

牧草盲蝽气味结合蛋白**OBP1**的 结构预测及功能分析

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汇报提纲

- 气味结合蛋白的研究进展
- 基因的克隆及分析
- 蛋白的表达纯化
- 蛋白的结合特性分析
- 三维建模及结合点预测
- 结合位点突变及功能验证
- 总结

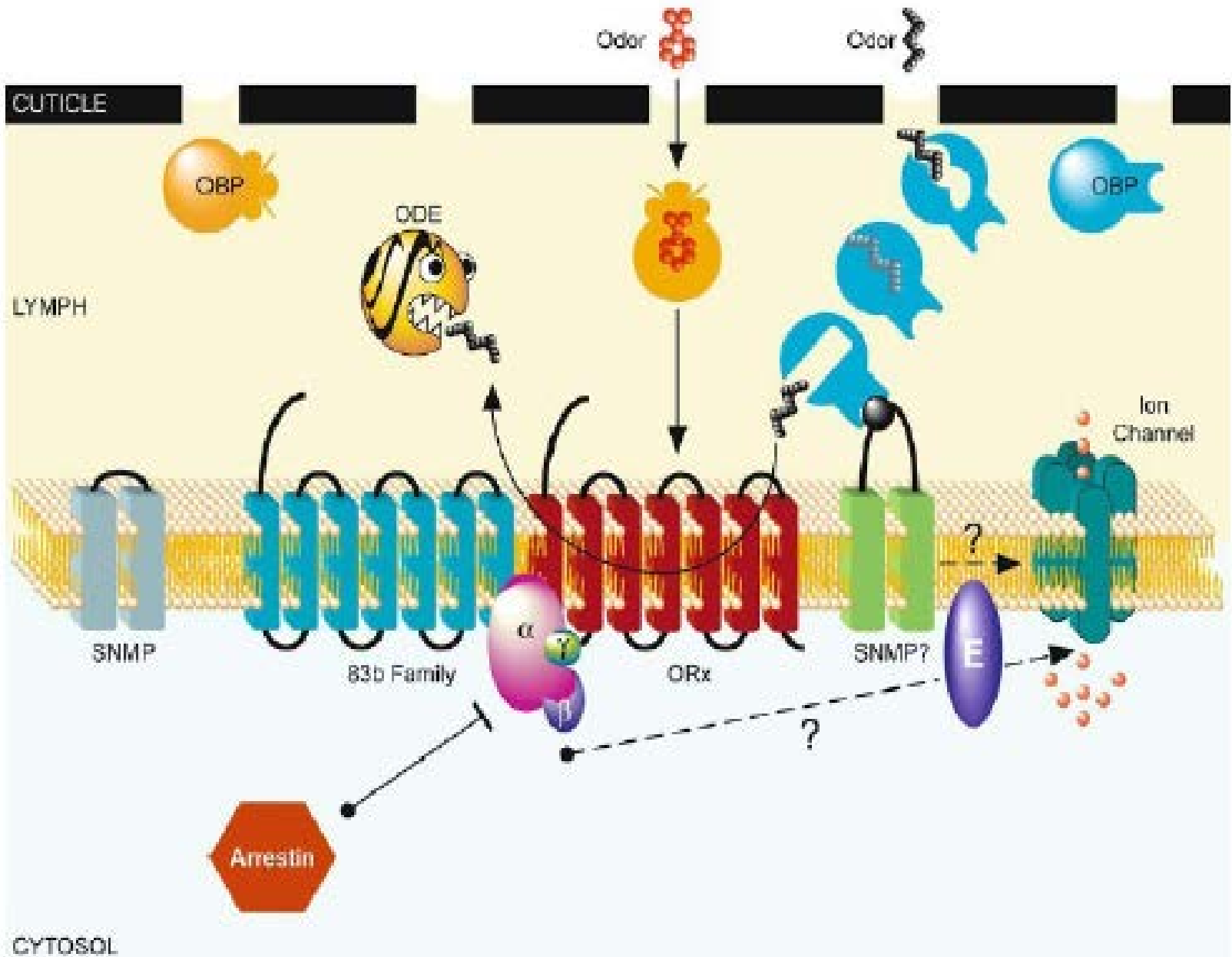
- 牧草盲蝽 *Lygus pratensis*(Linnaeus)属半翅目，盲蝽科。分布在内蒙古、宁夏、安徽、湖北、四川等地。为害玉米、小麦、棉花、豆类、蔬菜等作物。成、若虫刺吸嫩芽幼叶及叶片汁液，幼嫩组织受害后初现黑褐色小点，后变黄枯萎，展叶后出现穿孔、破裂或皱缩变黄。



气味结合蛋白的研究进展

- 昆虫在感受外界化学信号的过程包括化学信号的捕获、运输和嗅觉受体的激活，此过程中有一些重要的蛋白参与其中发挥作用。主要包括：气味结合蛋白（odorant-binding proteins, OBPs）、气味降解酶（odorant-degrading enzymes, ODEs）、嗅觉受体蛋白（olfactory receptor, OR）。昆虫特异性的识别外界气味物质的第一步是靠气味结合蛋白（odorant binding proteins, OBPs）实现的。

AIR



OBPS	解析方法	物种	PDB号
AaegOBP1	X-ray 1.85 Å	<i>A. aegypti</i>	3K1E
Agamobp1	X-ray 2.19 Å	<i>A. gambiae</i>	2ERB
Agamobp4	X-ray 2.00 Å	<i>A. Gambiae</i>	3Q8I
Agamobp7	X-ray 2.10 Å	<i>A. Gambiae</i>	3R1O
AtraPBP1	NMR	<i>A. Polyphemus</i>	2KPH
AmelOBP14	X-ray 1.24 Å	<i>A. mellifera</i>	3S0D

序列预测

Max ORF starts at AA pos 1(may be DNA pos 16) for 131 AA(393 bases), MW=14049

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      10      20      30      40      50      60
1      CTTCTCGTGGGCGAAATGAGGATTTTGGTTTTGTTCACTGCGGCACTTACGTGOGTTATG
1
      M R I L V L F T A A L T C V M

      70      80      90      100     110     120
61     GCGGGCGAACTACCGGAAGAAATGAGAGAAATGGCGCAGGGCCTGCATGAOGGCTGOGTA
16     A G E L P E E M R E M A Q G L H D G C V

      130     140     150     160     170     180
121    GAAGAAACCGGAGTAGACAATGGACTGATAGGACCATGTGCCAAAGGAAATTTGCTGAT
36     E E T G V D N G L I G P C A K G N F A D

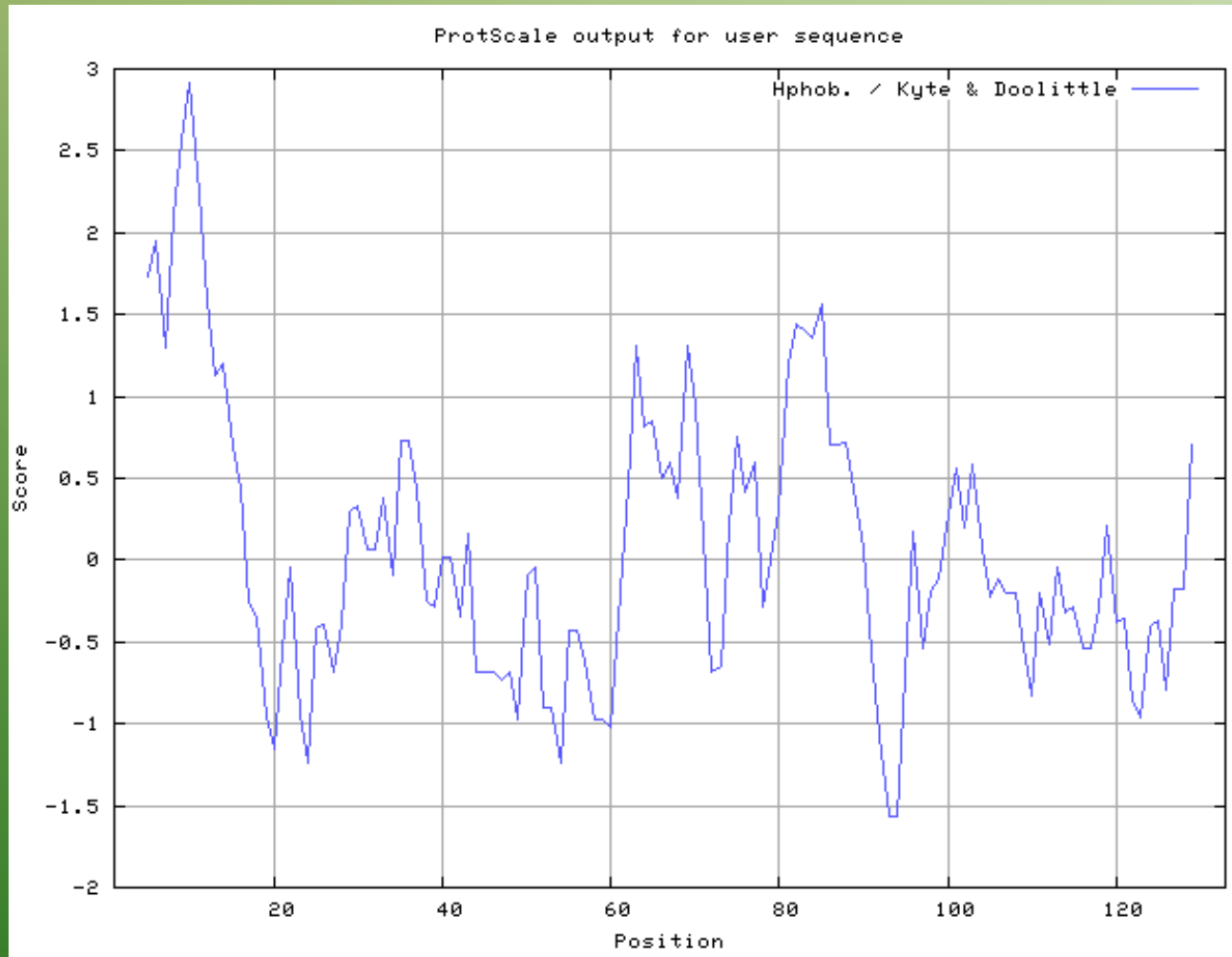
      190     200     210     220     230     240
181    GATCAAAAAGTGAAGTGCTACTTCAAATGTGTGTTGGTAAOC TCGGAGTGATTTCCGGAC
56     D Q K L K C Y F K C V F G N L G V I S D

      250     260     270     280     290     300
241    GAAGGCGAACTTGACGCAGAAAGCTTTTGGATCTATTTTGCCCTGACAACATGCAAGAACTC
76     E G E L D A E A F G S I L P D N M Q E L

      310     320     330     340     350     360
301    CTAOCTACCATCAGAGGCTGTGCTGGCACAAACGGGAGCAGATCCTTGGAACCTTGCAATG
96     L P T I R G C A G T T G A D P C E L A M

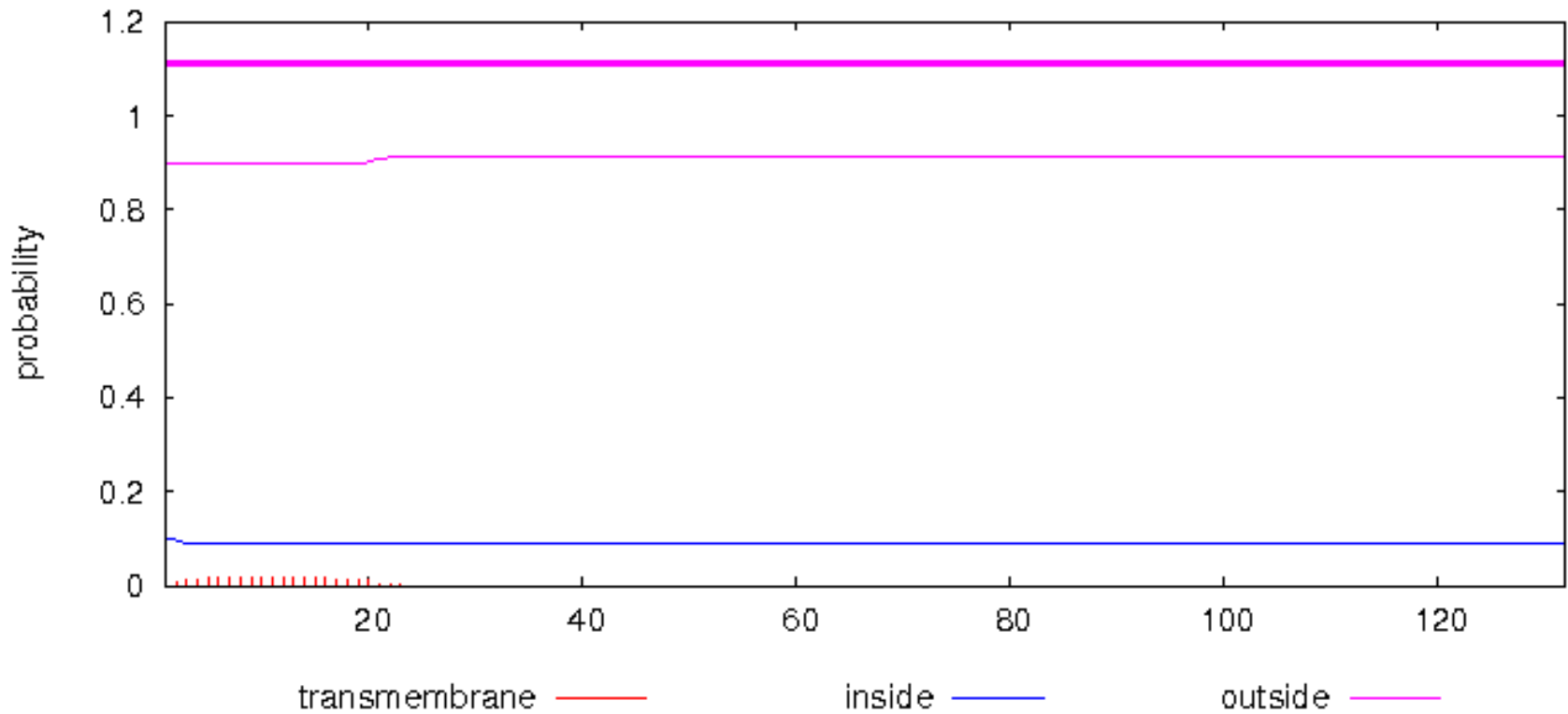
      370     380     390     400     410     420
361    AACTTCAACAAGTGCCTOCAGAAGGTGGACCCGTAAACTTCATGGTAATCTAATCTTAG
116    N F N K C L Q K V D P V N F M V I
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疏水性分析结果



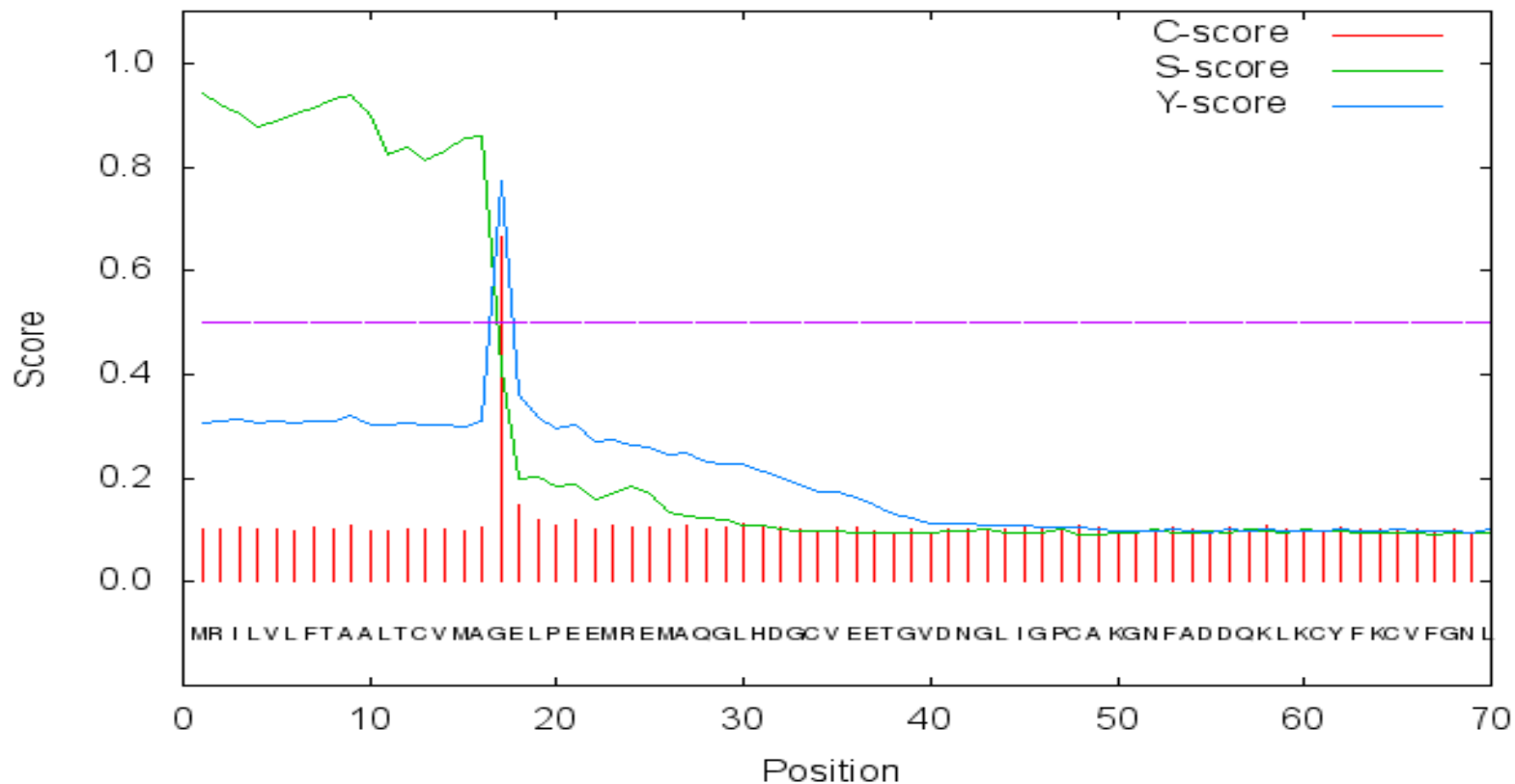
跨膜区预测

TMHMM posterior probabilities for AAC43033.1|



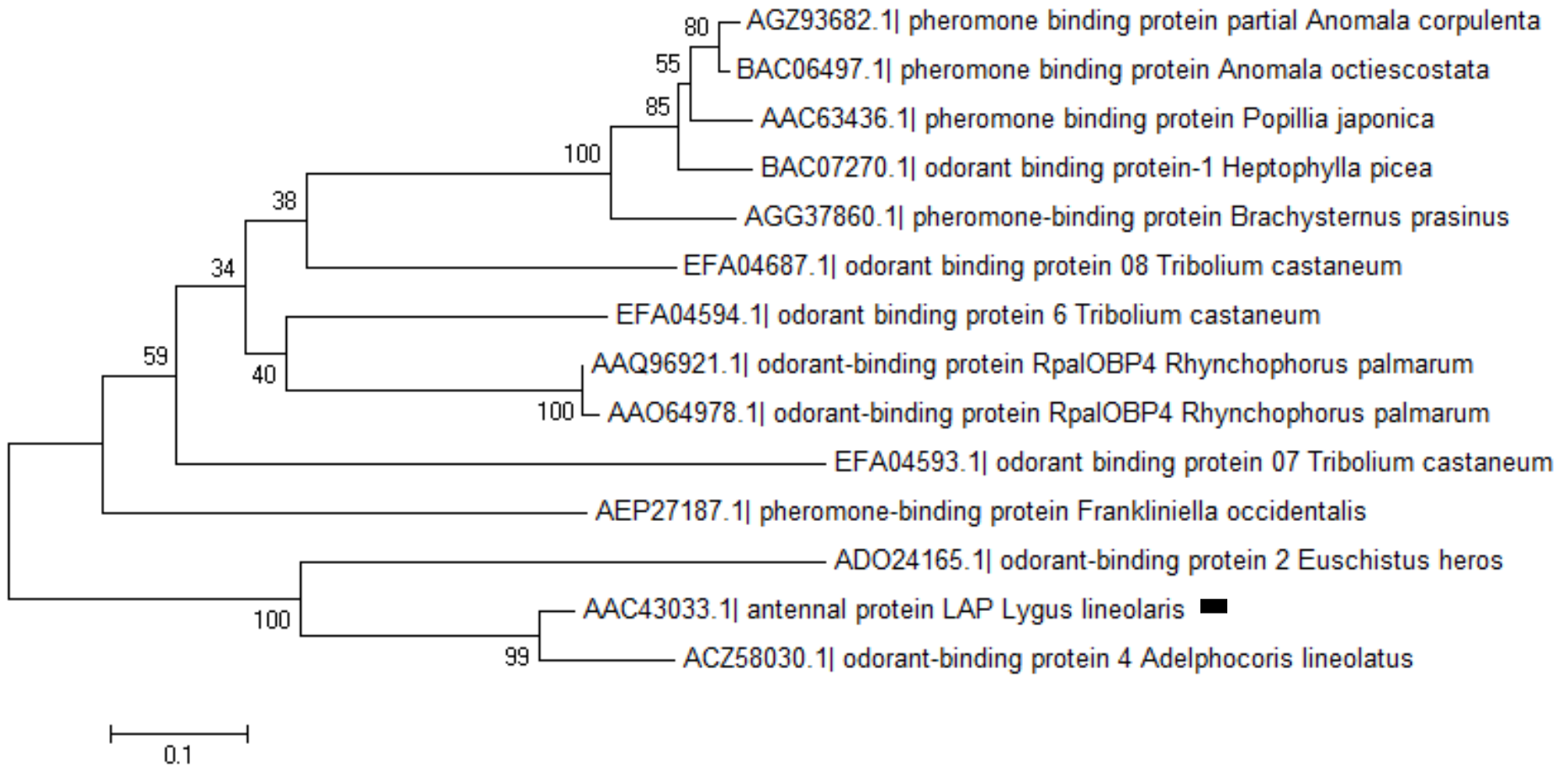
信号肽预测

SignalP-4.1 prediction (euk networks): AAC43033.1_

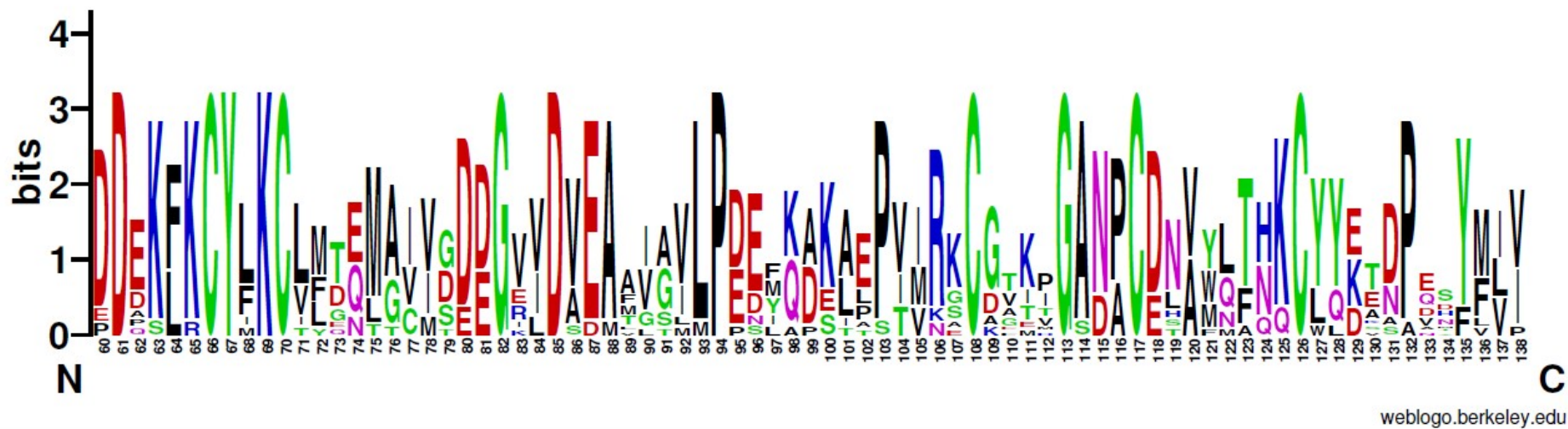
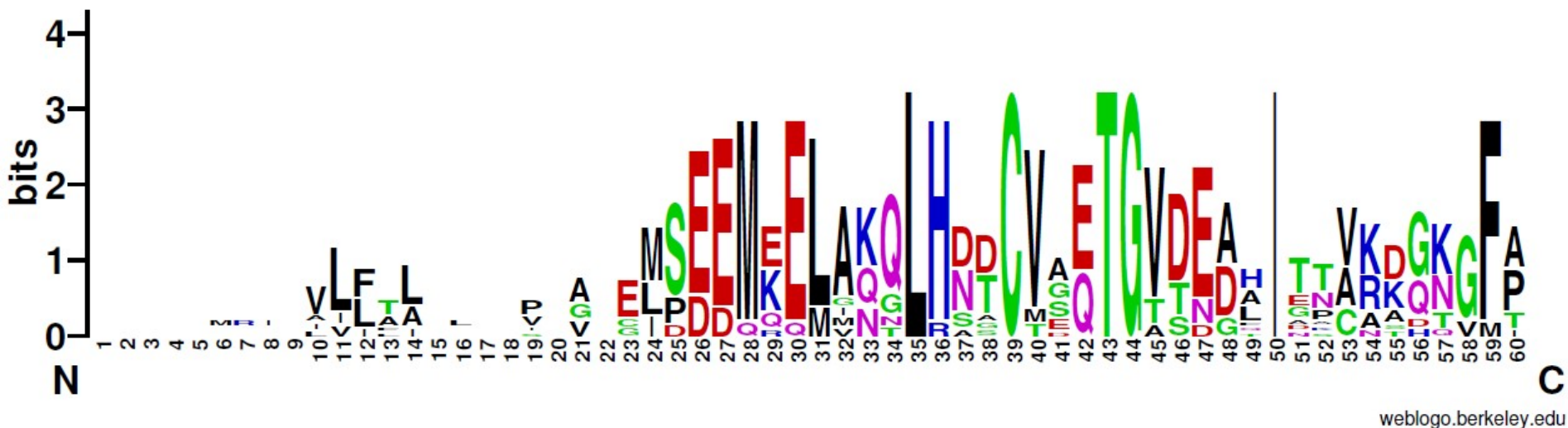


根据SignalP 4.0的分析结果，其N端前17个氨基酸残基为信号肽

同源性分析



运用Weblogo进行多序列比对



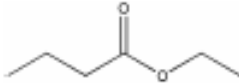
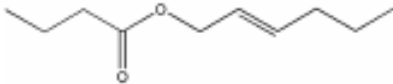
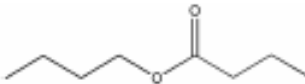
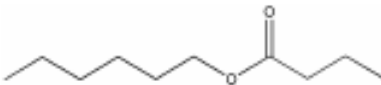
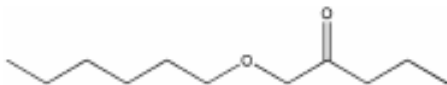



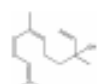
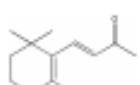

运用Clustal软件进行多序列比对

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                *           20           *           40           *           60           *
AAQ96921.1 : -----DEMKELAQQLHNTCVSETGTTEDAITNARAGT-FTDDEKFKCYLK : 44
AAO64978.1 : -----DEMKELAQQLHNTCVSETGTTEDAITNARAGT-FTDDEKFKCYLK : 44
EFA04594.1 : ----MSPLLLIFISCLFPRVFGISEEMQELANTLHATCVDETGVSEDAIESARKGN-FAPDDKCLKCYMK : 64
AGZ93682.1 : ----MLKLVLLTLGIYVPTVMCMSEEMEELAKQLHND CVAQTGVDEAHITTVKDKQKGFDPDEKFKCYLK : 66
BAC06497.1 : -----MSEEMEELAKQLHND CVAQTGVDEAHITTVKDKQKGFDPDEKFKCYLK : 47
AAC63436.1 : -----MSEEMEELAKQLHDDCVSQTGVDEAHITTVKDKQKGFDPDEKFKCYLK : 47
BAC07270.1 : -----EEMEELAKQLHDDCVGQTGVDEAHIGTVKDKQKGFDPDEKFKCYLK : 45
AGG37860.1 : -----MSEEMEELGKQLHDDCVGQTGVDEAFITTVKDKHKGFPDDEKFKCYLK : 47
EFA04687.1 : ----MIRYYIVLLLYFFAPP--ISEEMQELVNQLHSTCVAETGVSEDLINKVNSDKVMIDDEKCLKCYIK : 64
AEP27187.1 : MKTLLTLAAALLALCGLASAGVELSEDQKQLMKQLRSACMAETGVDEATIDACKTGQ-FADDPKCLKCYLK : 69
EFA04593.1 : -MYKTRVIYVLFALCLVEIFAEMDDDMKELINNLHNTCTGETGATDDQIENARKGN-FAEDDSFKCYFK : 68
AAC43033.1 : ----MRILVLFATAALTCVMAGELPEEMREMAQGLHDG CVEETGVDNGLIGPCA KGN-FADDQKCLKCYFK : 64
ACZ58030.1 : ----MRIFVIFTAALTCVMAGELPEEMKEMAQGLHDS CVEETGVDNGLIAPCA KGN-FADDAKLRCYFK : 64
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                80           *           100           *           120           *
AAQ96921.1 : CLLDQMAIVDEEGRIDVEAMIAVLPEEFQDSLPPVIRKCDTIIIGANACDNVWLTQQCYKKNPEHYFLI : 113
AAO64978.1 : CLLDQMAIVDEEGRIDVEAMIAVLPEEFQDSLPPVIRECDTIIIGANACDNVWLTQQCYKKNPEHYFLI : 113
EFA04594.1 : CIMEQMACIDDEGIIDVEATIAVLPEEYQAKAEPVIRKCGTKIGANACDNAFLTNCWYEEDPEDYFLV : 133
AGZ93682.1 : CLMTEMAIVGDDGVVDVEAAVGVL PDEYKAKAEPVIRKCGVKPGANPCDNVYQTHKCYDTPQSYMIV : 135
BAC06497.1 : CLMTEMAIVGDDGVVDVEAAVGVL PDEYKAKAEPVIRKCGVKPGANPCDNVYQTHKCYDTPQSYMIV : 116
AAC63436.1 : CLMTEMAIVGDDGVVDVEAAVGVL PDELKAKAEPIMRKCGFKPGANPCDNVYQTHKCYDTPQSYMIV : 116
BAC07270.1 : CLMTEMAIVGDDGVVDVEAAVGVL PDEFKDKAEPIMRKCGVKPGANPCDNVYQTHKCYDADPN SYMIV : 114
AGG37860.1 : CLMTEMAVVGDDGVVIDVEAAVGILPDDIKDKAEPVIRKCGAKVGANPCDHVYLTHKCYDTPETYMIV : 116
EFA04687.1 : CLLTETGCISDDGVVDVEATIALLPEDMKAKTTPVIRSCGAKMGANPCESAWLTHKCYLETSPADYVLI : 133
AEP27187.1 : CTYQQMTVMDDDGVVDADMLLTMLPEEIQPKAEPVVNACKEMRGADACDNAMMFNKCLYEKAPDYVMV : 138
EFA04593.1 : CVFDQMGCMTDDGKVDSEAVIAVMPPELADKIASTVRGC-TEVGANPCETAWLANKCYQKSNPDMYFVP : 136
AAC43033.1 : CVFGNLGVISDEGELDAEAFGSILPDNMQ-ELLPTIRGCAGTTGADPCELAMNFNKCLQKVDPVNFMI : 132
ACZ58030.1 : CVFGNLGVISDEGELDAEAFGSILPDSMQ-ELLPTIKSCGGTTGSDPCDLAMNFNKCLQKADPVNFLVI : 132
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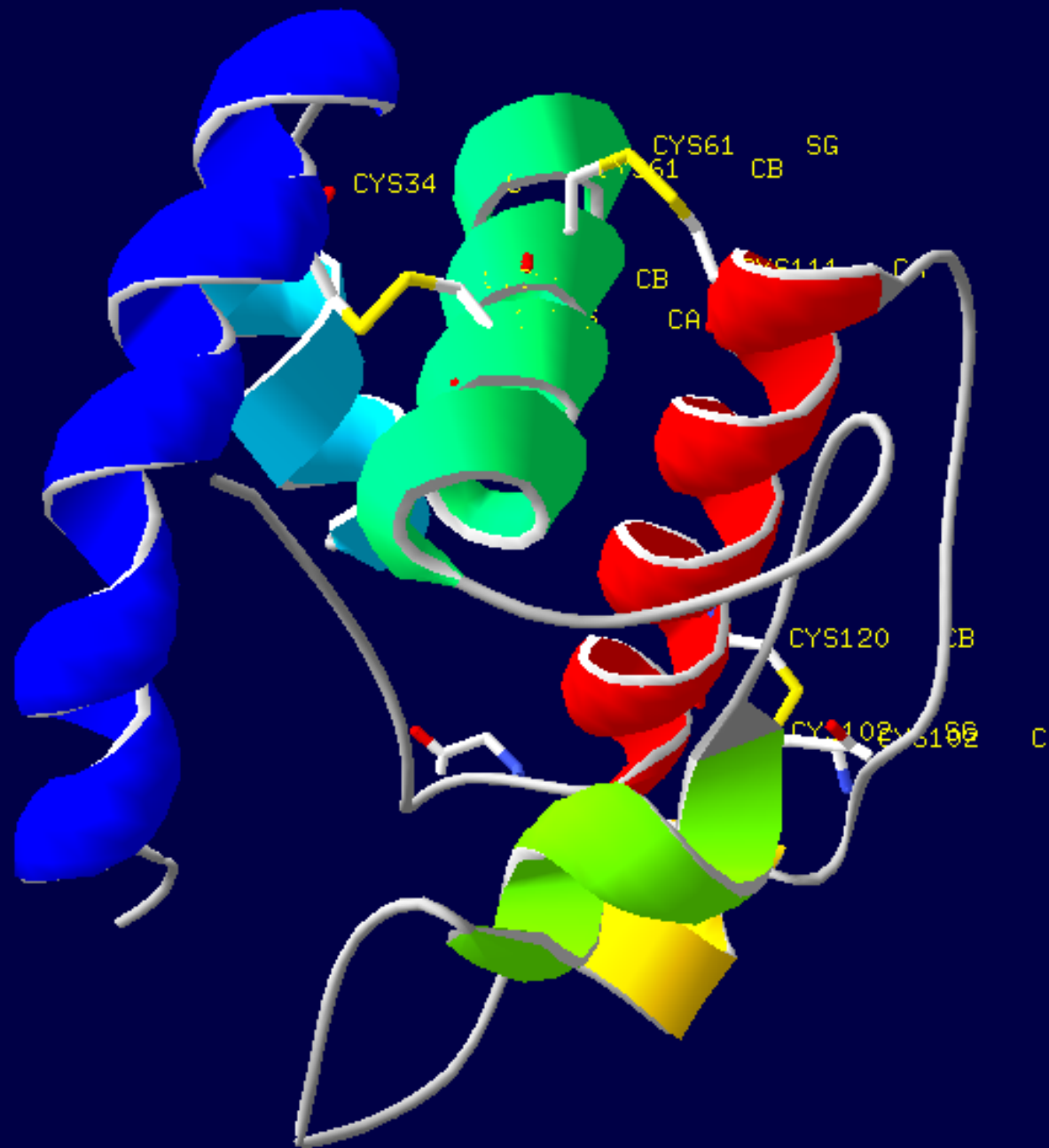
气味结合蛋白的功能研究

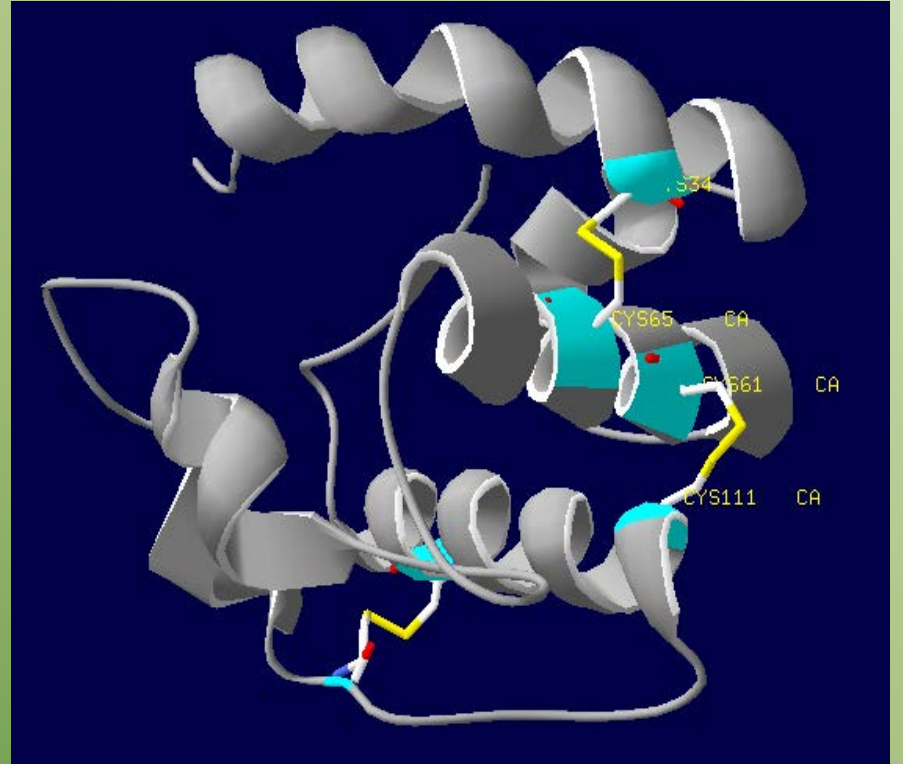
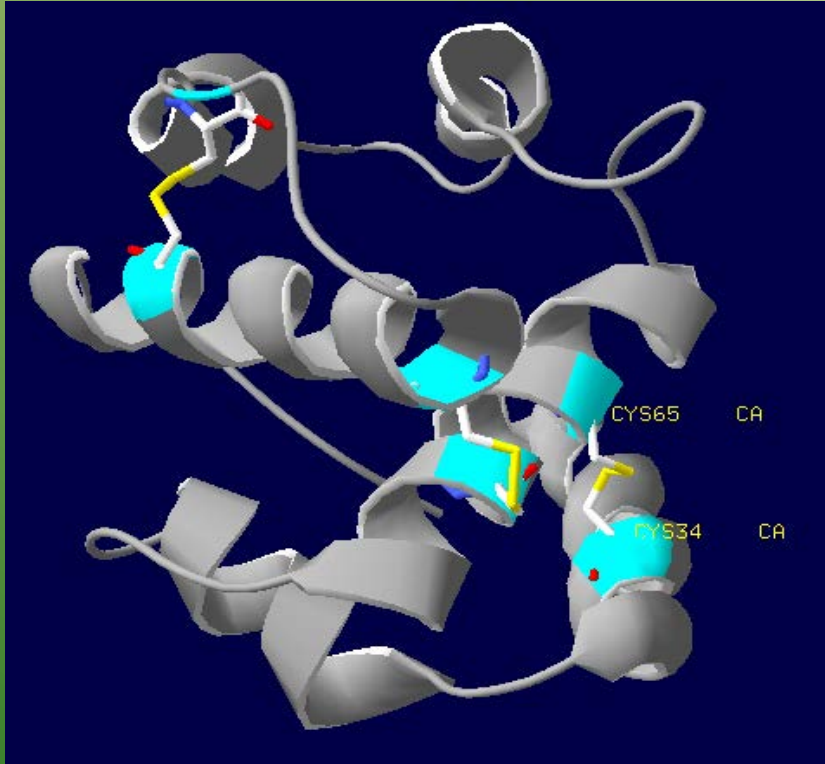
- 荧光结合实验可通过测量荧光强度间接地测定OBPs与气味分子的结合能力，方法简单、安全、快捷。
- 研究的流程为：设计引物 → 原核表达蛋白 → 蛋白纯化 → 竞争结合研究。

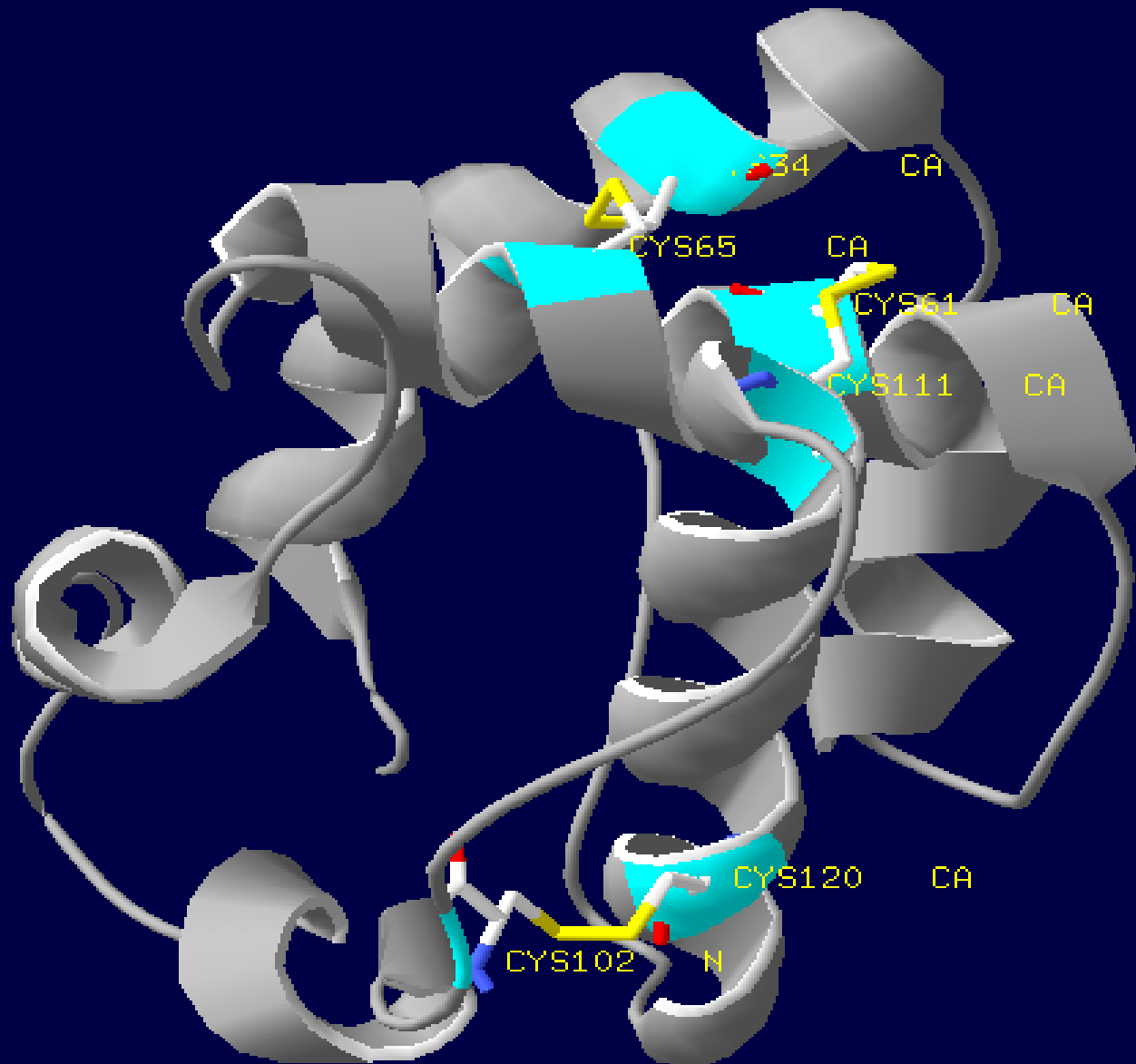
Ligands	Strukturformel	IC ₅₀ (μ M)	K _i (μ M)
丁酸乙酯 (Ethyl butyrate)*		-	-
丁酸反-2-己烯酯 (trans-2-Hexenyl butyrate)*		-	-
丁酸丁酯 (Butyl butanoate)*		-	-
丁酸己酯 (1-Hexyl butanoate)*		-	-
己酸己酯 (Hexyl hexanoate)*		-	-
4-氧代-反-2-己烯醛 (4-oxo-(E)-2-hexenal)*		-	-
α -石竹烯 (α -Caryophyllene)		-	-
β -石竹烯 (β -Caryophyllene)		32.75	22.73
橙花叔醇 (Nerolidol)		10.08	6.99
β -紫罗兰酮 (β -Ionone)		25.48	17.68
月桂酸乙酯 (Ethyl dodecanoate)		23.78	16.51

同源建模及结合位点分析

- 根据蚊子的OBP(3n7h-B)构建的结构模型显示，具有6个 α 螺旋，另外，Cys34与Cys65、Cys61与Cys111、Cys120与Cys102。形成三对二硫键。







同源建模及结合位点分析

- 125位的ASP与121位的LEU及129位的phe以氢键结合，有可能固定C末端，把C末端包于结合腔内，这可能与配体跟蛋白的结合与释放有关。
- 下一步就是对这些位点进行定点突变，然后再验证与化学物质的结合能力。

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Thank you !