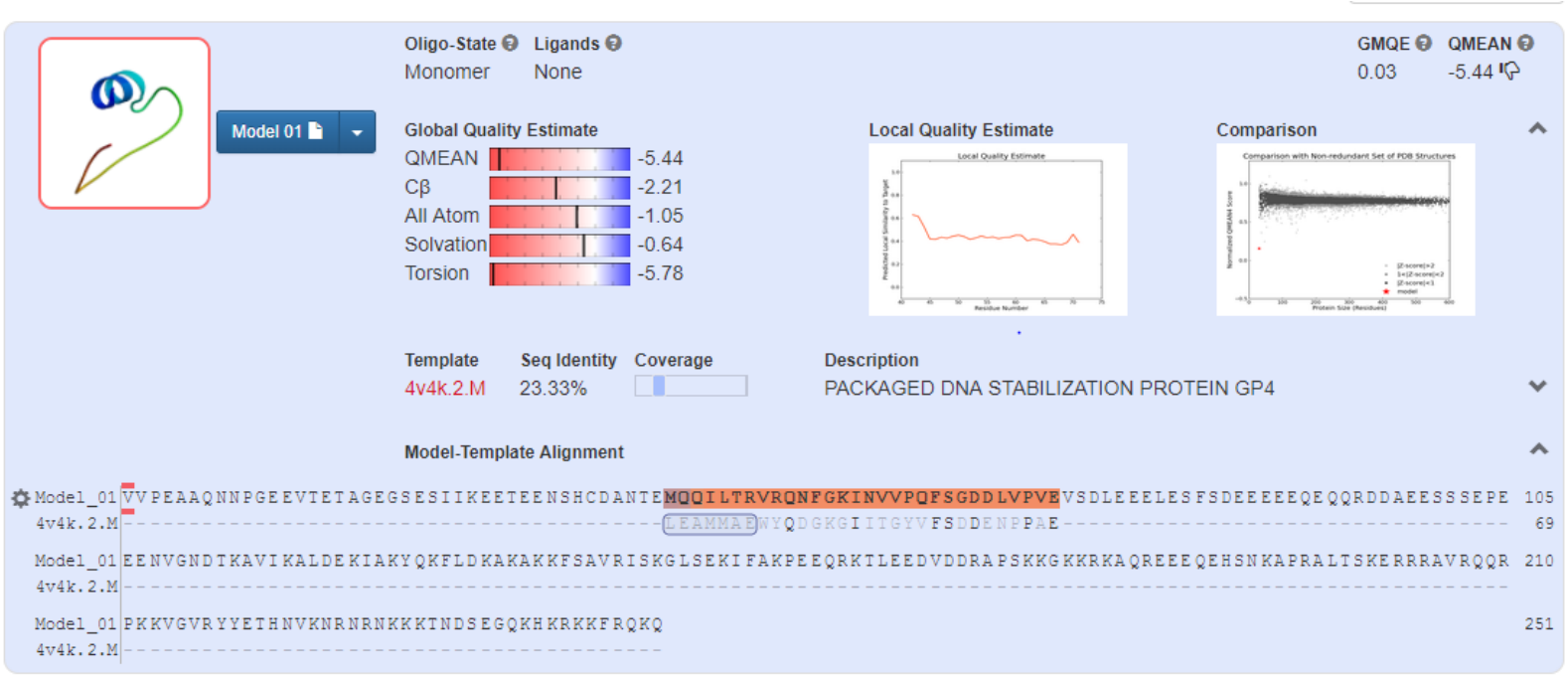
- NOG2蛋白的GTPase结构域为其功能结构域
- NOG2蛋白的GTPase结构域需要有互作蛋白才能行使其功能

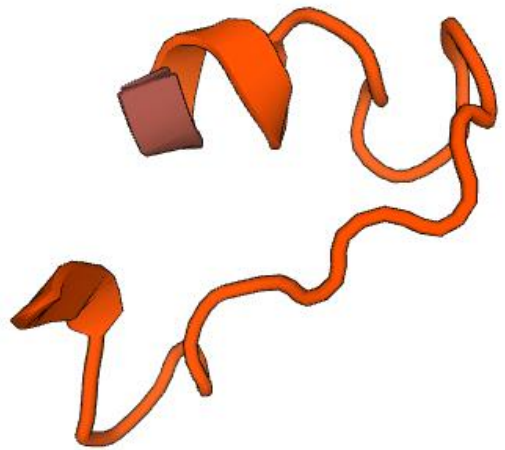
NOG2蛋白在60S ribosomal中的重要性



GTP结合位点！！！
NOG1, NOG2

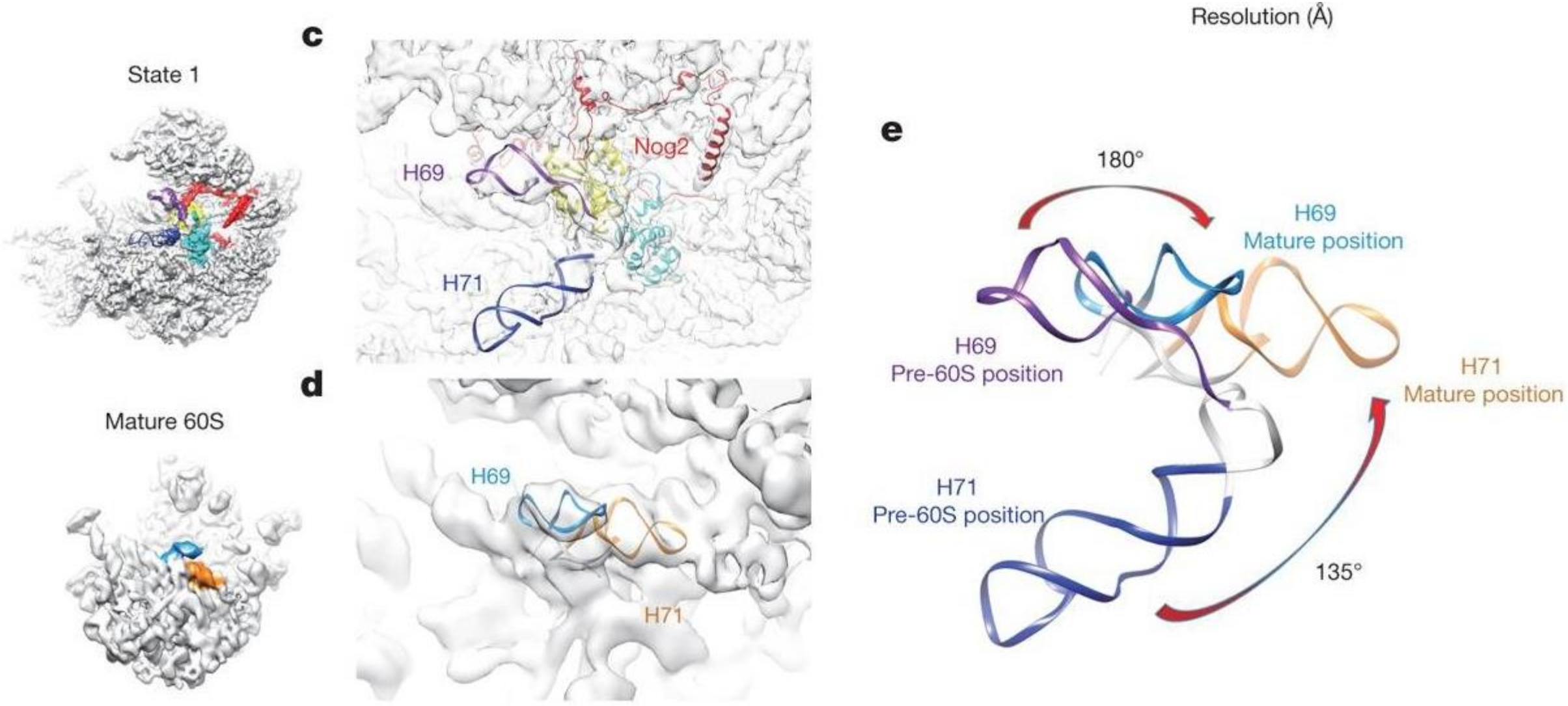
Swiss Model预测的人NOG2部分序列





- 从序列比对情况来看，NOG2_Human蛋白C‘端多了一个结构域，长度约200个氨基酸。
- 对这200个氨基酸进行单独 Swiss-Model结构模拟后，结果如图。

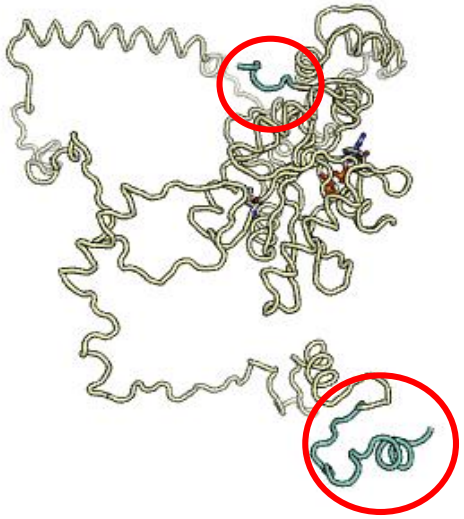
蛋白结构揭示互作机理



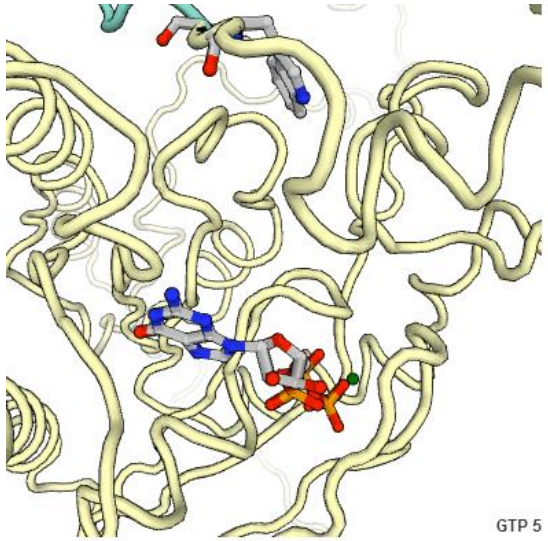
Ning Gao *et al*. Nature. 2016 Jun 2; 534(7605): 133–137.

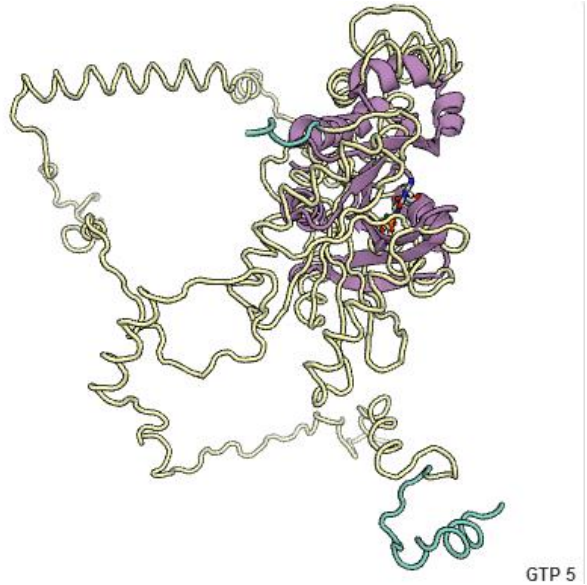
NOG2_Human预测结构图

54.78%



57.81%





- 多次预测结果显示出不同信息
- 下图表征GTP结合位点