



# 细胞色素P450 BM3蛋白的序列、结构和功能分析

The analysis of sequence, function and  
structure of cytochrome P450 BM3

**G12**

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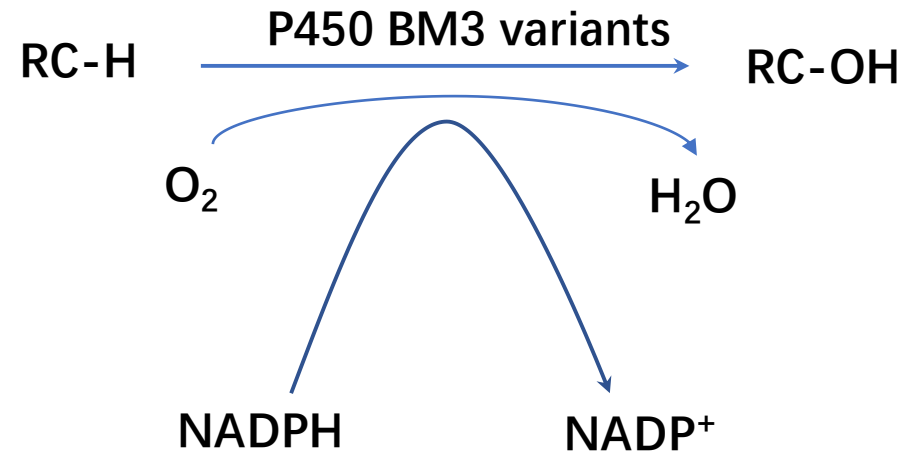
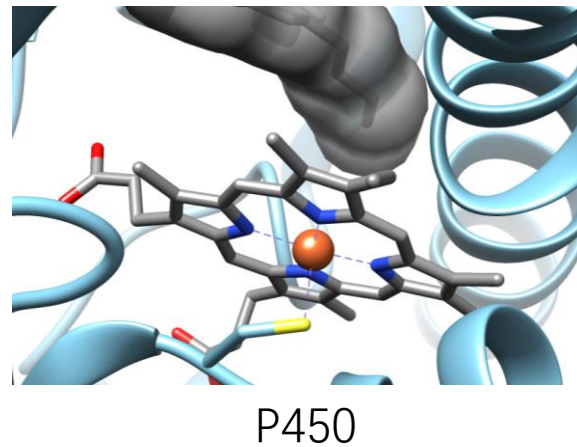
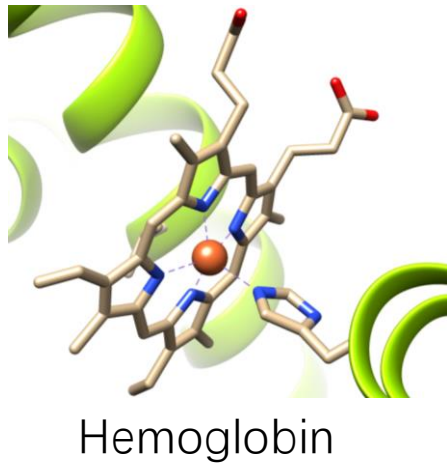
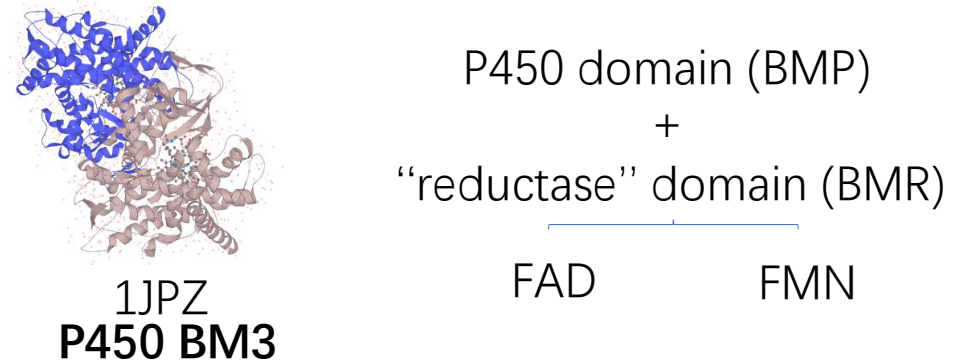
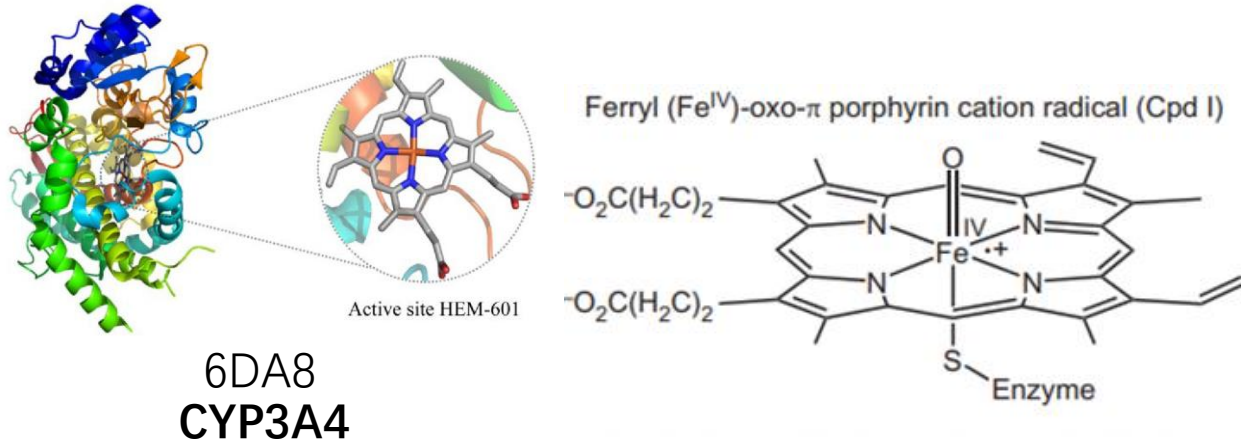
2022.1.16

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# P450单加氧酶背景介绍

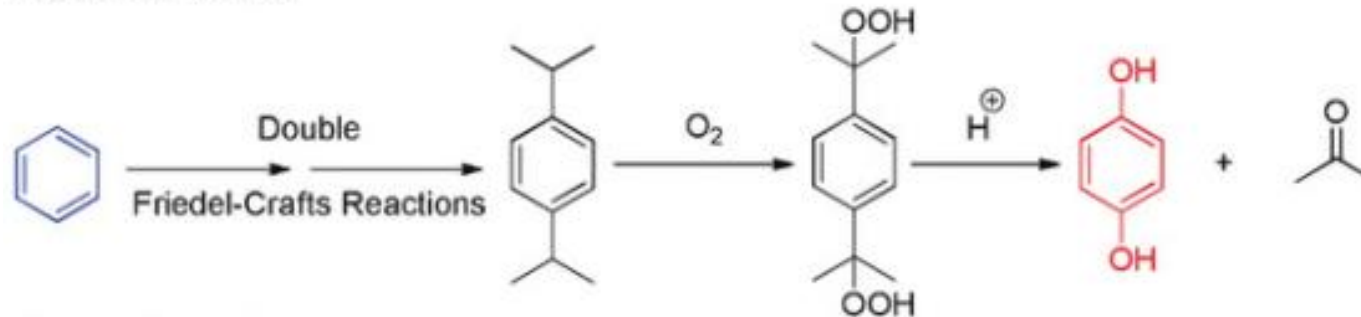


# P450单加氧酶背景介绍



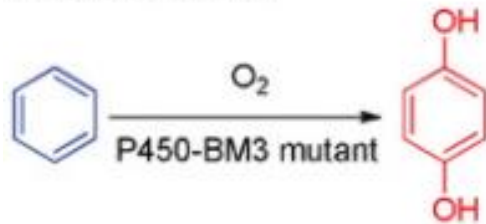
化学合成

Previous work:



酶催化

Present work:



# P450单加氧酶背景介绍

P450BM3

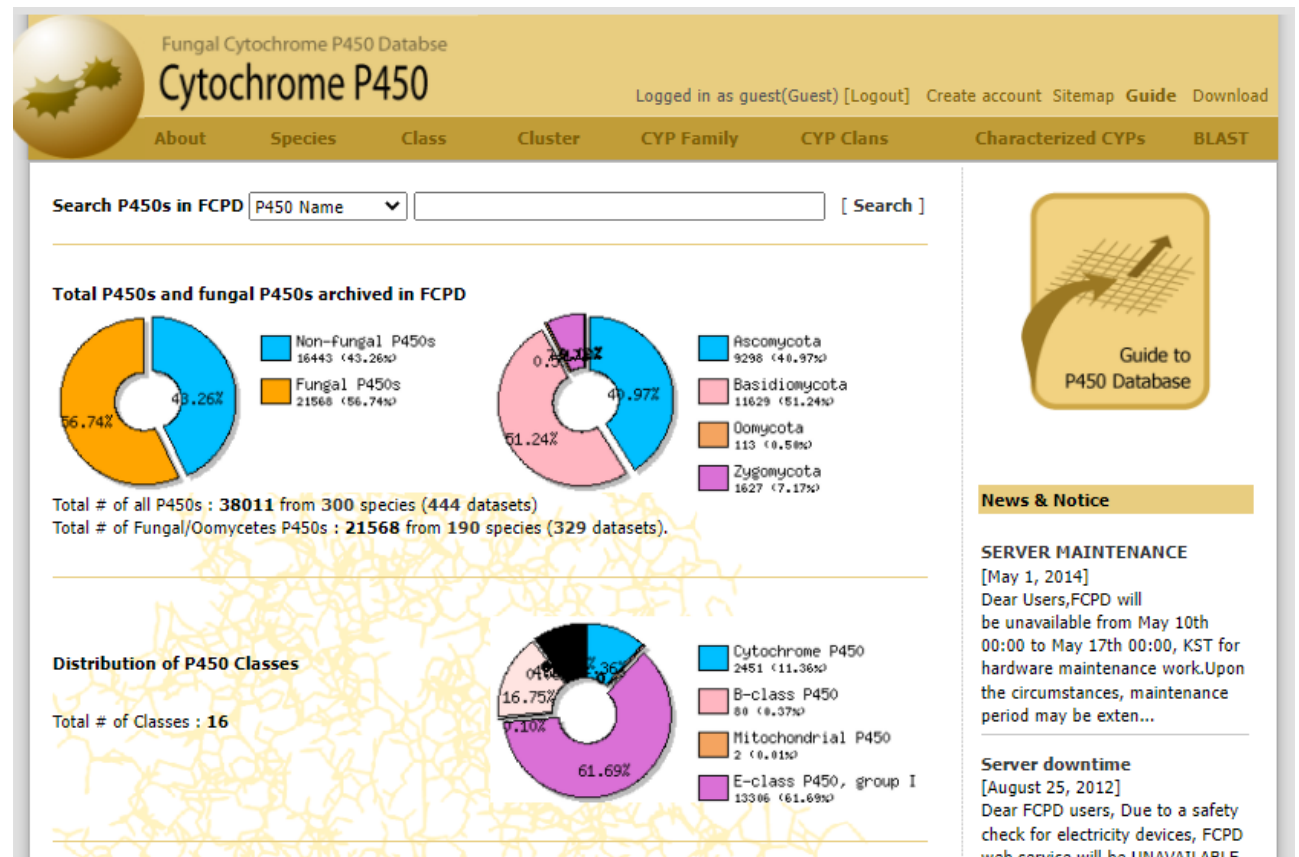
CYP102A1

102 : 家族 (40%以上的相似性)

A : 亚家族 (55%以上的相似性)

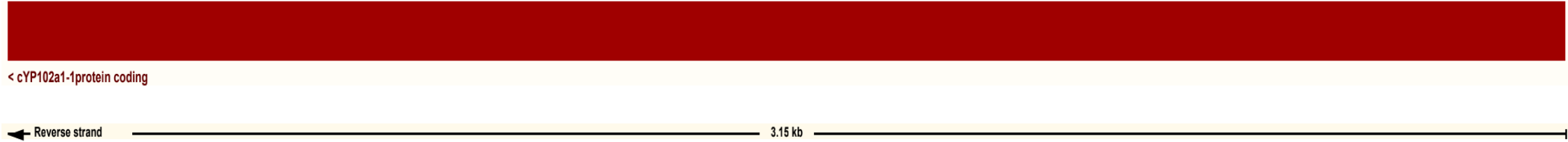
1 : 同工型

<http://p450.riceblast.snu.ac.kr/index.php?a=view>

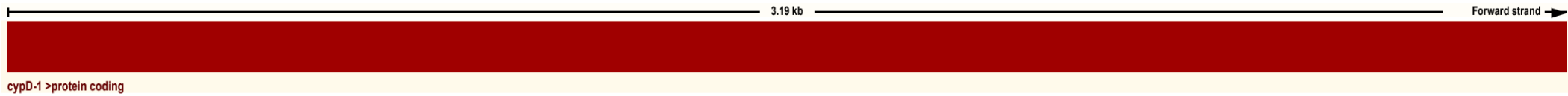


# cyp102A1、cyp102A2、cyp102A3

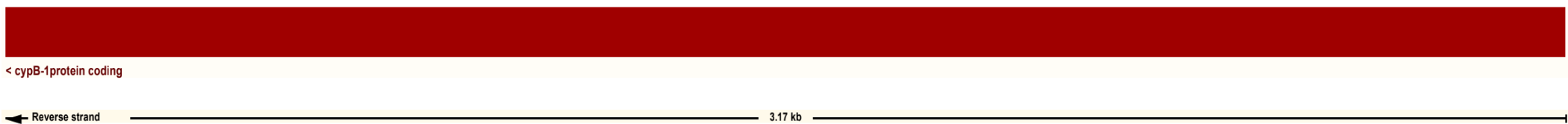
## cyp102A1



## cyp102A2



## cyp102A3



基因名	外显子数	长度
<b>cyp102A1</b>	<b>1</b>	<b>3.15kb</b>
<b>cyp102A2</b>	<b>1</b>	<b>3.19kb</b>
<b>cyp102A3</b>	<b>1</b>	<b>3.17kb</b>

# 多序列比对 (cyp102A1、cyp102A2、cyp102A3)

ClustalW:

```

cyp102A1 → sp|P14779|CPXB_BACMB  -MTIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLI 59
cyp102A2 → sp|008394|CYPD_BACSU  MKETSPQPQPKTFGPLGNLPLIDKDKPTLSLIKLAEEQGPIFQIHTPAGTTIVVSGHEL 60
cyp102A3 → sp|008336|CYPB_BACSU  MKQASAIQPQKTYGPLKNLPHLEKEQLSLSLWRIADELGPFRFDFPGVSSVSVSGHNL 60
      . :*****: * * * * : : : : : * : * : * * * : . * . : : * . : * :
      sp|P14779|CPXB_BACMB  KEACDESRFDKNLSQALKFVRDFAGDGLFTSWTHEKNWKAHNILLPSFSQQAMKGYHAM 119
      sp|008394|CYPD_BACSU  KEVCDEERFDKSIEGALEKVRAFSGDGLFTSWTHEPNWRKAHNILMPTFSQRAMKDYHEK 120
      sp|008336|CYPB_BACSU  AEVCDEKRFDKNLGKGLQKVREFGDGLFTSWTHEPNWQKAHRILLPSFSQKAMKGYHSM 120
      * . * * . * * * . : . * : * * * . * * * * * * * * * * * * * * * * * *
      sp|P14779|CPXB_BACMB  MVDIAVQLVQKWERLNADEHIEVPEDMTRLTLDITGLCGFNRYRFSFYRDQPHPFITSMV 179
      sp|008394|CYPD_BACSU  MVDIAVQLIQKWARLNPNNEAVDVPQDMTRLTLDITGLCGFNRYRFSYRETPHPFINSMV 180
      sp|008336|CYPB_BACSU  MLDIATQLIQKWSRLNPNEEIDVADDMTRLTLDITGLCGFNRYRFSFYRDSQHPFITSM 180
      * : * * * . * * : * * * * * * * * * * * * * * * * * * * * * * * *
      sp|P14779|CPXB_BACMB  RALDEAMNKLQRANPDDPAYDENKRQFQEDIKVMNDLVDKIIADRKASGEQ-SDDLTHM 238
      sp|008394|CYPD_BACSU  RALDEAMHMQRLDVQDKLMVRTKRQFRYDIQTMFSLVDSIIAERRANGDQDEKDLLARM 240
      sp|008336|CYPB_BACSU  RALKEAMNQSKRLGLQDKMMVKTKLQFQKDIQVMSLVDRMIAERKANPDENIKDLLSLM 240
      * * * . * * * : : * . : * * . * * * * * * * * * * * * * * * * * *
      sp|P14779|CPXB_BACMB  LNGKDPETGEPLDDENIRYQIITFLIAGHETTSGLLSFALYFLVKNPHVLQKAAEEAARV 298
      sp|008394|CYPD_BACSU  LNVEDPETGEKLDDEENIRFQIITFLIAGHETTSGLLSFATYFLLKHPDKLKKAYEEVDRV 300
      sp|008336|CYPB_BACSU  LYAKDPVTGETLDDENIRYQIITFLIAGHETTSGLLSFAIYCLLTHPELKKAAQEEADR 300
      * : * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
      sp|P14779|CPXB_BACMB  LVDPVPSYKQVKQLKYVGMVLNEALRLWPTAPAFSLYAKEDTVLGGEYPLEKGDLEMLVI 358
      sp|008394|CYPD_BACSU  LTDAAPTYKQVLELTYIRMI LNESLRLWPTAPAFSLYPKEDTVIGGKFPITNDRISVLI 360
      sp|008336|CYPB_BACSU  LTDDTPEYKQIQQLKYIRMVNLNETLRLYPTAPAFSLYAKEDTVLGGEYPIKGGQPVTVLI 360
      * . * . * * * * : : * . * : * * * * * * * * * * * * * * * * * *
      sp|P14779|CPXB_BACMB  PQLHRDKTIWGDDVEEFRPERFENPSAIPQHAFKPFNGGQRACIGQQFALHEATLVLGMM 418
      sp|008394|CYPD_BACSU  PQLHRDRDAWGKDAEEFRPERFEHQDQVPHHAYKPFNGGQRACIGMQFALHEATLVLGMI 420
      sp|008336|CYPB_BACSU  PKLHRDQNAWGPDAEDFRPERFEDPSSIPHHAYKPFNGGQRACIGMQFALQEAATMVLGLV 420
      * : * * * * : * * * . * . * : * * * * * * * * * * * * * * * * *
      sp|P14779|CPXB_BACMB  LKHDFDFEDHTNYELDIKETLTLKPEGFVVKAKSKKIPLGGIPSPSTE-----QSACK 470
      sp|008394|CYPD_BACSU  LKYFTLIDHENYELDIKQTLTLKPGDFHISVQSRHQEAIHADVQAAEKAAPDEQKEKTEA 480
      sp|008336|CYPB_BACSU  LKHFEINHTGYELKIKEALTIKPDFKITVVKPRKTAAINVQRKEQADI-----KAET 473
      * * * * : * * . * * * . * * * * * * * * * * * * * * * * * *
  
```

三条序列在1-475位点之间均含有细胞色素P450家族的高度保守氧化结构域

# 多序列比对 (cyp102A1、cyp102A2、cyp102A3)

ClustalW:

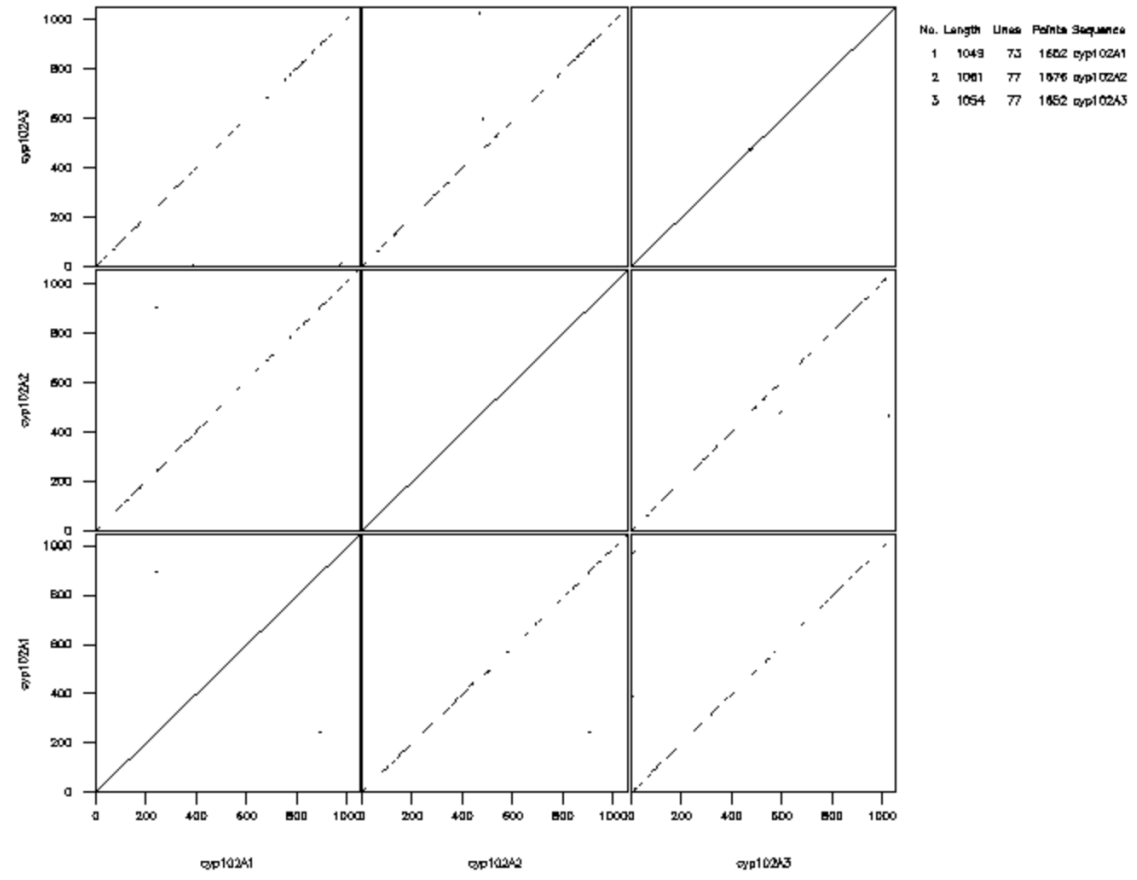
cyp102A1	→	sp P14779 CPXB_BACMB	VRKKAENAHNTPLLVLVYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSHAGNLPREGAV	530
cyp102A2	→	sp 008394 CYPD_BACSU	KGASVIGLNNRPLLVLVYGSDDTGTAEVARELADTASLHGVRTKTAPLNDRIKLPKEGAV	540
cyp102A3	→	sp 008336 CYPB_BACSU	KPKETKPKHGTPLLVLFGSNLGTAEGIAGELAAQGRQMGFTAETAPLDDYIGKLP EEGAV	533
			.. :. *****: ** : ***** * : ** . * . : * * : . * : * : * : * : * : * : *	
		sp P14779 CPXB_BACMB	LIVTASYNGHPPDNAKQFVDWLDQASADEVKGVRYSVFGCGDKNWATTYQKVP AFIDE TL	590
		sp 008394 CYPD_BACSU	VIVTSSYNGKPPSNAGQFVQWLQEIKPGELEGVHYAVFGCGDHNWASTYQYVPRFIDEQL	600
		sp 008336 CYPB_BACSU	VIVTASYNGAPPDAAAGFVWELKELEEGLKGVSYAVFGCGNRSWASTYQRI PR LID DMM	593
			: * * : * * * : * * . * * * : * * * : * * * : * * * : * * * : * * * : * * * : *	
		sp P14779 CPXB_BACMB	AAKGAENIADRGEADSDDFEGTYEEWREHMSDVAAYFNLDIE-NSEDNKSTLSLQFVD	649
		sp 008394 CYPD_BACSU	AEKGATRF SARGEGDVSGDFEGQLDEWKKSMWADA IKA FGL ELNENADKERSTLSLQFVR	660
		sp 008336 CYPB_BACSU	KAKGASRLTAIGEGDAADD FESHRESWENRFWKETMDAFDINEIA-QKEDRPSLSITFLS	652
			* * * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *	
		sp P14779 CPXB_BACMB	SAADMPLAKMHGAFSTNVVASKELQPGSARSTRHLEIELPKEASYQEGDHLGVIPRNYE	709
		sp 008394 CYPD_BACSU	GLGESPLARSYEASHASIAENRELQSADSDRSTRHIEIALPPDVEYQEGDHLGVLPKNSQ	720
		sp 008336 CYPB_BACSU	EATETPVAKAYGAFEGIVLENRELQTA ASTRSTRHIEIIPAGKTYKGDHIGILPKNSR	712
			: * * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *	
		sp P14779 CPXB_BACMB	GIVNRVTARFGLDASQQIRLEAE E E KLAHLPLAKTVSVEELLQ-YVELQDPVTRTQLRAM	768
		sp 008394 CYPD_BACSU	TNVSRI LHRFGLKGT DQVTLSASGRSAGHLPLGRPVSLHDLLSYSEVQEAA TRAQ IREL	780
		sp 008336 CYPB_BACSU	ELVQRVLSRFGLQSNHVIVKVS GS-AHMAHLPMDRP I KVV D L L S S Y V E L Q E P A S R L Q R E L	771
			* * : * * * . . . . : : . . . . * * * : * : * : * * . * * * : * * * * : *	
		sp P14779 CPXB_BACMB	AAKTVCPPHKVELEALL-EKQAYKEQVLAKRLTMLLELEKYPACEMKFSEFIALLPSIRP	827
		sp 008394 CYPD_BACSU	ASFTVCPPHRRELEEL-SAEGVYQEILKKRISMLDLEKYEACDMPFERFLELLRPLKP	839
		sp 008336 CYPB_BACSU	ASYTVCPPHQKELEQLVSDDGTYKEQVLAKRLTMDLFLEDYPACEMPFERFLALLPSLKP	831
			* : * * * * : * * * * . * * * * * * : * * * * : * * * * * * * * * * : * * * * *	
		sp P14779 CPXB_BACMB	RYYSISSSPRVDEKQASITVSVVSGEAWSGYGEYKGIASNYLAELQEGDTITCFISTPQS	887
		sp 008394 CYPD_BACSU	RYYSISSSPRVNPRQASITVGVVRGPAWSGRGEYRGVASNDLAERQAGDDVVMFIRTPES	899
		sp 008336 CYPB_BACSU	RYYSISSSPKVHANIVSMTVGVVKASAWSGRGEYRGVASNLYLAELNTGDAACFIRTPQS	891
			* * * * * * * * : * . * * * * * . * * * * * * : * * * * * * * * : * * * * *	
		sp P14779 CPXB_BACMB	EFTLPKDPETPLIMVGP GTGVAPFRGFVQARKQLKEQGQSLGEAHL YFGCRSPHEDYLYQ	947
		sp 008394 CYPD_BACSU	RFQLKDPETPIIMVGP GTGVAPFRGF LQARDVLKREGKTLGEAHL YFGCRN-DRDFIYR	958
		sp 008336 CYPB_BACSU	GFQMPNDPETPMIMVGP GTGIAPFRGFIQARSVLKKEGSTLGEALLYFGCRRPDHDDL YR	951
			* : * : * * * * : * * * * * * : * :	
		sp P14779 CPXB_BACMB	EELNAQSEGIITLHTAFSRMPNPKTYVQHVMEQDGKKLIELLDQGAHFYICGDGSQMA	1007
		sp 008394 CYPD_BACSU	DELERFEKDGIVTVHTAFSRKEGMPKTYVQHLMDAQADTLISILDRGGRLYVCGDGSQMA	1018
		sp 008336 CYPB_BACSU	EELDQAEQDGLVTIRRCYSRVENEPKGYVQHLLKQDTQKLMTLIEKGAHIYVCGDGSQMA	1011
			: * * : . : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *	
		sp P14779 CPXB_BACMB	PAVEATLMKSYADVHQVSEADARLWLQOLEEKGRYAKDVWAG-	1049
		sp 008394 CYPD_BACSU	PDVEAALQKAYQAVHGTGEQEAQNWLRLHLDQTMGYAKDVWAGI	1061
		sp 008336 CYPB_BACSU	PDVERTLRLAYEAEKAASQEEASAVWLQKLQDQRRYVKDVWVTGM	1054
			* * * * : * * * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *	

三条序列在475-1061位点之间均含有细胞色素P450家族的高度保守还原结构域



# 多序列比对 (cyp102A1、cyp102A2、cyp102A3)

Poly dotplot of /var/lib/emboss-explorer/output/445642/...  
Thu 13 Jan 2022 14:40:38

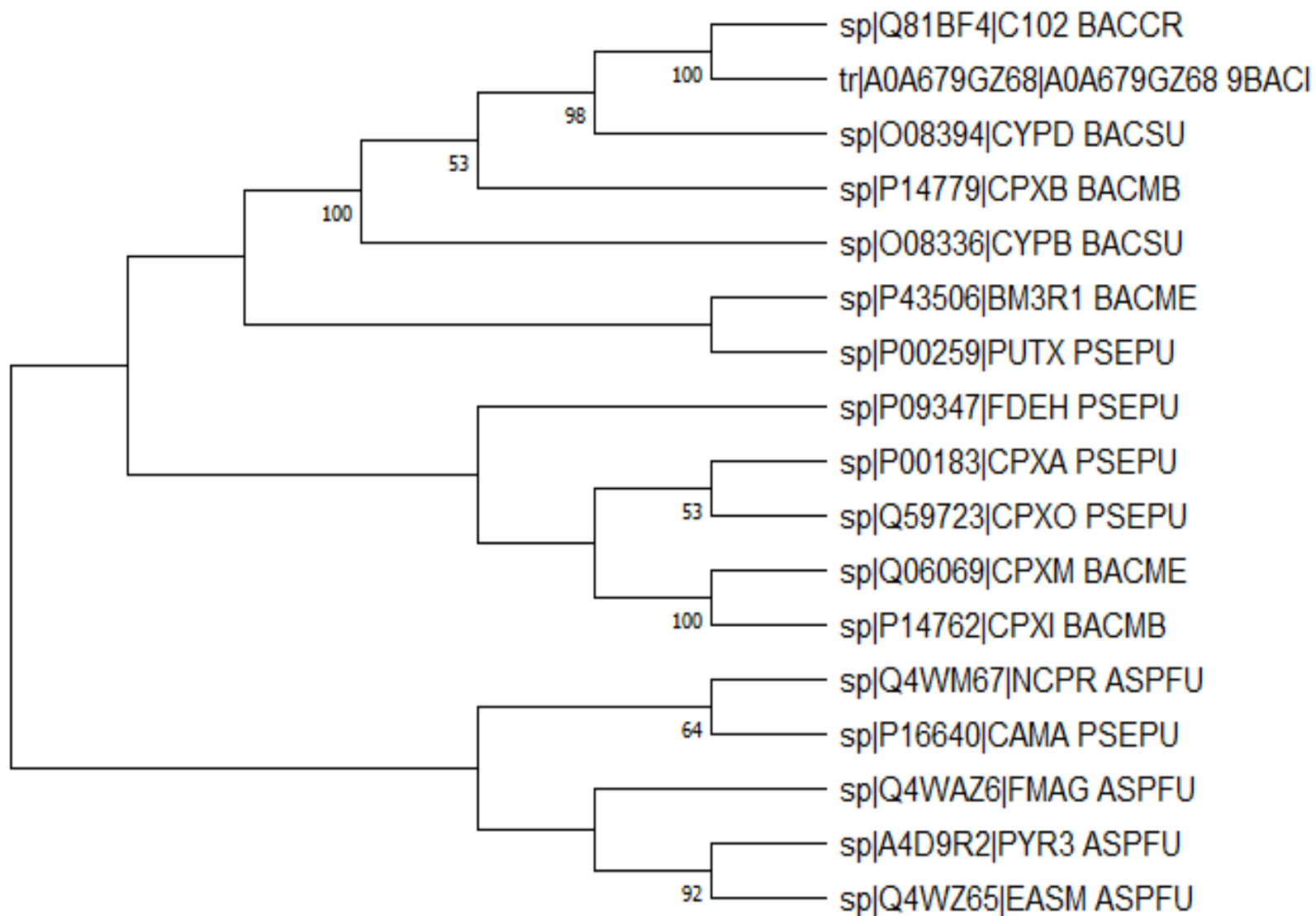


三条序列均包含氧化结构域与还原结构域

# 蛋白序列氨基酸残基的分布

AA		A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
SwissProt*	%	7.9	1.5	5.3	6.7	4	6.9	2.3	5.9	5.9	9.7	2.4	4.1	4.9	4	5.4	6.9	5.4	6.7	1.1	3
cyp102A1	%	9.2	0.9	6.1	8.7	4.1	6	2.7	4.2	6.7	9.8	2.7	3.4	5.3	5.2	4.2	5.6	4.9	5.8	1.1	3.3
cyp102A2	%	7.9	0.7	6.5	7.9	3.9	7.1	3.1	5.1	5.3	9.5	2.1	2.8	5.2	4.7	6.6	5.5	5.4	5.8	1.1	5
cyp102A3	%	7.9	1	5.6	7.8	3.7	6.6	2.3	5.2	6.7	10	2.8	3	5.4	4.9	5.5	5.8	5.3	5.2	1.1	3.3

# P450进化分析



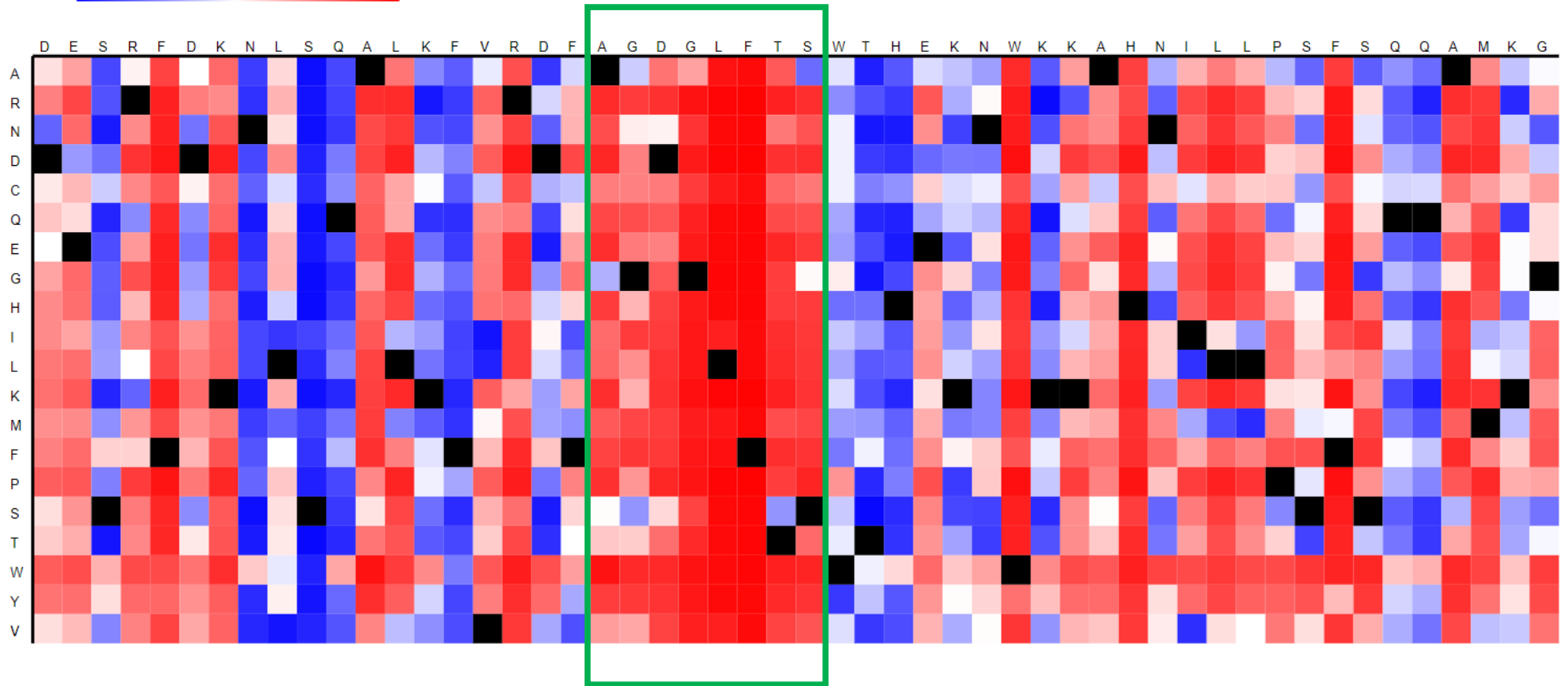
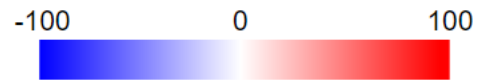
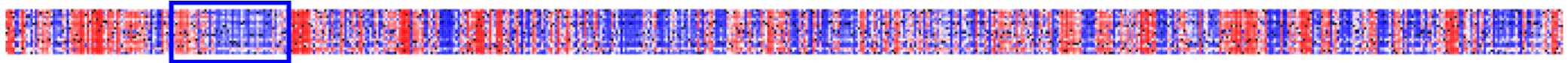
# MEME 分析



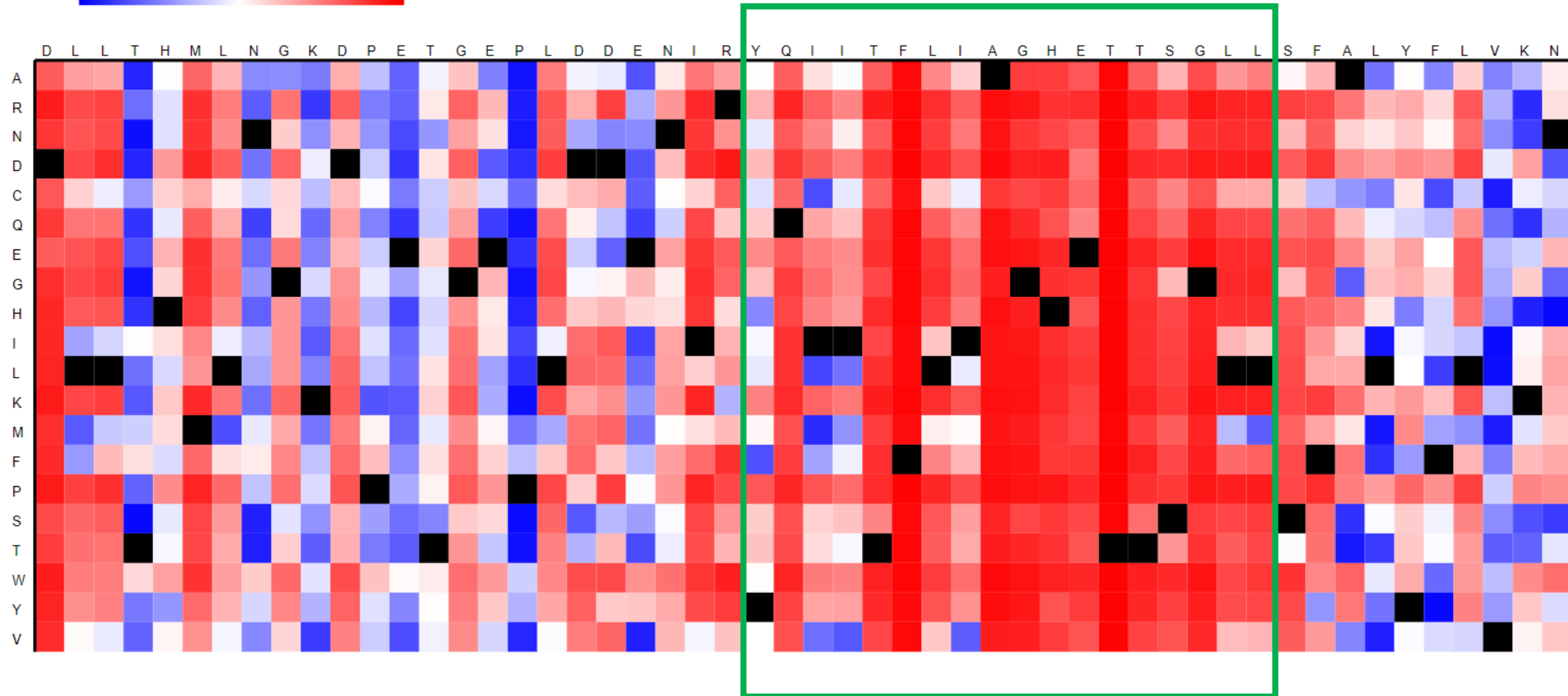
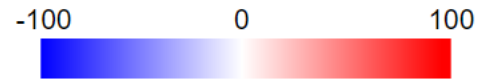
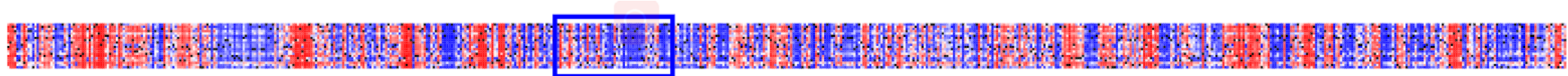
Name	p-value	Motif Locations
sp P14779 CPXB_BACMB	0.00e+0	
sp O08336 CYPB_BACSU	0.00e+0	
sp O08394 CYPD_BACSU	0.00e+0	
sp Q81BF4 C102_BACCR	0.00e+0	
tr A0A679GZ68 A0A679GZ68_9BAC1	0.00e+0	

Motif	Symbol	Motif Consensus
1.		FTSWTHEPNWKKAHNILMPTFSQRAMKDYHAMMVDIAVQLVQKWARLNPNEVDVPEDMTRLTLDITGLCGFNRYRFNSFY
2.		NIRFQIITFLIAGHETTSGLLSFAIYFLLKNPDKLKKAYEEVDRVLTDPPTYKQVMKLYIRMLNESLRLWPTAPAFS
3.		VLI PQLHRD KDAWGDDVEEFRPERFEFVPHHAYKPFNGQRACIGMQFALHEATLVLMGMJLKHFE LIDHQN YELD I K
4.		GEYRGVASN YLAERHNGDEI ICFIRTPQSNFZLPKDPETPIIMVGPGTGIAPFRGFLQARRVLKQEGKTLGEAHL YFGCR
5.		BDRIGSLPKEGAVLIVTSSYNGKPPSNAGQFVQWLLEELKPDDELKGVYAVFGCGDHNWASTYQRI PRFIDEQMAQKGATR
6.		HPKTYVQH L I KQDR I KLI SLLDNGAHL Y I CGDGSKMAPDVEDTLCKAYQE I HEVSEQEARNWLQRLQDEGRYAKDVW

# Motif 1



# Motif 2







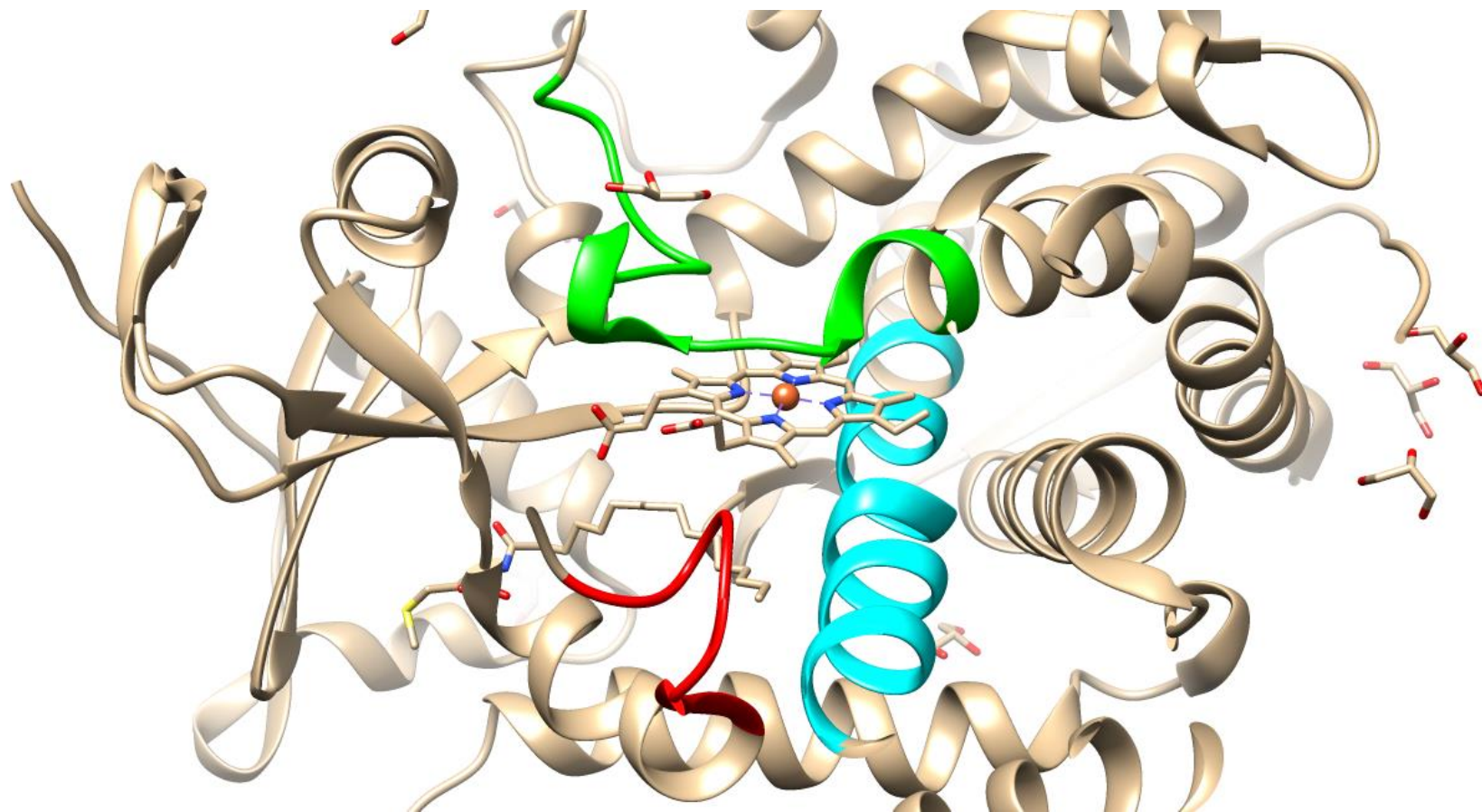
# MEME 分析

Name

sp|P14779|CPXB\_BACMB

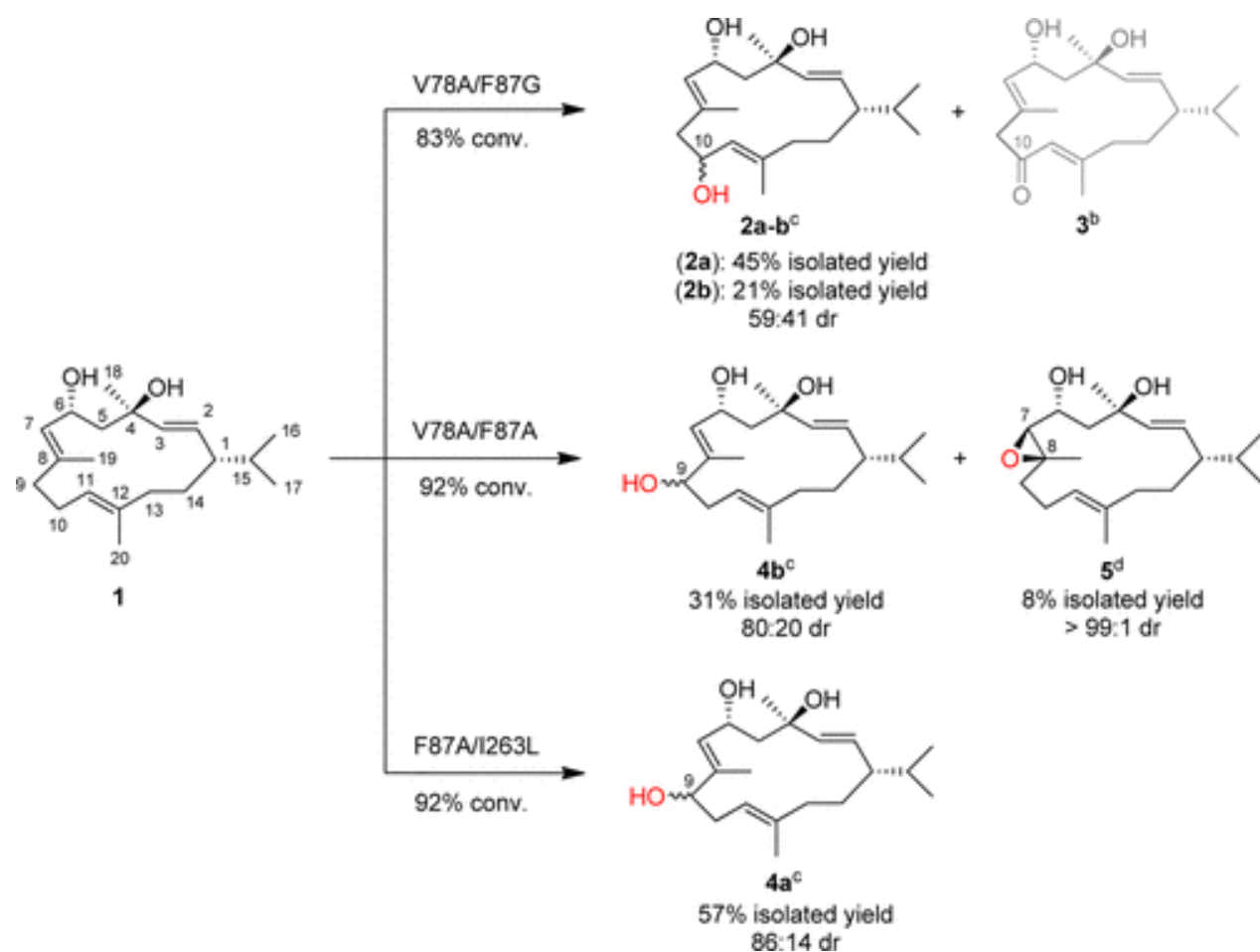
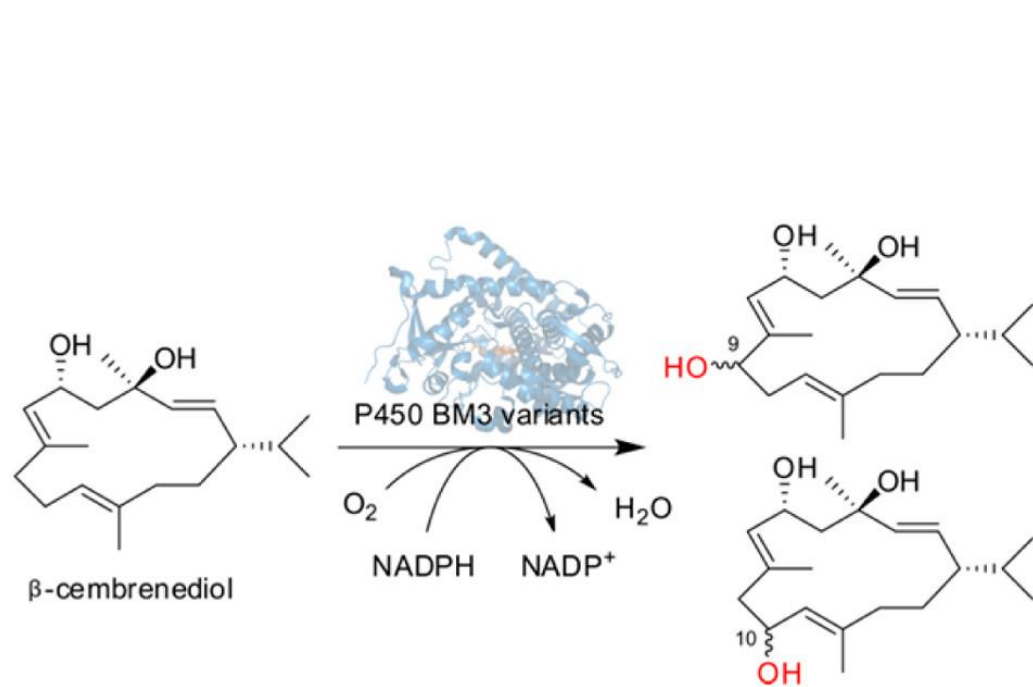
p-value Motif Locations

0.00e+0

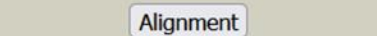
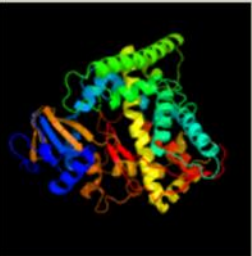
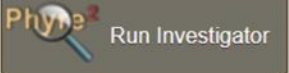





# P450 BM3突变体催化效率



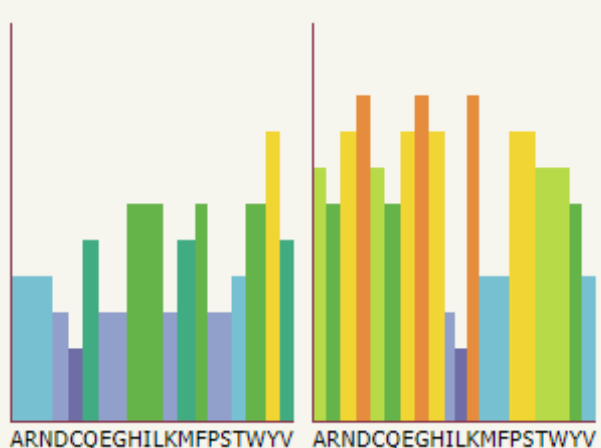
# 用Phyre<sup>2</sup>分析突变位点

#	Template	Alignment Coverage	3D Model	Confidence	% i.d.	Template Information
1	<a href="#">d2ij2a1</a> <input type="radio"/> <input type="checkbox"/>	 Alignment		100.0	98	<b>Fold:</b> Cytochrome P450 <b>Superfamily:</b> Cytochrome P450 <b>Family:</b> Cytochrome P450 <b>PDB entry:</b> <a href="#">PDBe</a> <a href="#">RCSB</a> <a href="#">PDBj</a> 

## Residue: LEU 76


Sequence profile 

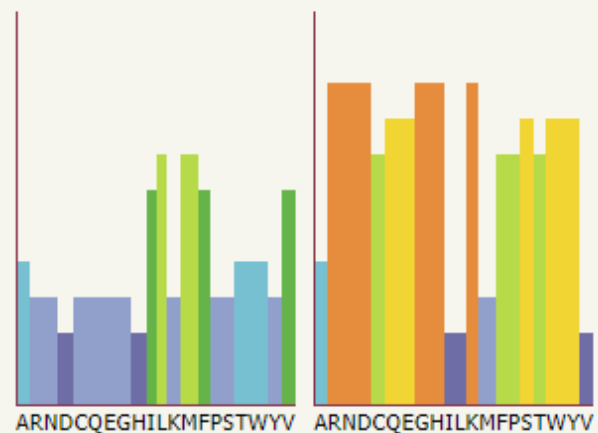
Mutations 



## Residue: VAL 79

Sequence profile 

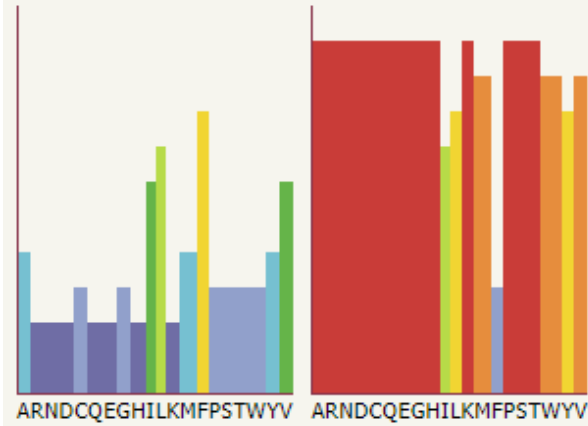
Mutations 




## Residue: PHE 88

Sequence profile 

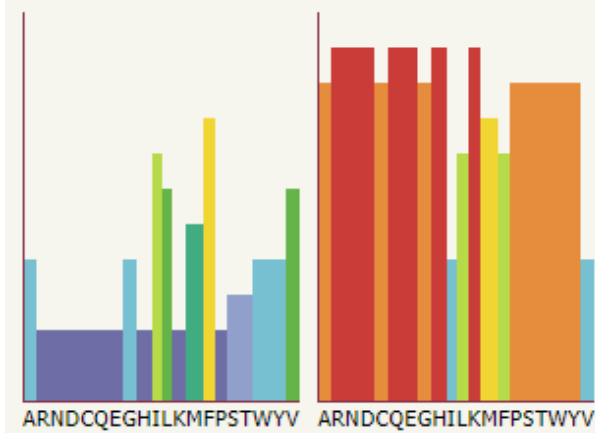
Mutations 



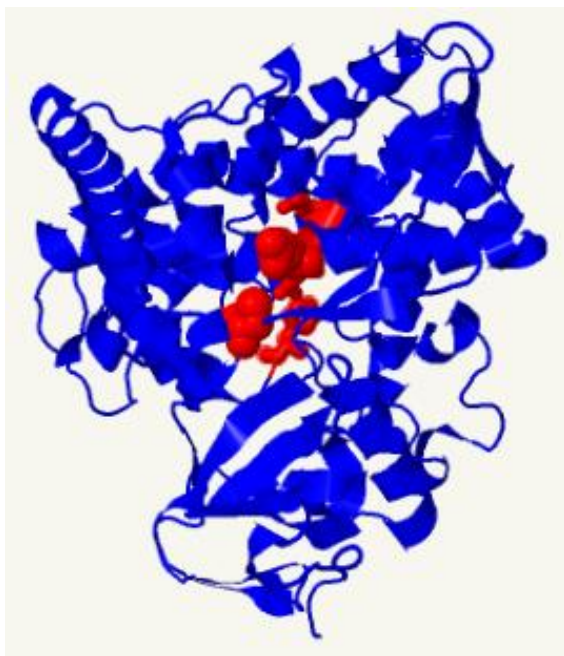
## Residue: ILE 264

Sequence profile 

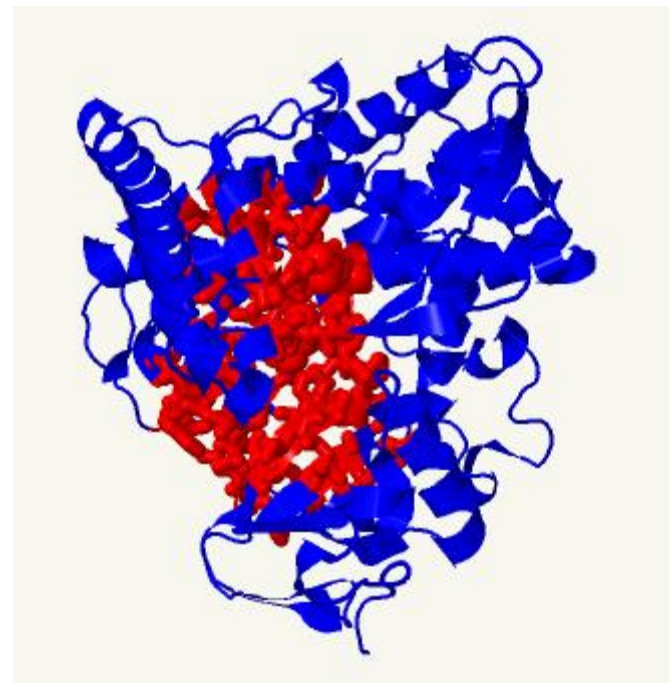
Mutations 



## 用Phyre<sup>2</sup>预测催化位点和结合口袋



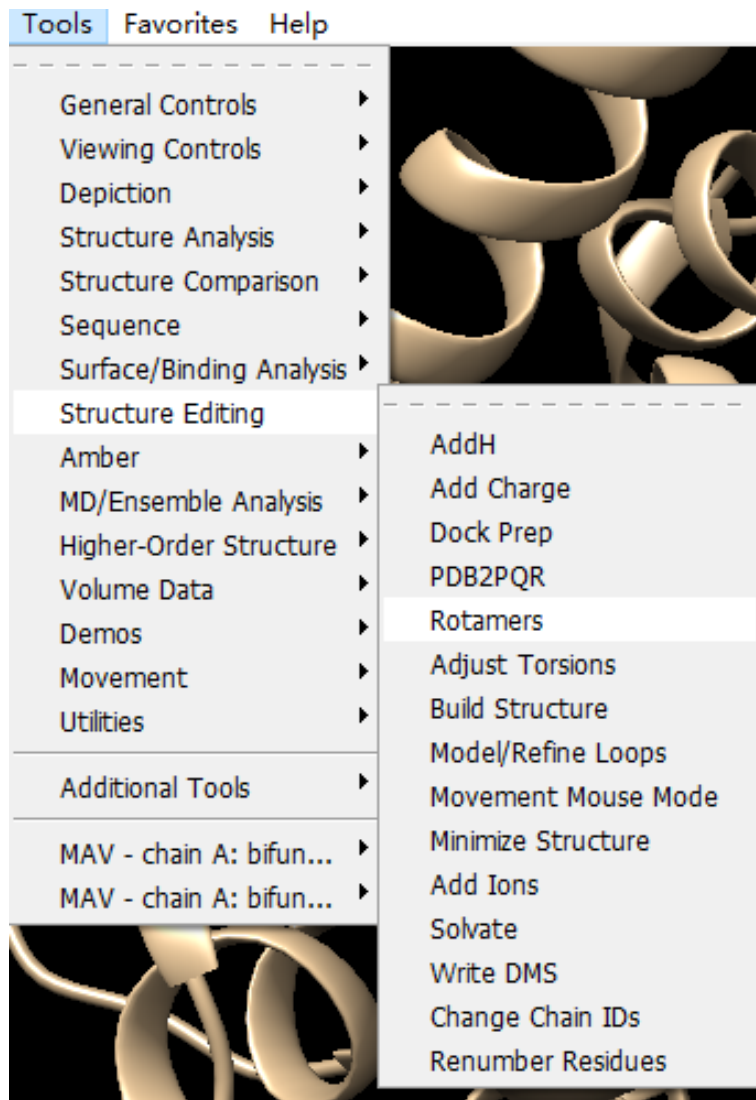
Catalytic sites  
278-270 ETT  
394F  
401C  
410E



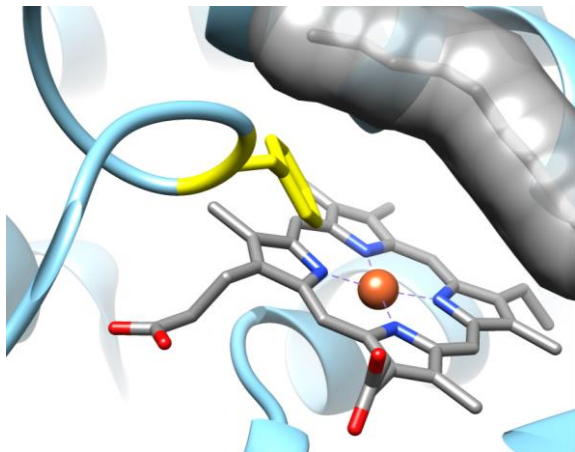
Pocket detection

59 62 63 65 68 69 70 71 72 73 76 87 88 90 91 92 97 101  
108 113 116 154 157 158 178 179 182 183 189 234 258  
261 262 263 264 265 266 267 268 269 270 329 330 331  
332 333 334 355 356 358 361 392 393 394 395 396 398  
399 400 401 402 403 406 407 437 438 439

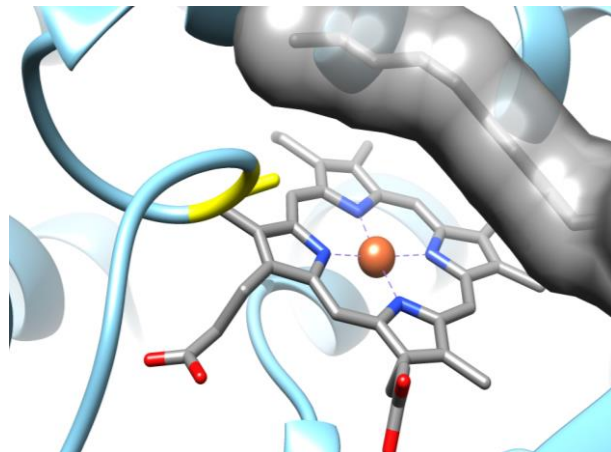
# Chimera 展示 P450 BM3突变体结构的改变



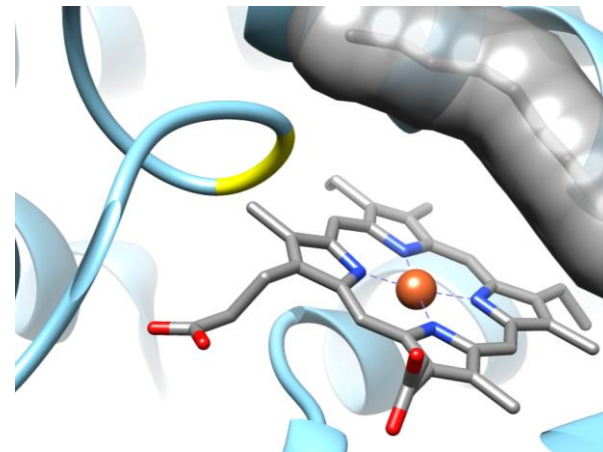




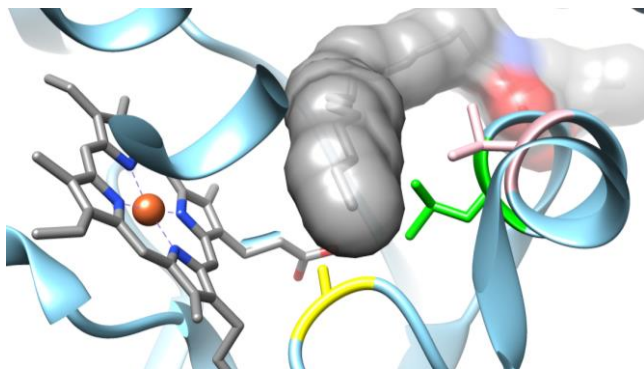
F87



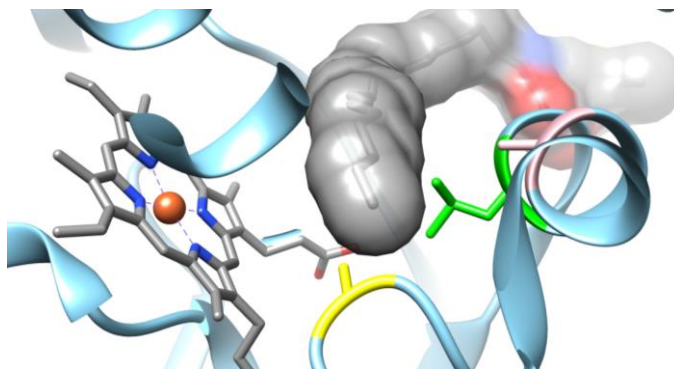
F87A



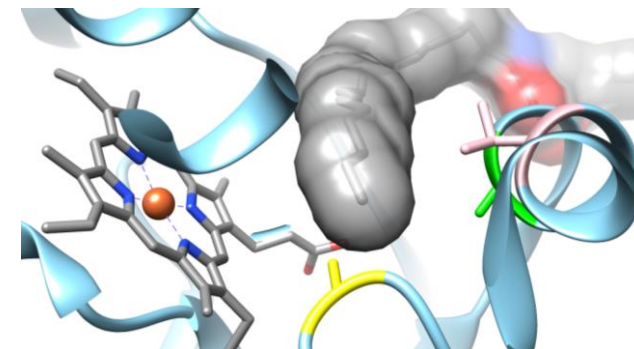
F87G



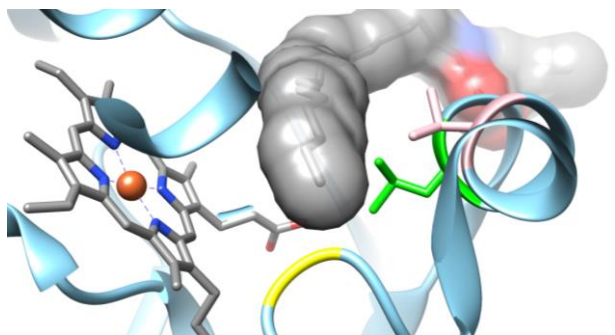
F87A L75 V78



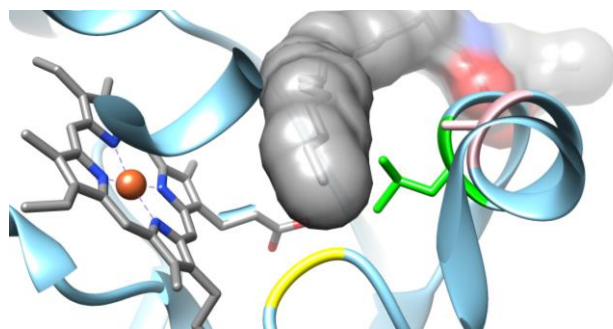
F87A L75 V78A



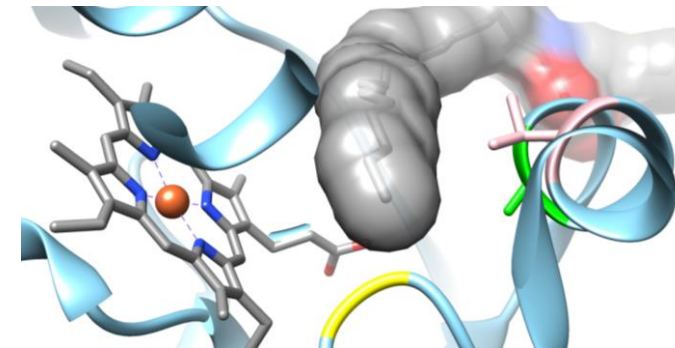
F87A L75A V78



F87G L75 V78



▲ F87G L75 V78A



F87G L75A V78



**Thank you for listening!**

张雪洁 李煜荣 余丽华

2022.1.16