

磁受体蛋白的 序列相似性比对 同源模建与分析

组长：马奔

组员：高雪凌 王秋月 曾维倩

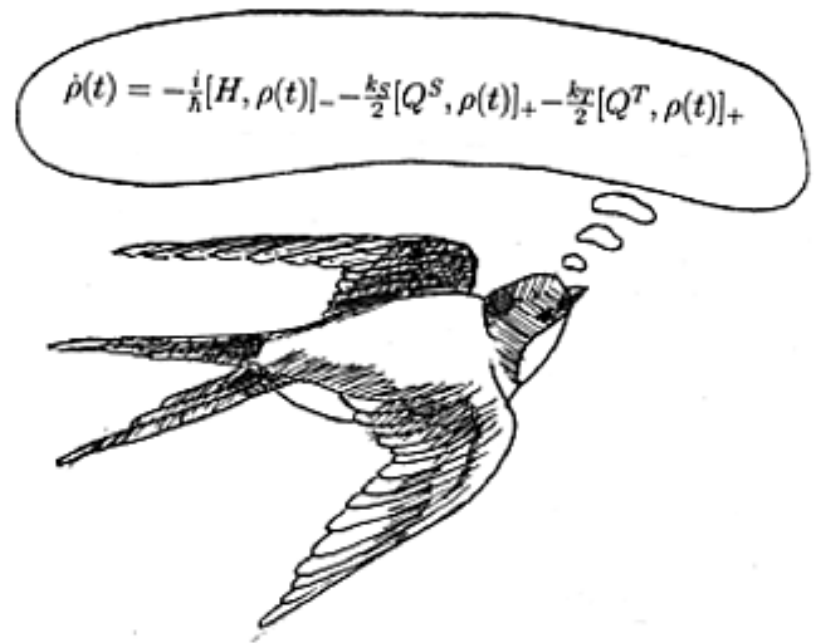
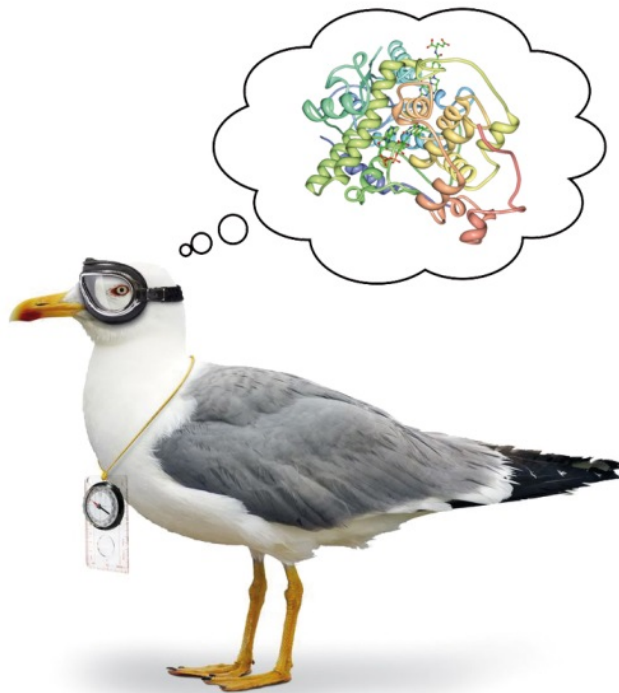
报告人：曾维倩

2013-06-21

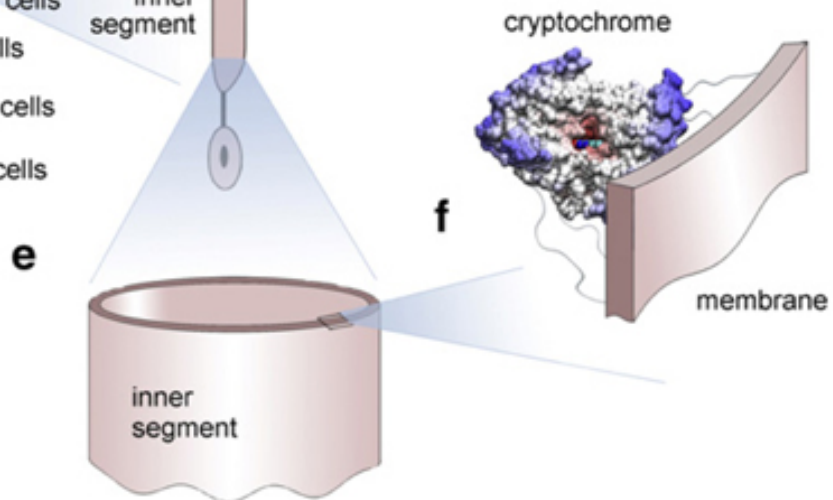
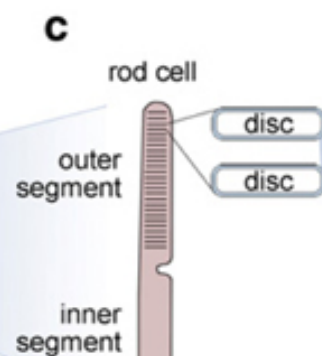
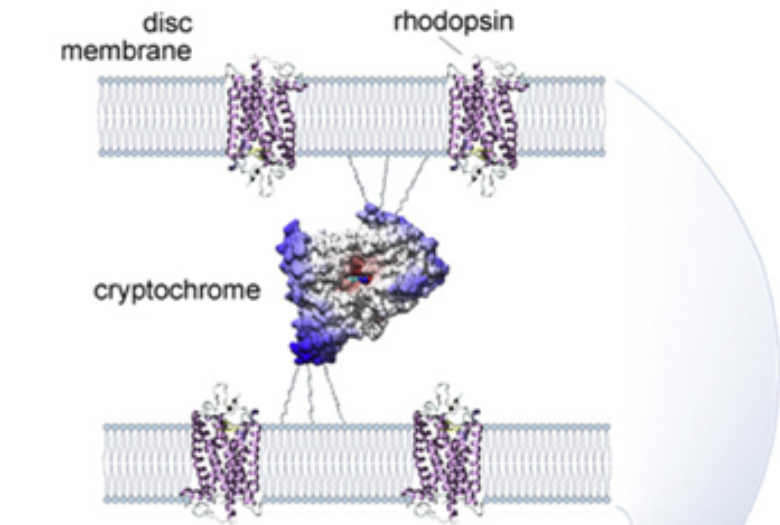
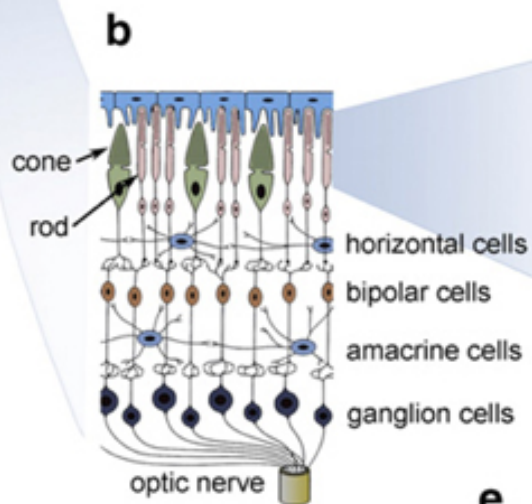
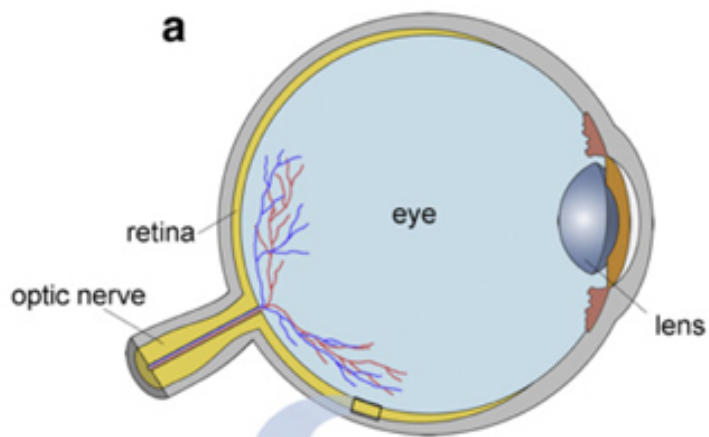
项目背景

- 目的：构建磁感应酵母（合成生物学）
- 理论依据：Radical Pair Mechanism（欧洲罗宾鸟）
- 实验依据：蛋白相互作用与信号通路，分子结构与作用机制（拟南芥、果蝇等）

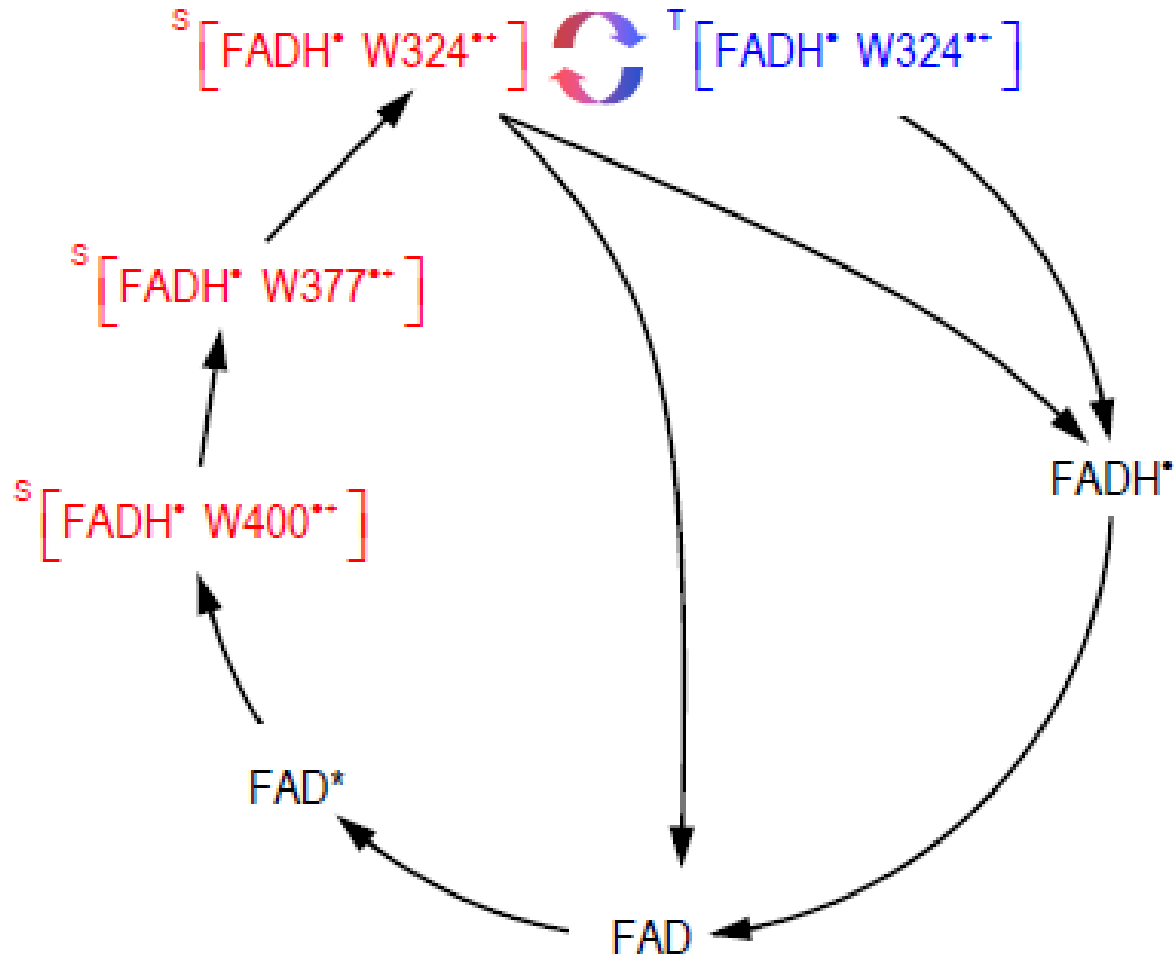
量子统计对于Radical Pair的研究很多，其中“罗宾鸟感受磁场”是整个理论体系中的一个代表

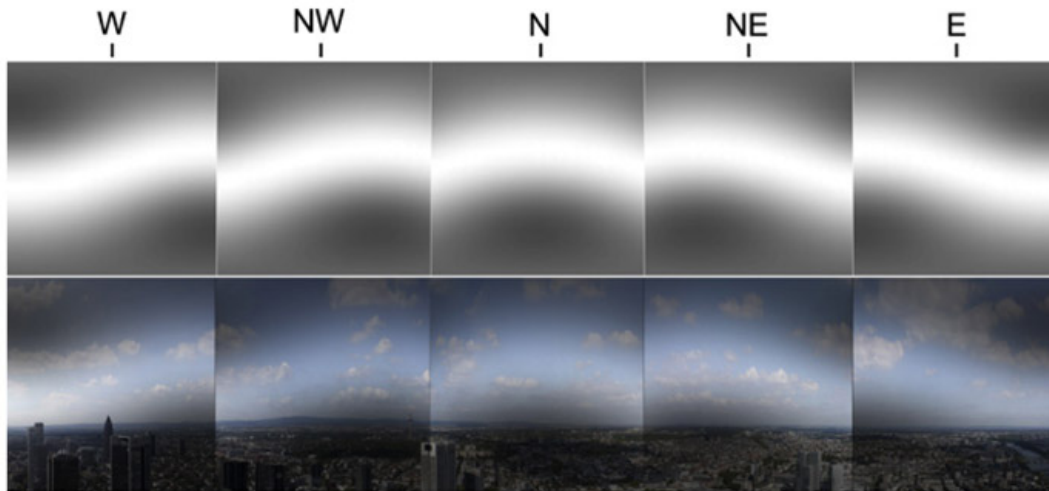


Picture from:
www.ks.uiuc.edu & pitp.physics.ubc.ca

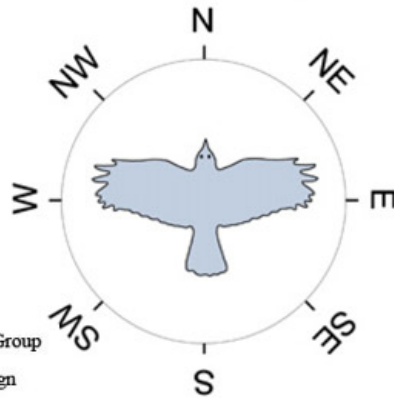


Radical-Pair Mechanism

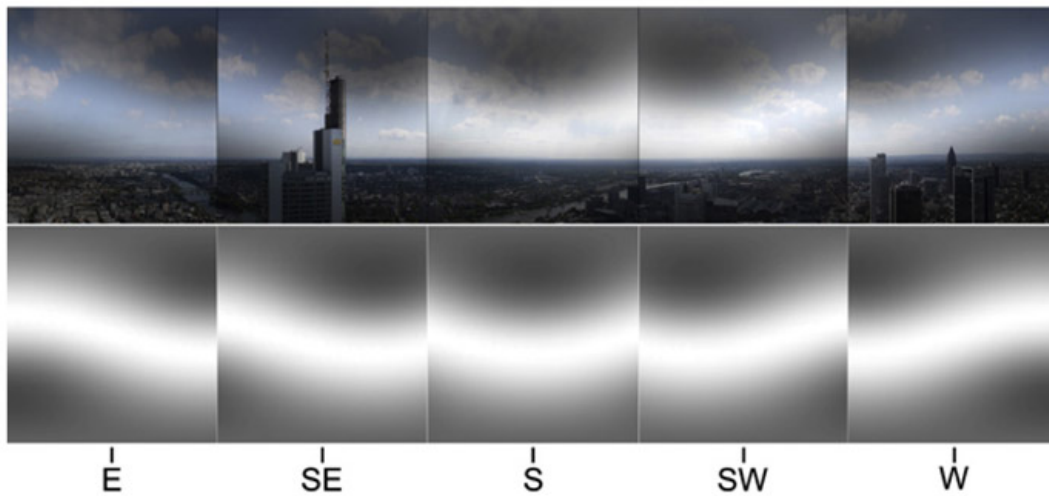
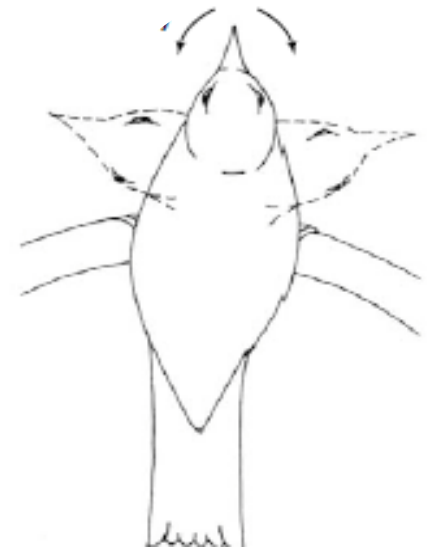




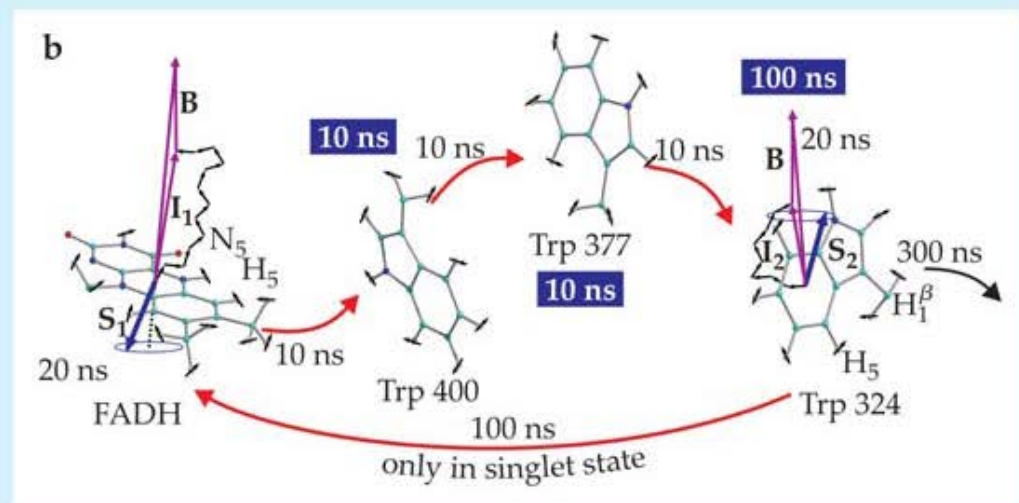
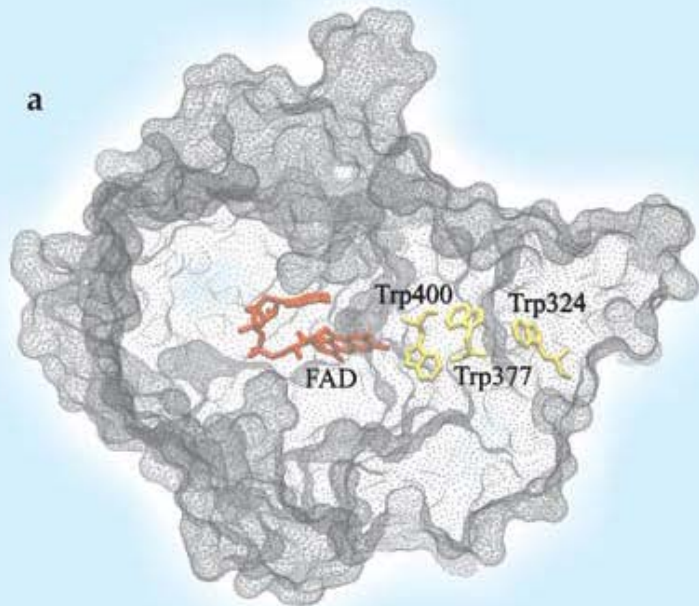
Scanning
movement



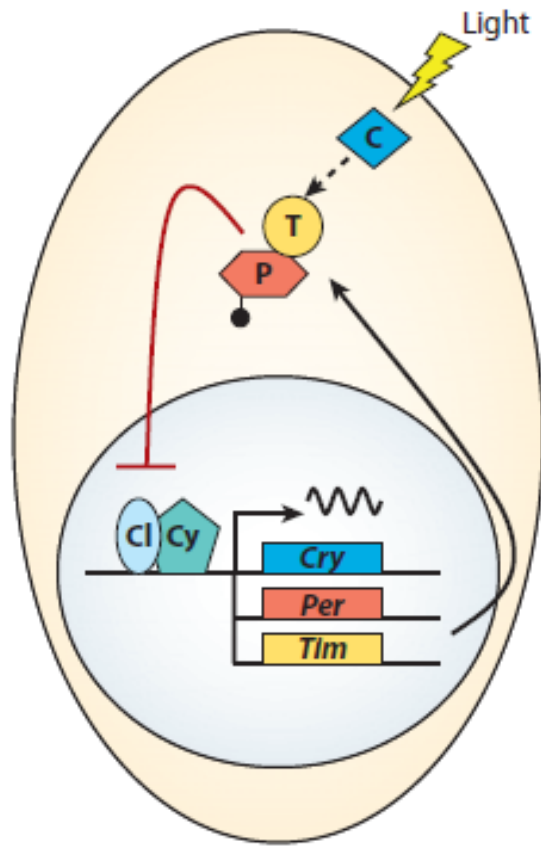
Theoretical and Computational Biophysics Group
Beckman Institute
University of Illinois at Urbana-Champaign



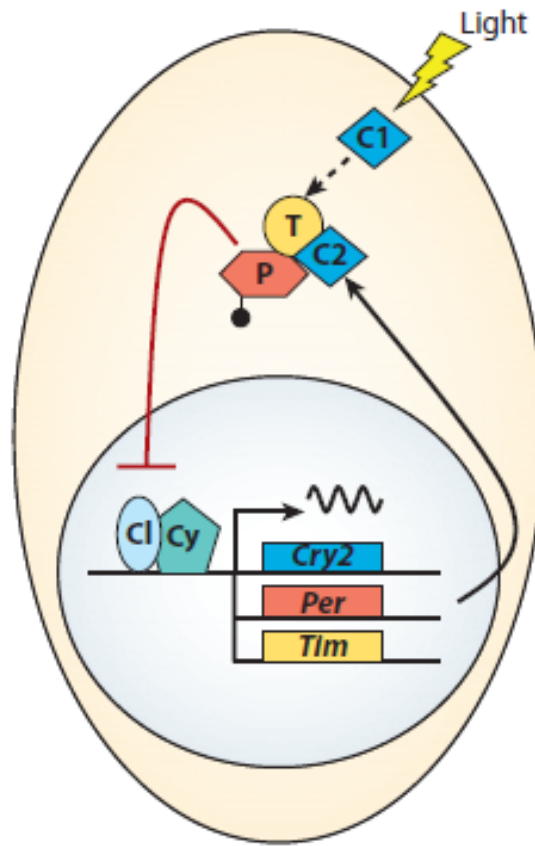
Supports for cryptochrome as the magnetoreceptor



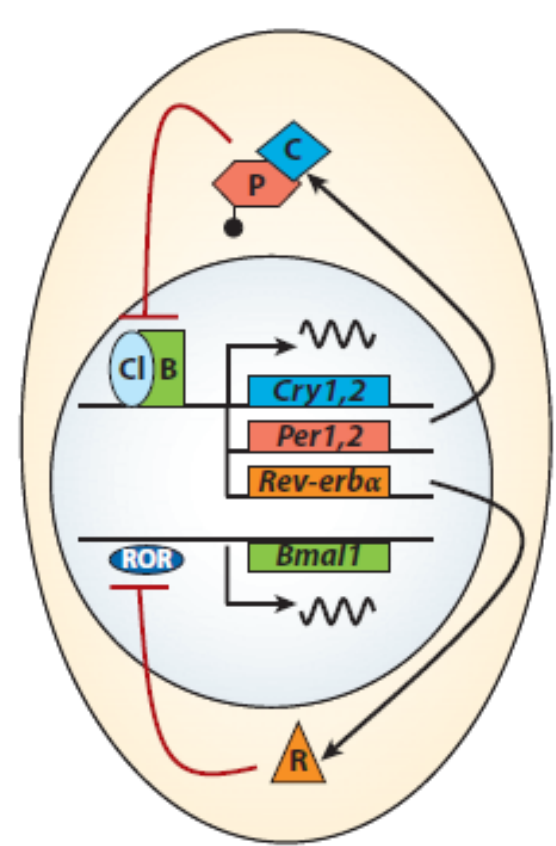
Cryptochrome1 of Arabidopsis Thaliana (PDB ID: 1U3D)
Phys. Today 61(3), 29 (2008); doi: 10.1063/1.2897947




Type I



Type I + II



Type II

- 
- PDB中的10个真核中的cryptochrome中没有鸟类的。所以需要同源模建得到鸟类的蛋白结构。



Taxonomy

- Eukaryota (10)
- Bacteria (2)



Organism

- Arabidopsis thaliana (6)
- Mus musculus (3)
- Homo sapiens (2)
- Drosophila melanogaster (1)

1、序列相似性比对以及 cryptochrome 起源分析



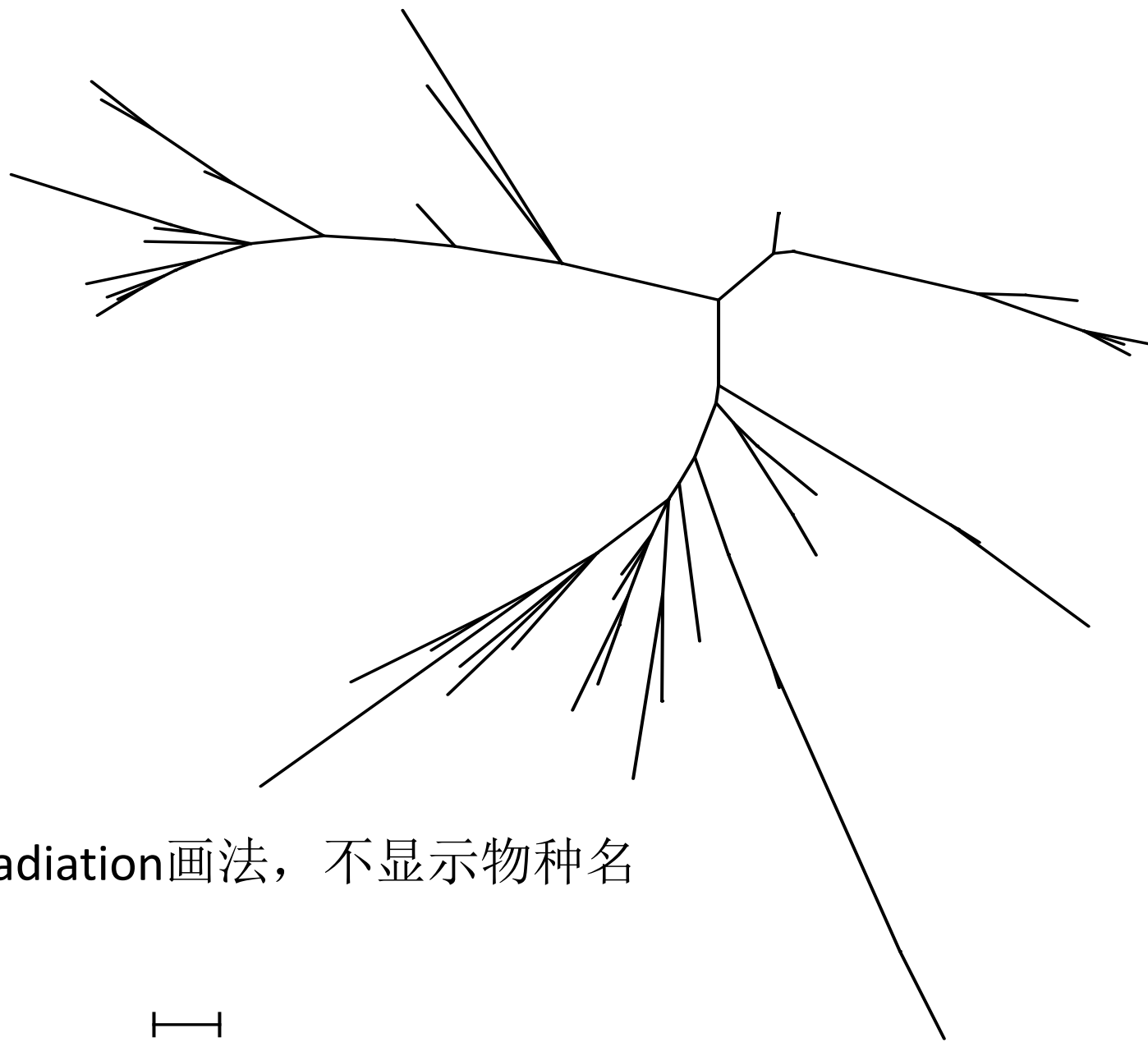
Protein Sequences

Species/Abbrv

1.	gi 118572249 sp Q5IZC5.2 CRY1 ERIRU:4-505	RecName: FullCryptochrome-1
2.	gi 82091801 sp Q6ZZY0.1 CRY1 SYLBO:4-505	RecName: FullCryptochrome-1
3.	gi 82104700 sp Q8QG61.1 CRY1 CHICK:4-505	RecName: FullCryptochrome-1
4.	gi 81882230 sp P97784.1 CRY1 MOUSE:4-505	RecName: FullCryptochrome-1
5.	gi 74735764 sp Q16526.1 CRY1 HUMAN:4-505	RecName: FullCryptochrome-1
6.	gi 75048080 sp Q8WP19.1 CRY1 MACFA:4-505	RecName: FullCryptochrome-1
7.	gi 118572250 sp Q32Q86.1 CRY1 RAT:4-505	RecName: FullCryptochrome-1
8.	gi 81864807 sp Q70AD6.1 CRY1 SPAJD:4-505	RecName: FullCryptochrome-1
9.	gi 81882058 sp Q9R194.1 CRY2 MOUSE:22-523	RecName: FullCryptochrome-2
10.	gi 118572251 sp Q8QG60.2 CRY2 CHICK:14-514	RecName: FullCryptochrome-2
11.	gi 118572252 sp Q49AN0.2 CRY2 HUMAN:23-524	RecName: FullCryptochrome-2
12.	gi 81867613 sp Q923I8.1 CRY2 RAT:22-523	RecName: FullCryptochrome-2
13.	gi 306756349 sp O48652.2 UVR3 ARATH:26-525	RecName: Full(6-4)DNA photolyase AltName: FullP
14.	gi 122106526 sp Q17DK5.1 CRY1 AEDAE:4-517	RecName: FullCryptochrome-1
15.	gi 97048023 sp P77967.2 CRYD SYNY3:7-482	RecName: FullCryptochrome DASH
16.	gi 81834455 sp Q7NMD1.1 CRYD GLOVI:5-492	RecName: FullCryptochrome DASH
17.	gi 97047983 sp Q7UJB1.2 CRYD RHOBA:3-488	RecName: FullCryptochrome DASH
18.	gi 205696380 sp Q7PYI7.4 CRY1 ANOGA:4-517	RecName: FullCryptochrome-1 ShorttagCRY1
19.	gi 97047702 sp Q4KML2.2 CRYD DANRE:6-481	RecName: FullCryptochrome DASH AltName: FullProte
20.	gi 82239825 sp Q75WS4.1 CRYD XENLA:9-500	RecName: FullCryptochrome DASH
21.	gi 205696352 sp Q293P8.2 CRY1 DROPS:7-517	RecName: FullCryptochrome-1
22.	gi 74960862 sp O77059.1 CRY1 DROME:7-517	RecName: FullCryptochrome-1 ShortDmCRY1 Shortdcry
23.	gi 306756329 sp Q0E2Y1.1 UVR3 ORYSJ:16-511	RecName: Full(6-4)DNA photolyase
24.	gi 97047786 sp Q3IPX9.1 CRYD NATPD:3-470	RecName: FullCryptochrome DASH
25.	gi 130152 sp P00914.1 PHR ECOLI:3-470	RecName: FullDeoxyribodipyrimidine photo-lyase AltNa
26.	gi 110825715 sp Q38JU2.2 CRYD SOLLC:80-547	RecName: FullCryptochrome DASH chloroplastic/mit
27.	gi 3913379 sp Q96524.2 CRY2 ARATH:6-497	RecName: FullCryptochrome-2 AltName: FullBlue ligh
28.	gi 2499552 sp Q55081.1 PHR SYNY3:20-484	RecName: FullDeoxyribodipyrimidine photo-lyase Alt
29.	gi 20141633 sp P25078.2 PHR SALTY:3-471	RecName: FullDeoxyribodipyrimidine photo-lyase Alt
30.	gi 118595452 sp P05327.4 PHR SYN6:6-477	RecName: FullDeoxyribodipyrimidine photo-lyase Al
31.	gi 97047673 sp Q84KJ5.2 CRYD ARATH:86-526	RecName: FullCryptochrome DASH chloroplastic/mit
32.	gi 75322263 sp Q651U1.1 CRYD ORYSJ:86-579	RecName: FullCryptochrome DASH chloroplastic/mit
33.	gi 11133758 sp P57386.1 PHR BUCAI:3-479	RecName: FullDeoxyribodipyrimidine photo-lyase Alt
34.	gi 730324 sp P40115.1 CRY1 SINAL:6-498	RecName: FullCryptochrome-1 AltName: FullBlue light
35.	gi 81858027 sp Q9KNA8.1 PHR VIBCH:3-468	RecName: FullDeoxyribodipyrimidine photo-lyase Alt
36.	gi 298286845 sp Q04449.2 PHR BACPE:10-475	RecName: FullDeoxyribodipyrimidine photo-lyase A
37.	gi 130155 sp P27526.1 PHR NEUCR:137-636	RecName: FullDeoxyribodipyrimidine photo-lyase Alt
38.	gi 47117035 sp Q9HQ46.2 PHR HALSA:3-478	RecName: FullDeoxyribodipyrimidine photo-lyase Alt

最大似然度法构树

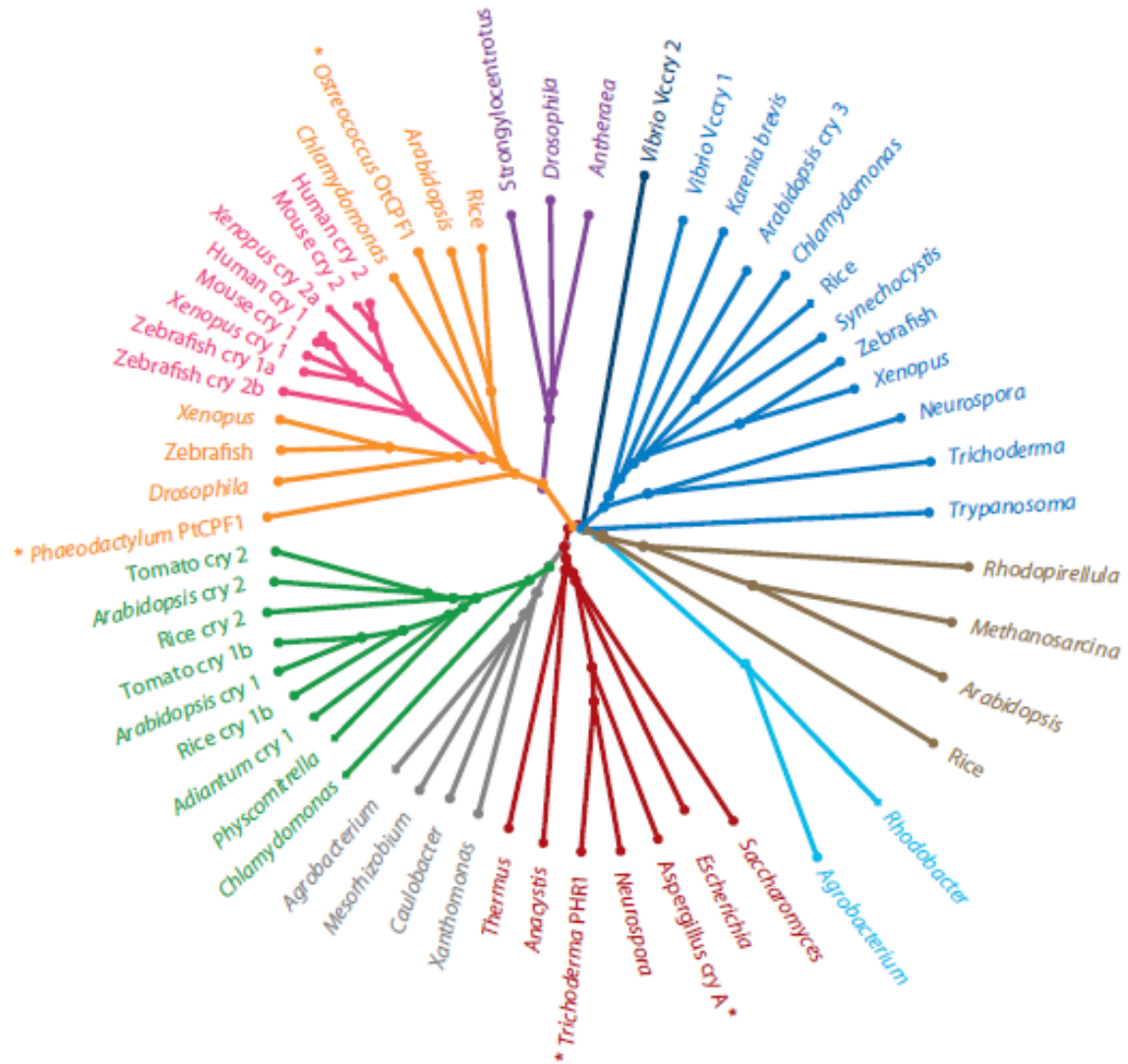




radiation画法，不显示物种名

0.2

根据文献中建
的树可以知道这个
蛋白家族进化上最
早可能分为三支。




Key	
● (Purple)	Animal cryptochrome
● (Blue)	DASH-related cryptochrome
● (Orange)	DASH cryptochrome
● (Brown)	CPD photolase class II
● (Red)	CPD photolase class I
● (Grey)	CPD photolase class III
● (Green)	Plant cryptochrome
● (Light Blue)	Additional bacterial protein
● (Orange with star)	6-4 photolase
★	Dual-type

2、同源模建及模型分析

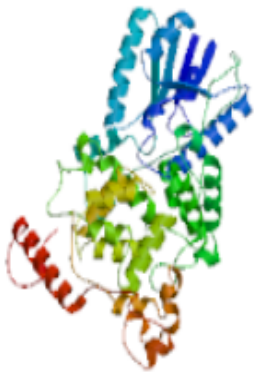
方法一：Swiss-Model

Workunit: P000002 RobinCRY1 - Overview



Print/Save this page as 

Model Summary



Model information:

Modelled residue range: 4 to 505
Based on template: [4i6jA] (2.70 Å)
Sequence Identity [%]: 80.68
Evaluate: 0.00e-1

Quality information: [details]▼

QMEAN Z-Score: -1.4



Quaternary structure information: [details]▼

Template (4i6j): HETERO TRIMER
Model built :SINGLE CHAIN

Ligand information: [details]▼

Ligands in the template: none.
Ligands in the model: none.

logs: [Templates]▼ [Alignment]▼ [Modelling]▼

display model: as [pdb]▼ - as [DeepView project]▼ - in [AstexViewer]▼

download model: as [pdb]⚡ - as [Deepview project]⚡ - as [text]⚡

- 模板:

A ubiquitin ligase-substrate complex

DOI:10.2210/pdb4i6j/pdb

4I6J

- Display Files ▾
- Download Files ▾
- Share this Page ▾

Primary Citation

SCFFBXL3 ubiquitin ligase targets cryptochromes at their cofactor pocket.

Xing, W.¹, Busino, L.¹, Hinds, T.R.¹, Marionni, S.T.¹, Saifee, N.H.¹, Bush, M.F.¹, Pagano, M.¹, Zheng, N.¹

Journal: (2013) Nature 496: 64-68

PubMed: 23503662 [↗](#)

PubMedCentral: PMC3618506 [↗](#)

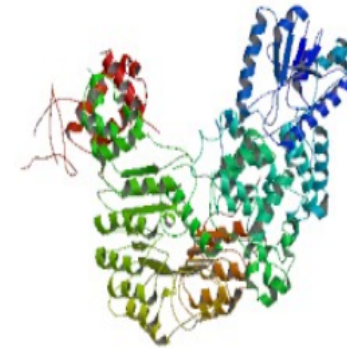
DOI: 10.1038/nature11964 [↗](#)

Search Related Articles in PubMed [↗](#)

PubMed Abstract:

The cryptochrome (CRY) flavoproteins act as blue-light receptors in plants and insects, but perform light-independent functions at the core of the mammalian circadian clock. To drive clock oscillations, mammalian CRYs associate with the Period proteins (PERs) and together inhibit the transcription of their own genes. The SCF(FBXL3) ubiquitin ligase complex controls this negative feedback loop by promoting CRY ubiquitination and degradation. However, the molecular mechanisms of their interactions and the functional role of flavin adenine dinucleotide (FAD) binding in CRYs remain poorly understood. Here we report crystal structures of mammalian CRY2 in its apo-, FAD-bound and FBXL3-SKP1-complexed forms. Distinct from other

Biological Assembly ?



[View in 3D](#)

[More Images...](#)

Biological assembly 1 assigned by authors and generated by PISA (software)

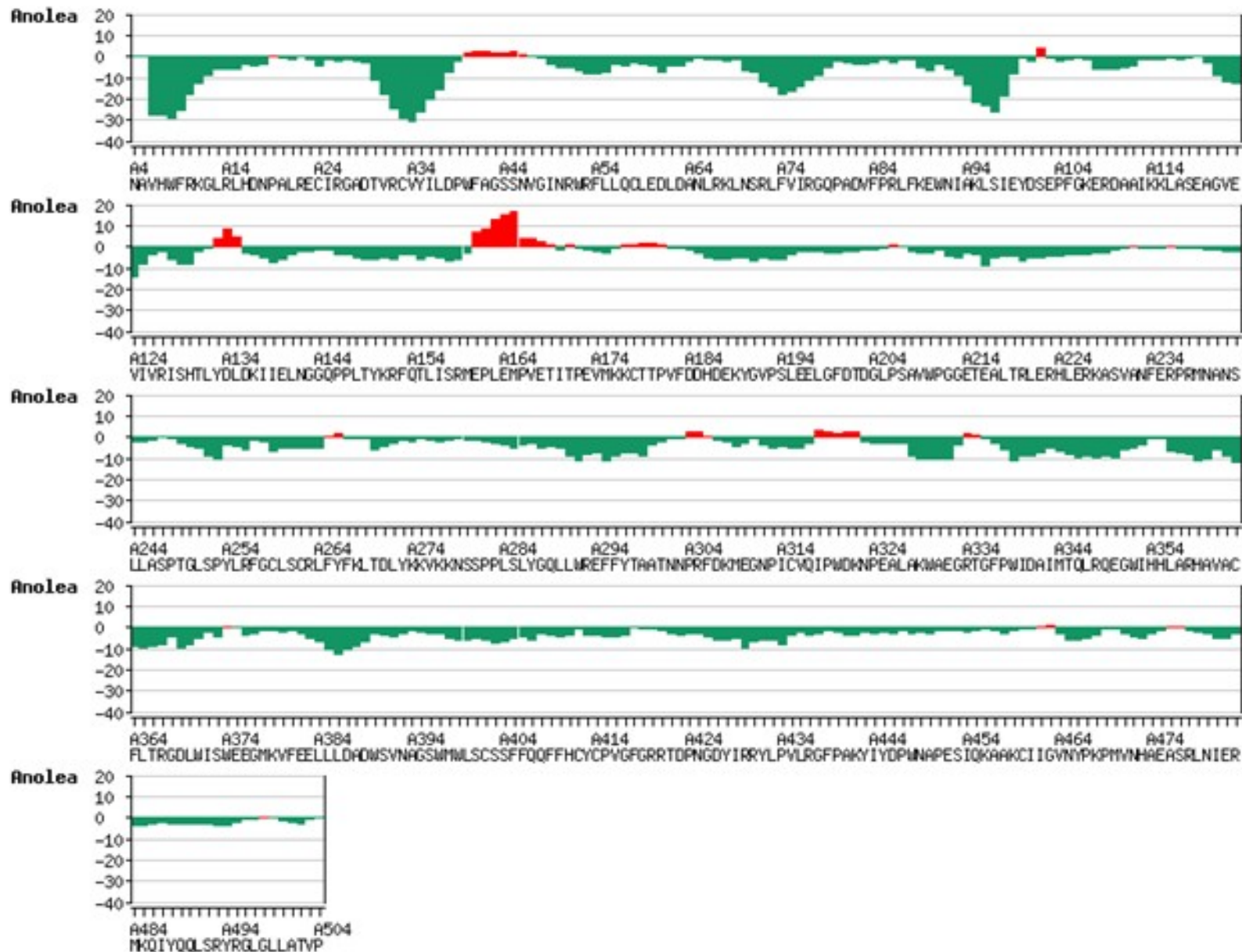
Downloadable viewers:

• 模型评价与分析

TARGET	348	GWIHHLARHA	VACFLTRGDL	WISWEEGMKV	FEELLLDADW	SVNAGSWMWL
4i6jA	369	gwihhlarha	vacfltrgdl	wvswesgvrv	fdelllldadf	svnagswmwl
TARGET		hhhhhh	hhhhh	hhhhhh	hhh	h hhhhhhhhhh
4i6jA		hhhhhh	hhhhh	hhhhhh	hhhh	h hhhhhhhhhh
TARGET	398	SCSSFFQQFF	HCYCPVGFGR	RTDPNGDYIR	RYLPVLRGFP	AKYIYDPWNA
4i6jA	419	scsaffqqff	hcycpvgfgr	rtdspgdyir	rylpklkgfp	sryiyepwna
TARGET		h	hhhh	hh	hhhh	
4i6jA		h	hhhh	hh	hhhh	
TARGET	448	PESIQKAAKC	IIGVNYPKPM	VNHAEASRLN	IERMKQIYQQ	LSRYRGLGLL
4i6jA	469	pesvqkaakc	iigvdyprpi	vnhaetsrln	iermkqiyqq	lsryrglcll
TARGET		hhhhhhh		hhhhhhhh	hhhhhhhhhh	hh h
4i6jA		hhhhhhh		hhhhhhhh	hhhhhhhh	h h
TARGET	498	ATVPS ---				
4i6jA	519	asvpsscved				
TARGET		h				
4i6jA		h				

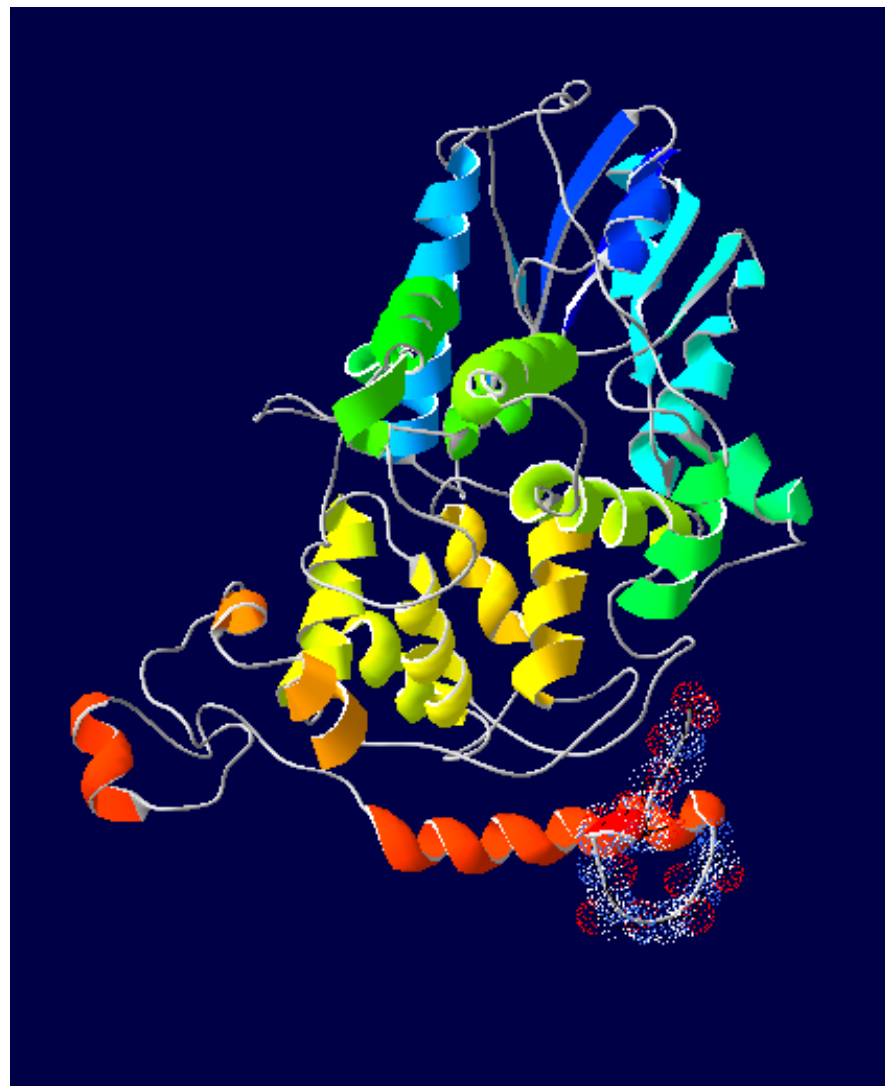
QMEAN4 score为 0.674

通过Anolea计算蛋白链上每个氨基酸的能量

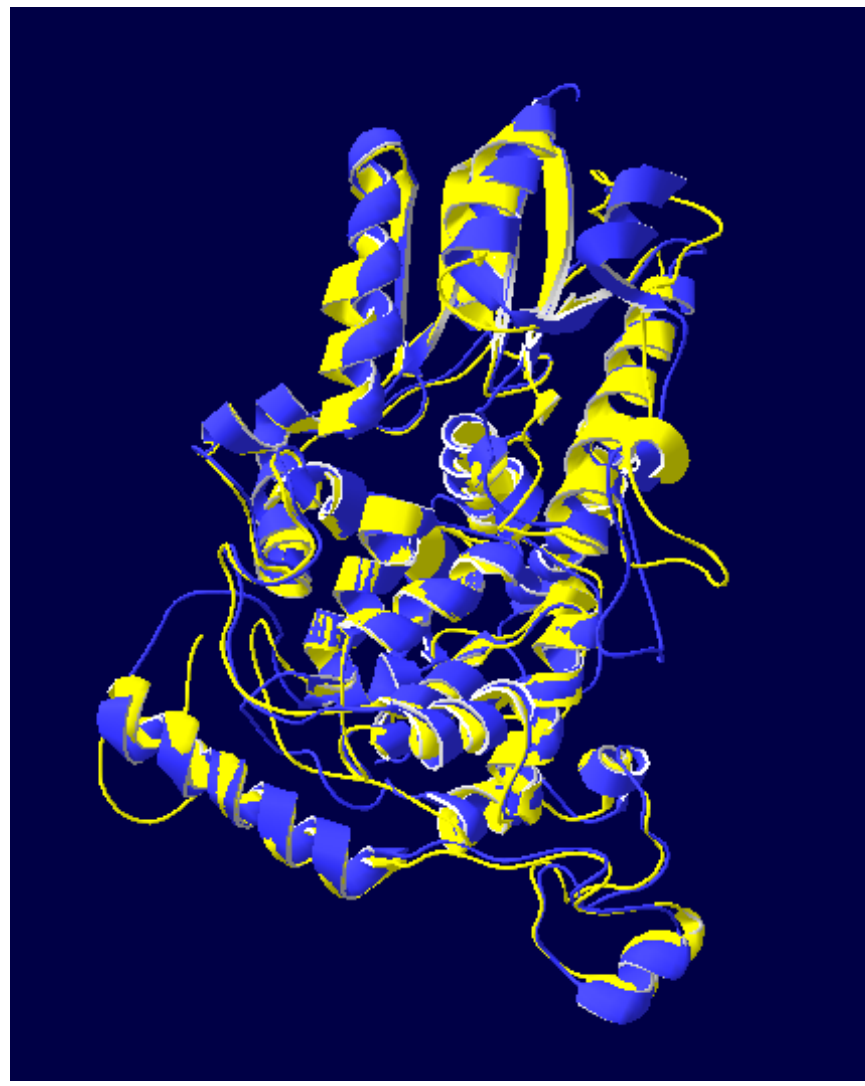




Swiss Model反馈的罗宾鸟CRY1蛋白结构



罗宾鸟CRY1与果蝇CRY1结构比较



方法二: modeller

- 通过序列相似性选模板

Alignments Download GenPept Graphics Distance tree of results Multiple alignment

	Description	Max score	Total score	Query cover	E value	Max ident	Accession
<input type="checkbox"/>	Chain A, A Ubiquitin Ligase-substrate Complex	888	888	81%	0.0	81%	4I6J_A
<input type="checkbox"/>	Chain A, A Vertebrate Cryptochrome >pdb 4I6G A Chain A, A Vertebrate Cryptochrome With Fad >pdb 4I6G B Chain B, A Vertebrate Cryptochrome W	871	871	79%	0.0	81%	4I6E_A
<input type="checkbox"/>	Chain A, Drosophila Melanogaster (6-4) Photolyase Bound To Ds Dna With A T-T (6-4) Photolesion >pdb 3CVY A Chain A, Drosophila Melanogaste	542	542	78%	0.0	53%	3CVU_A
<input type="checkbox"/>	Chain A, Drosophila Melanogaster (6-4) Photolyase H365n Mutant Bound To Ds Dna With A T-T (6-4) Photolesion And Cofactor F0	540	540	78%	0.0	53%	3CVW_A
<input type="checkbox"/>	Chain A, Drosophila Melanogaster (6-4) Photolyase H369m Mutant Bound To Ds Dna With A T-T (6-4) Photolesion	539	539	78%	0.0	53%	3CVX_A
<input type="checkbox"/>	Chain A, (6-4) Photolyase Crystal Structure >pdb 3FY4 B Chain B, (6-4) Photolyase Crystal Structure >pdb 3FY4 C Chain C, (6-4) Photolyase Crysta	512	512	78%	5e-175	50%	3FY4_A
<input type="checkbox"/>	Chain A, Structure Of Full-length Drosophila Cryptochrome >pdb 4GU5 B Chain B, Structure Of Full-length Drosophila Cryptochrome	371	371	79%	3e-120	40%	4GU5_A



Bioinformatics Toolkit

Max-Planck Institute for Developmental Biology

- Search
- Alignment
- Sequence Analysis
- 2ary Structure
- 3ary Structure
- Classification
- Utils

bFit HHfrag Modeller SamCC

Help

Modeller

Input

Paste multiple alignment

```
>sp|Q5IZC5|CRY1_ERIRU Cryptochrome-1 OSErithacus rubecula GNCRY1 PI
-----MGVNAVHWFRKGLRLHDNPALRECIRGADTVR-----CV
>4I6J|A
-----MAAAAVVAATVPAQSMGADGASSVHWFRKGLRLHDNPALLAAVRGARCVR-----CV
>4I6E|A
```

The first sequence in the multiple alignment must be the query sequence, the other sequences are used as templates. In each template the first word in the header has to be the PDB- or SCOP-identifier. [Paste example sequence.](#)

or upload a local file

Select input format

FASTA

Options

Enter own alignment identifier

(must be the identifier from the alignment above!)

upload PDB files

MAX-PLANCK-GESELLSCHAFT

Show results of job:

Show results

Recent jobs:

↕ ↕ ↕

Select all Deselect all

Clear sel. Jobs

Delete sel. Jobs

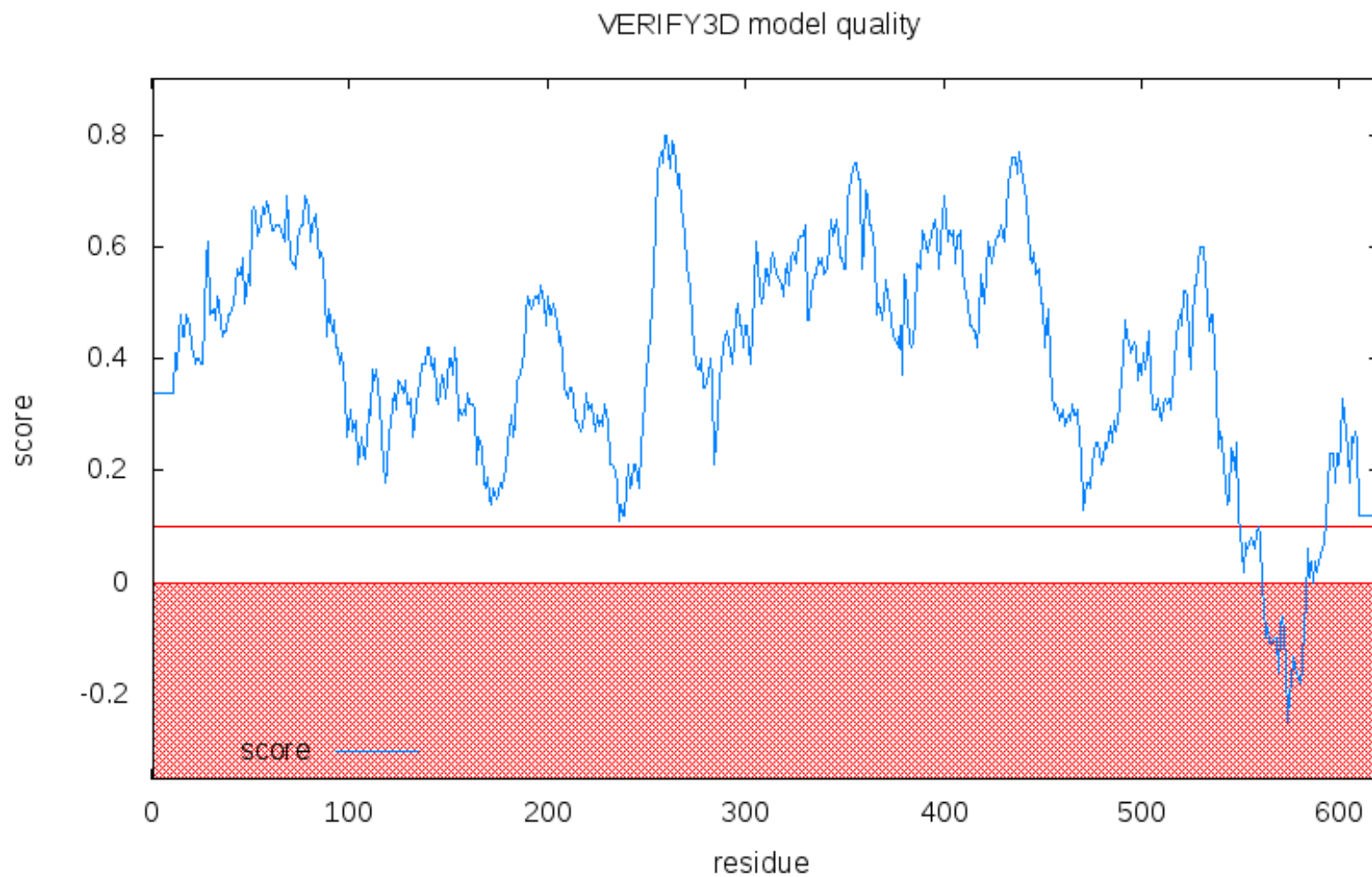
queued

running

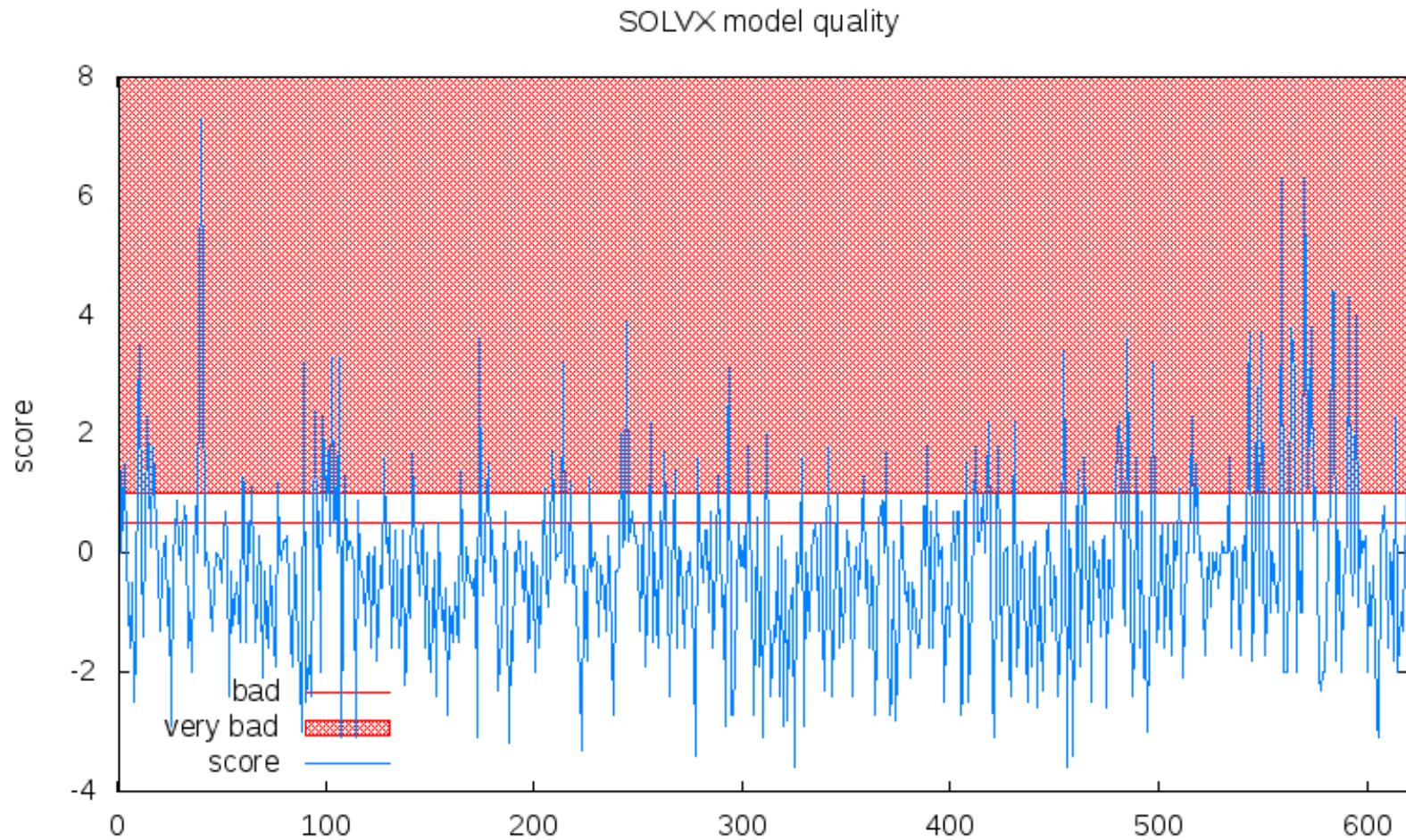
done

error

VERIFY3D-模型评估

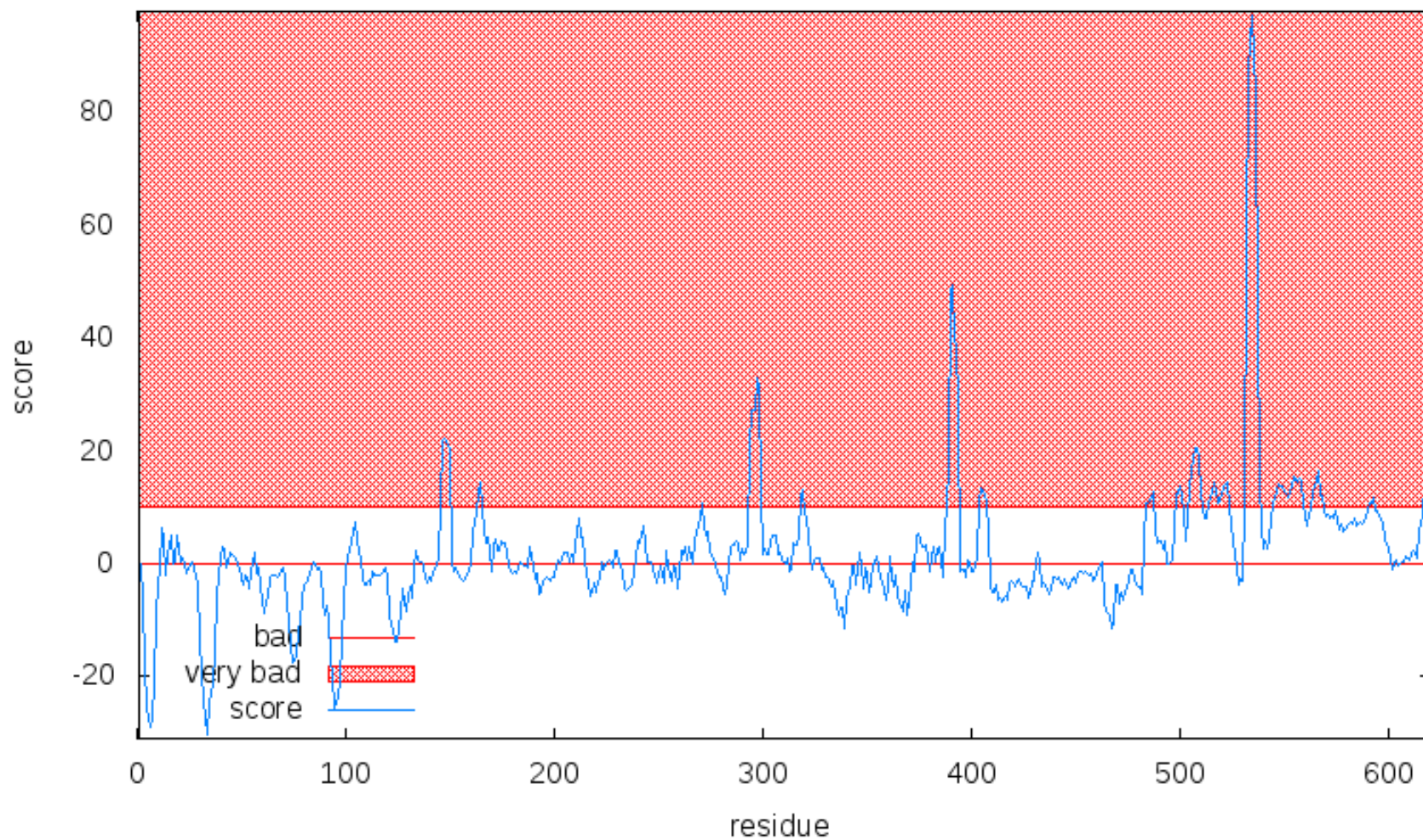


SOLVX-模型评估



ANOLEA-模型评估

ANOLEA model quality

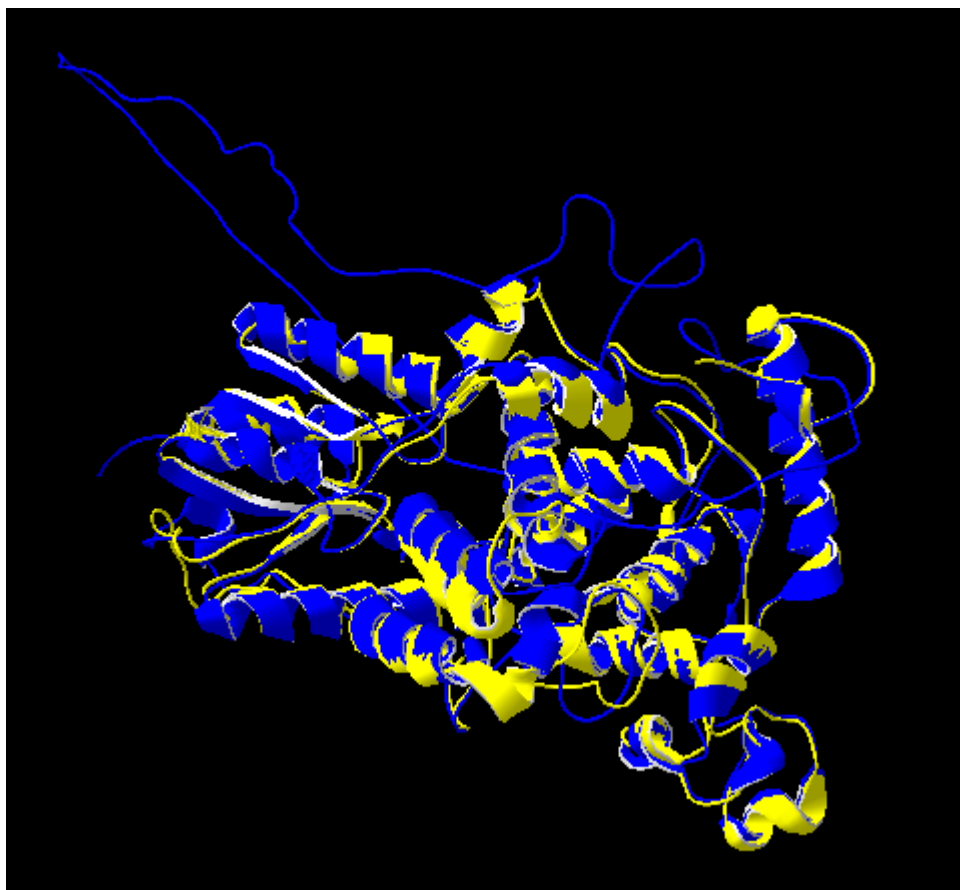




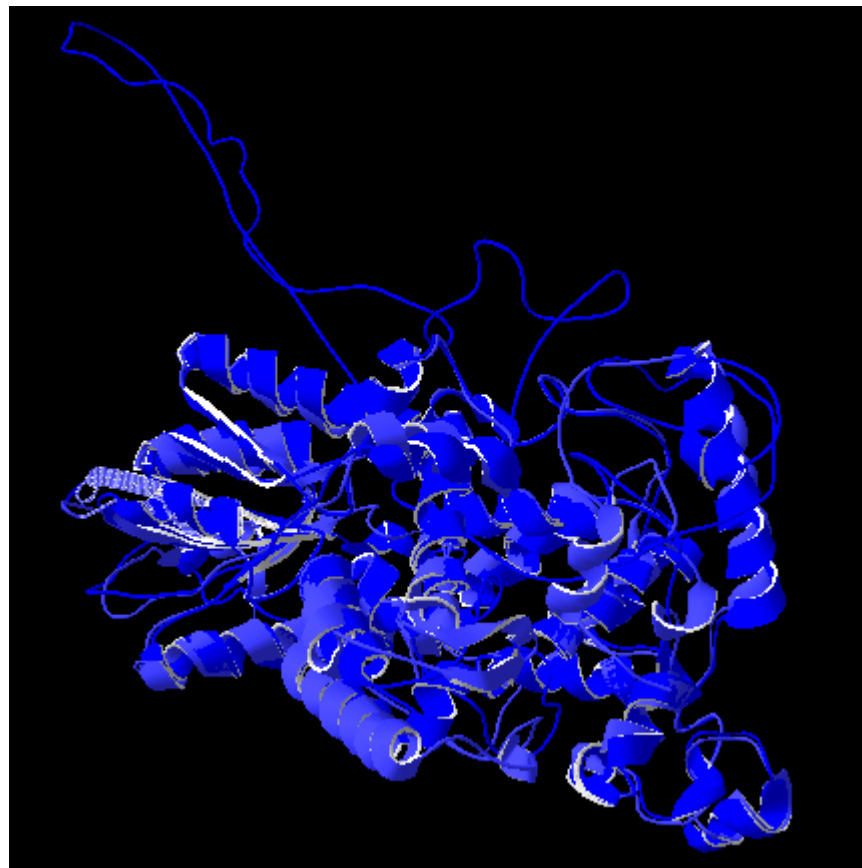
总结

- 前505A.A.建模效果较好
- 最后的505~620A.A.建模效果较差

蛋白质结构比较



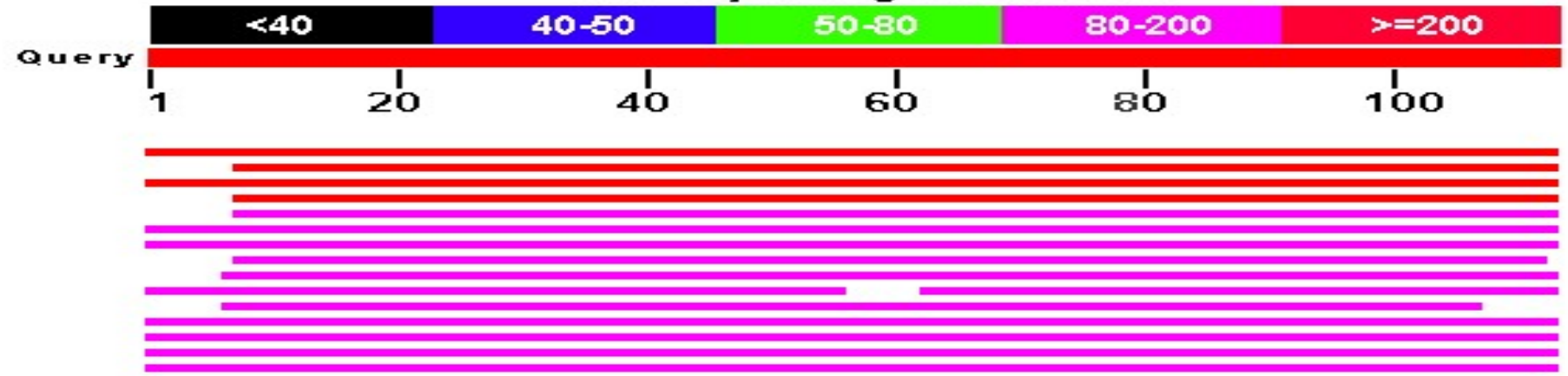
magic fit modller (蓝色) 和Swiss model模建 (黄色) 结果




magic fit modller (深蓝色) 与果蝇中的cryptochrome 4GU5-B (浅蓝)



Color key for alignment scores



	Description	Max score	Total score	Query cover	E value	Max ident
<input type="checkbox"/>	RecName: Full=Cryptochrome-1 >gb AAW48290.1 cryptochrome-1a [Erithacus rubecula]	231	231	100%	4e-70	100%
<input type="checkbox"/>	RecName: Full=Cryptochrome-1 >emb CAG14931.1 cryptochrome 1 [Sylvia borin]	217	217	93%	5e-65	95%
<input type="checkbox"/>	Cryptochrome-1, partial [Anas platyrhynchos]	212	212	100%	2e-63	91%
<input type="checkbox"/>	Cryptochrome-1, partial [Columba livia]	206	206	93%	3e-61	93%
<input type="checkbox"/>	cryptochrome-1 [Gallus gallus] >sp Q8QG61.1 CRY1_CHICK RecName: Full=Cryptochro	192	192	93%	8e-56	85%
<input type="checkbox"/>	cryptochrome 1 (photolyase-like) [Xenopus laevis] >gb AAH72120.1 Cry1-A protein [Xeno	136	136	100%	1e-35	61%
<input type="checkbox"/>	cryptochrome 1 [Xenopus laevis]	136	136	100%	2e-35	61%
<input type="checkbox"/>	cryptochrome 1 [Podarcis siculus]	134	134	92%	7e-35	63%
<input type="checkbox"/>	cryptochrome 1 (photolyase-like) [Xenopus (Silurana) tropicalis] >gb AAI61038.1 Unknow	129	129	94%	1e-32	59%
<input type="checkbox"/>	cryptochrome-1b [Erithacus rubecula]	123	123	49%	8e-31	100%
<input type="checkbox"/>	cryptochrome 1 [Xenopus (Silurana) tropicalis]	120	120	89%	9e-30	59%
<input type="checkbox"/>	cryptochrome-1 [Mus musculus] >sp P97784.1 CRY1_MOUSE RecName: Full=Cryptochr	119	119	100%	4e-29	54%
<input type="checkbox"/>	Cryptochrome-1 [Bos grunniens mutus]	116	116	100%	3e-28	52%

- 
- PDB中的10个真核中的cryptochrome中没有鸟类的



Taxonomy

- Eukaryota (10)
- Bacteria (2)

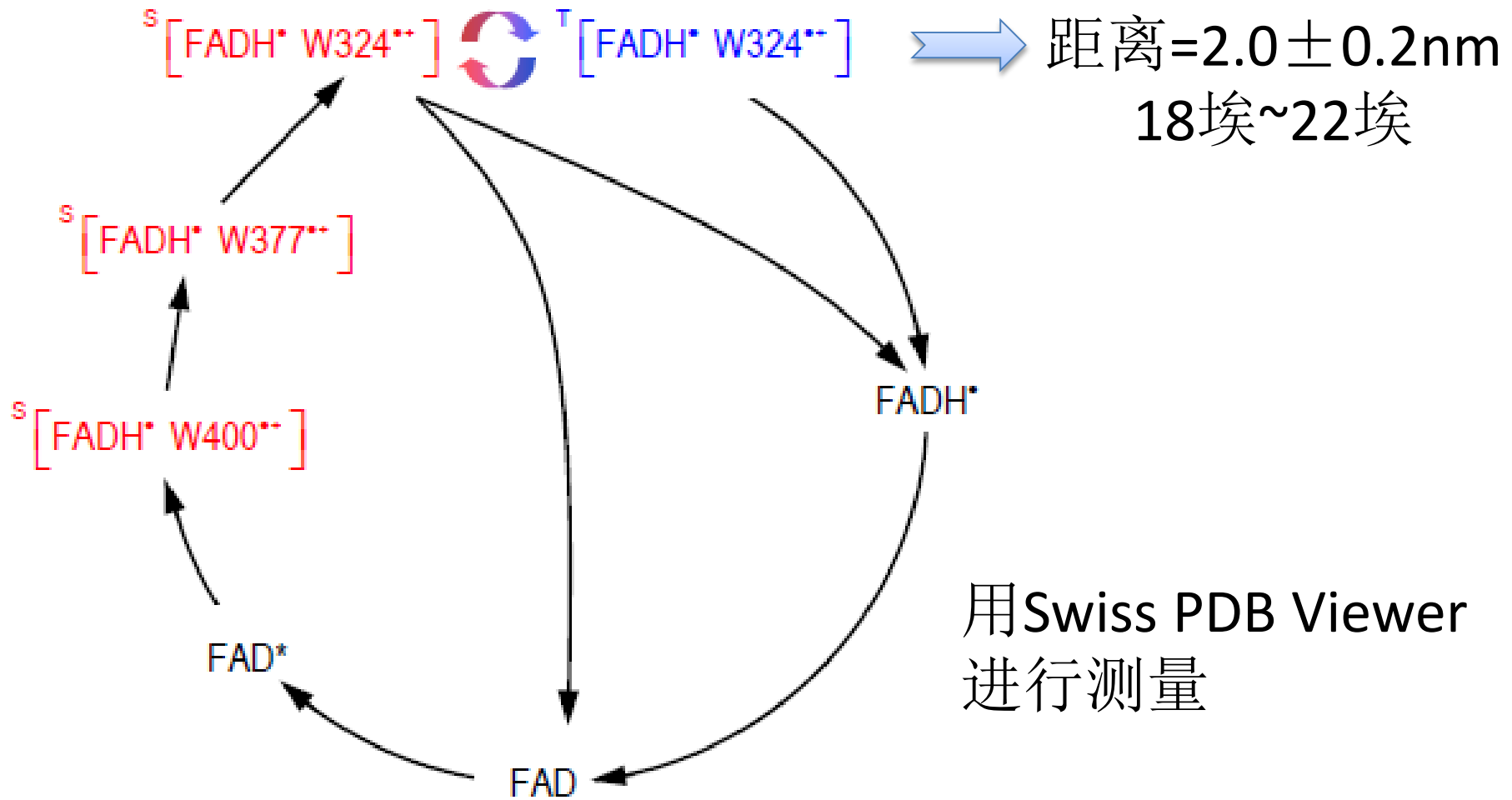


Organism

- *Arabidopsis thaliana* (6)
- *Mus musculus* (3)
- *Homo sapiens* (2)
- *Drosophila melanogaster* (1)

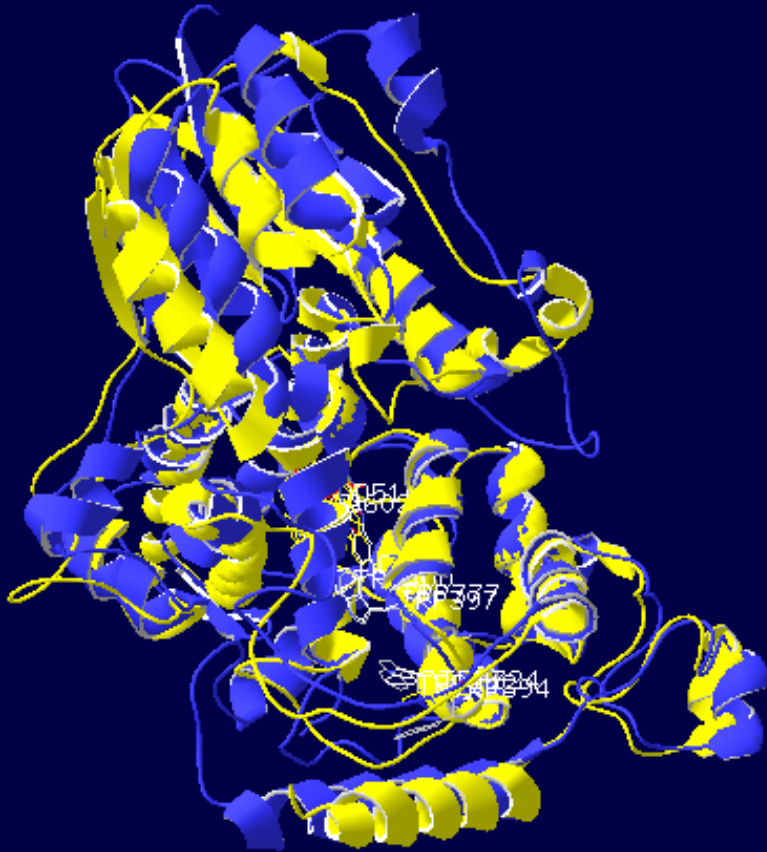
3、测量

根据Radical-Pair 机制的理论计算
要求:



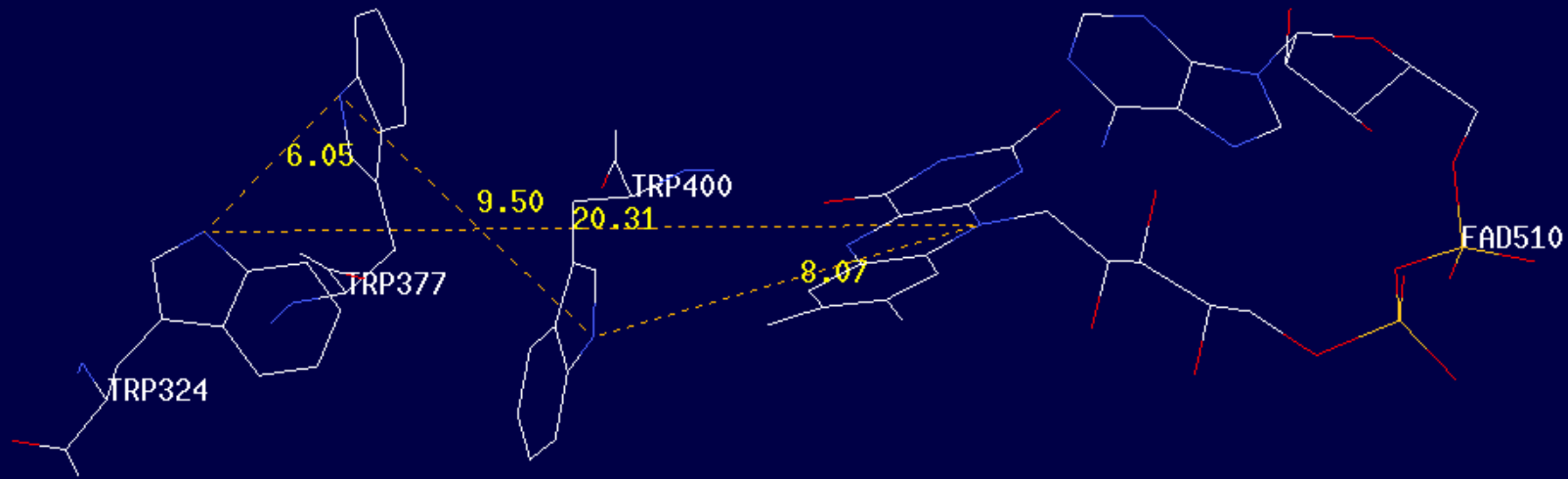
用Swiss PDB Viewer
进行测量

FADH-W(3)

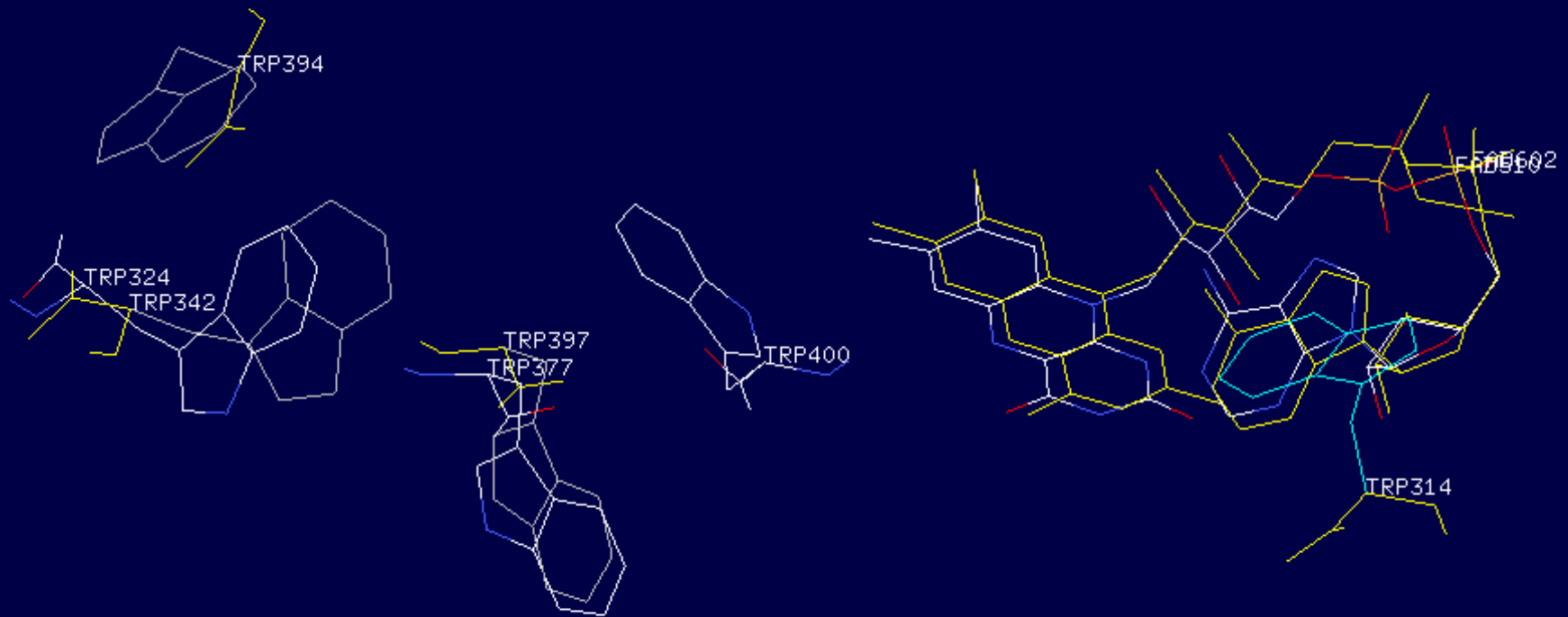


- 文献确定果蝇和拟南芥CRY中三个色氨酸的编号和位置
- Magic fit确定罗宾鸟CRY中色氨酸的编号和位置
- 清除视野，测量距离

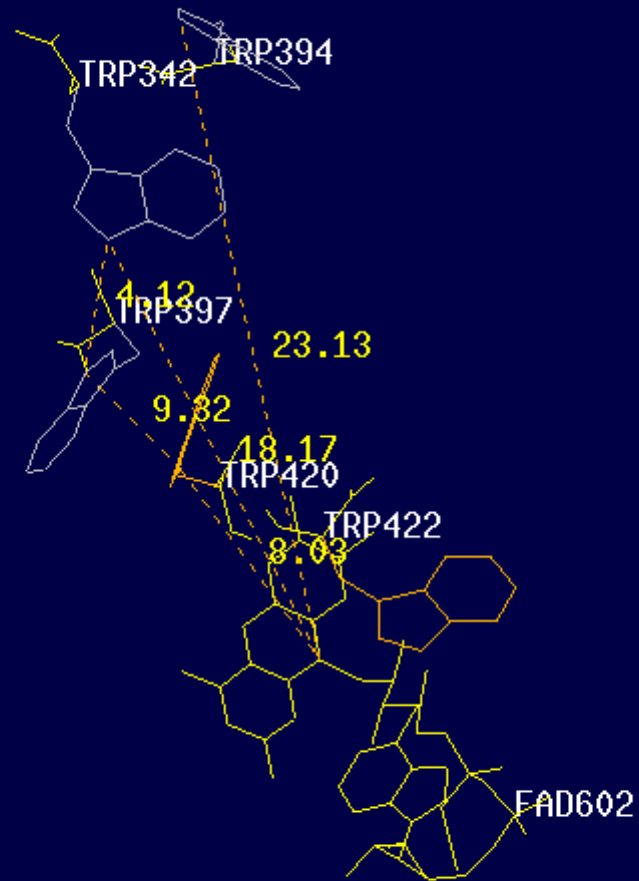
拟南芥CRY1中FAD与三个色氨酸的相对距离



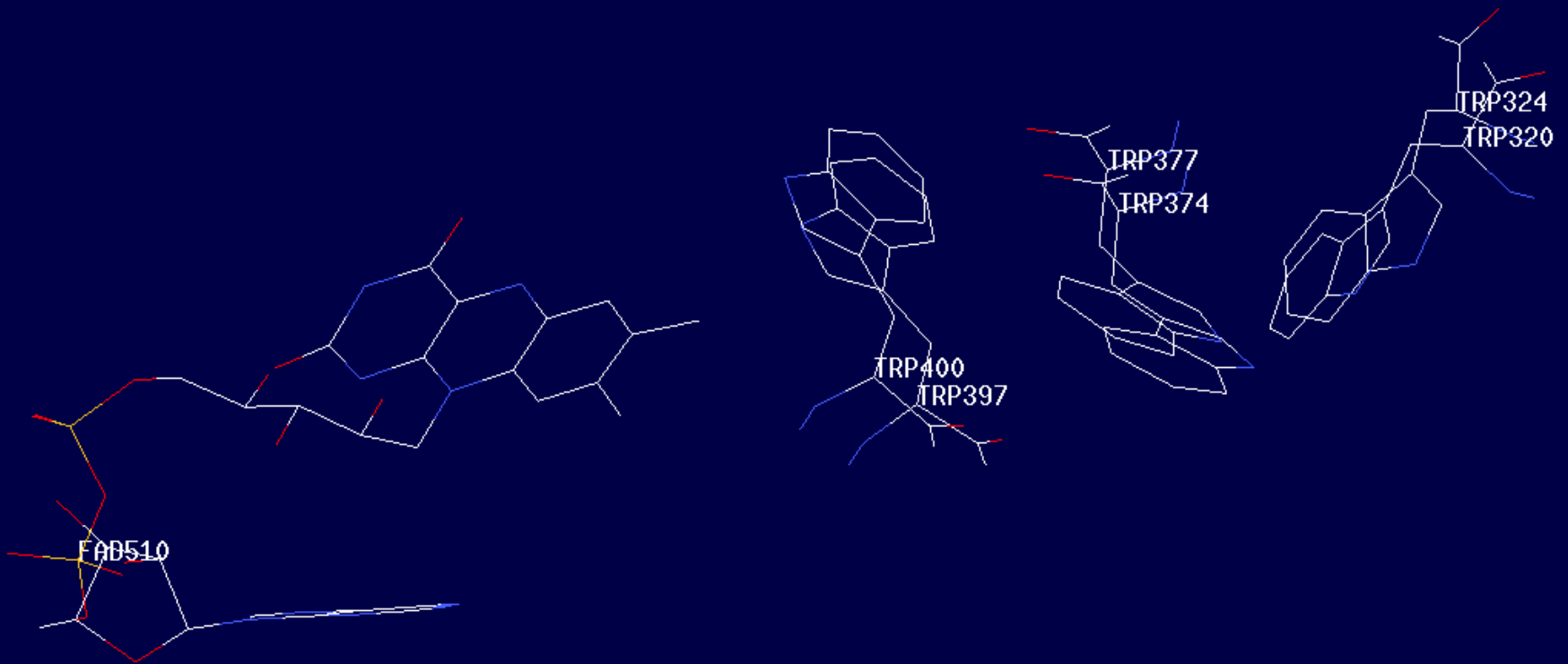
拟南芥和果蝇CRY1中的FAD与三个色氨酸 magic fit之后的相对位置



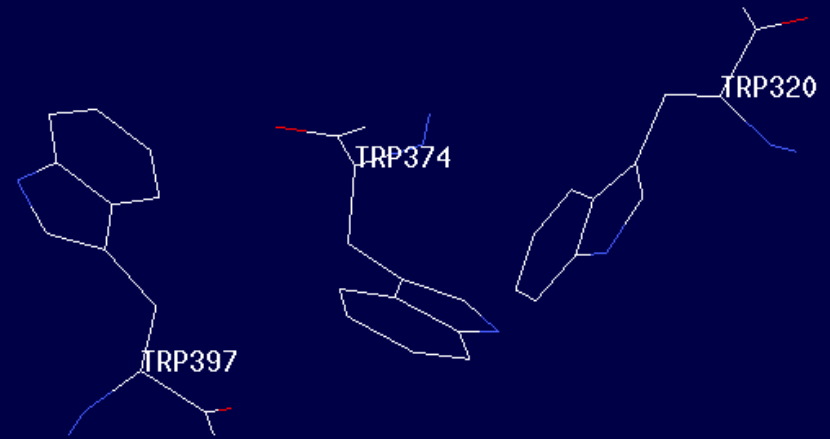
果蝇CRY1中FAD与三个色氨酸的相对距离



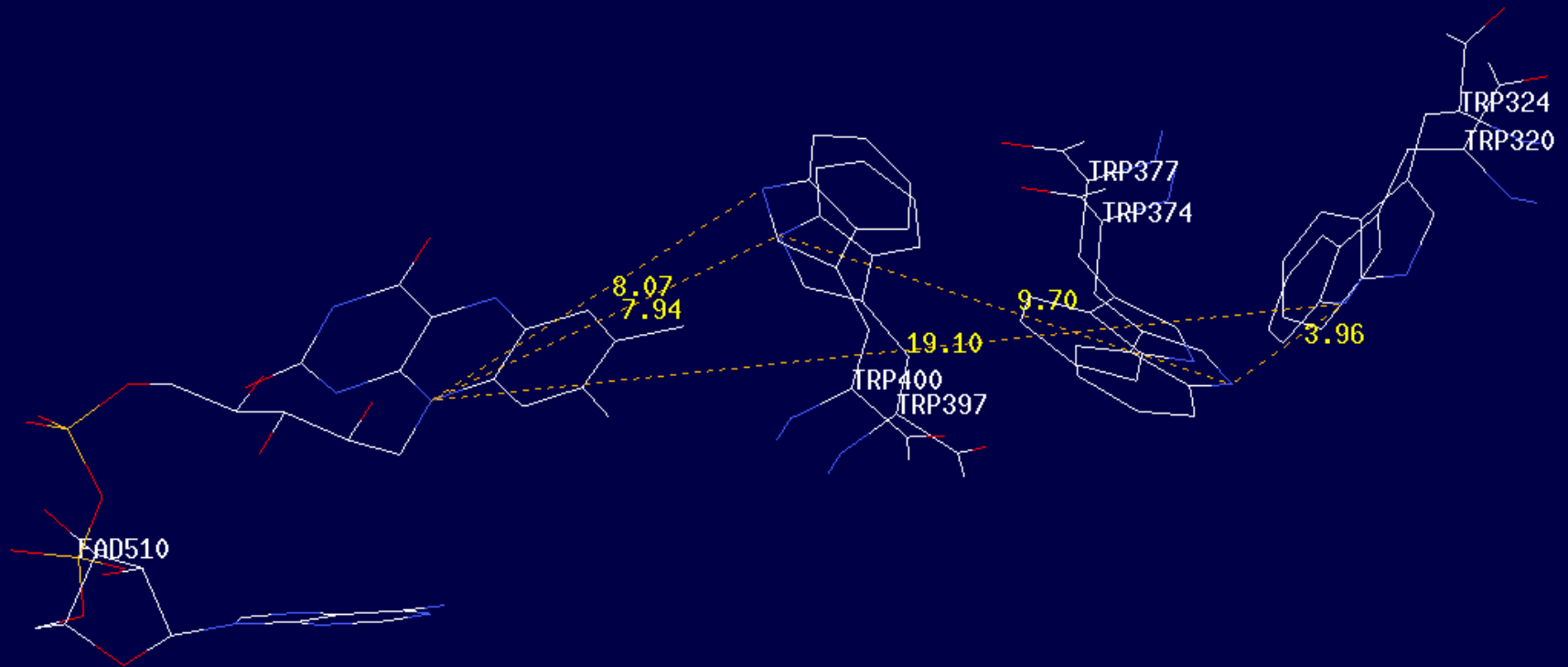
拟南芥和罗宾鸟CRY1中的FAD与三个色氨酸 magic fit之后的相对位置



Swiss Model建模得到的罗宾鸟CRY蛋白没有FAD的信息，所以测量距离时要借助拟南芥的FAD



罗宾鸟CRY1中FAD与三个色氨酸的相对距离



距离基本符合