

# NZ2114及衍生肽分子设计、 理化参数分析与结构预测

Molecular design, physical /chemical  
parameters analysis and structural  
prediction of NZ2114 and derived peptides

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报告日期：2018年6月23日



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# 1. 背景介绍

1

抗菌肽（AMP）是具有抗菌活性少于100个氨基酸残基的多肽，通常带正电荷（+2 – +9），富含精氨酸（Arg）和赖氨酸（Lys），具有两亲性等特征。

2

AMP广泛存在于动植物及微生物中，是宿主先天防御系统主要组分，具有抗细菌，抗真菌，抗寄生虫，抗肿瘤，抗病毒等功能，是新型抗生素来源和人类攻克其它疾病的药物资源库。



3

饲料中的抗生素大量使用导致**耐药性病原菌**在临床和畜牧业等相关领域广泛产生，直接和间接威胁着人类健康。抗菌肽以其**独特杀菌机制、不易耐药、抗菌活性强、杀菌速度快**和**来源广泛而安全**等优点，成为传统抗生素理想替代品。

4

一些具有良好应用价值的天然抗菌肽具有**细胞毒性**，**抗菌谱过广**，还有一些抗菌肽**杀菌活性不足**，**对生物膜无作用**等。因此，对天然抗菌肽进行有目的改造克服不足在抗菌肽药物与饲用抗生素替代品开发中具有重要意义。



UniProtKB - Q53I06 (PLECT\_PSENR)

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Entry

Protein | Plectasin

Publications

Gene | DEF

Feature viewer

Organism | *Pseudoplectania nigrella* (Ebony cup)

Plectasin是新世纪初Mygid等从北欧松林地表腐生子囊菌中分离到的一种具有抗菌功能的多肽，为首例真菌防御素，对金黄色葡萄球菌、肺炎链球菌和猪链球菌具有高效杀菌作用。属于无脊椎动物防御素家族。

Plectasin基因编码一个95AA的前体肽，经分泌过程加工处理，信号肽（1-23AA）及前体片段（24-55AA）被信号肽酶切除，分泌出含40AA残基的成熟肽（56-95AA）即Plectasin。

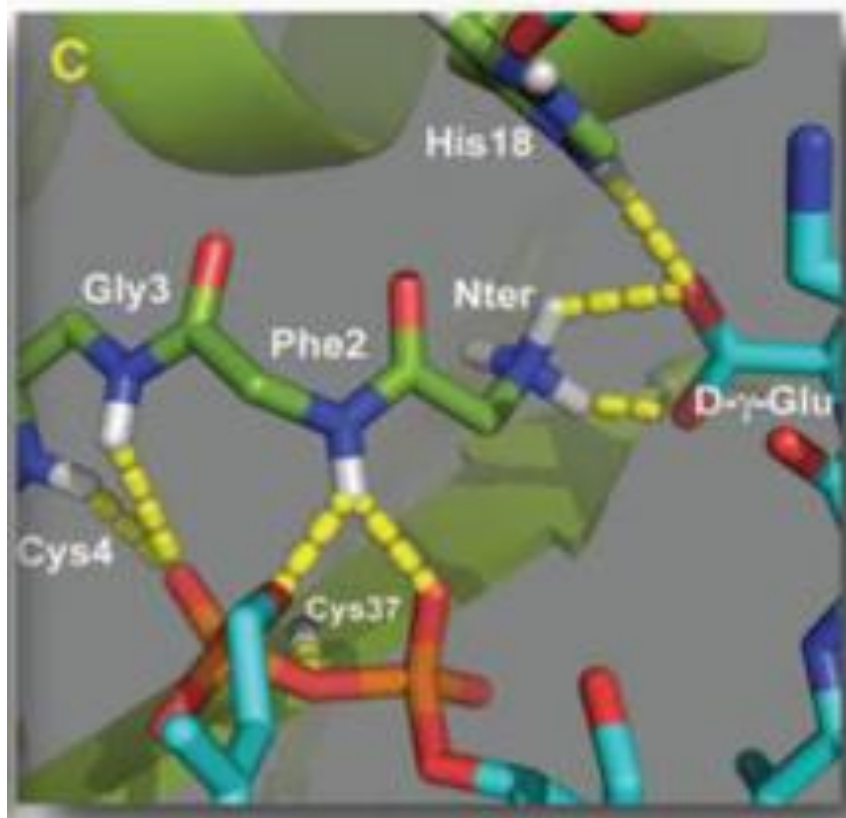
## 6 Plectasin杀菌机理

Plectasin杀菌机理是通过其N端2, 3, 4及37位氨基酸残基与lipidII焦磷酸盐形成氢键，而His18与lipidII的D- $\gamma$ -谷氨酸形成盐桥，最终阻碍细胞壁合成。

## 7 NZ2114

NZ2114是Plectasin通过高通量突变筛选到的对金葡具有强烈抑菌活性的突变体。

NZ2114是将Plectasin 第9, 13, 14位氨基酸Asp, Met, Gln突变为Asn, Leu, Arg。



## 2. 分子设计

### 1 Plectasin序列比对和结构模型



APD数据库（<http://aps.unmc.edu/AP/main.php>）：  
抗菌肽数据库目前收录了来自6个国家的2981种抗菌肽序列及功能信息，包括抗菌肽来源、氨基酸组成、净电荷数、多肽长度、疏水残基比例等信息。

APD ID: AP00549  
Name/Class: Plectasin (fungi, fungal defensin; UCSS1a; 3S=S, fungii; BBW; Derivatives: NZ2114)  
Source: *Pseudoplectania nigrella*  
Sequence: **GFGCNGPWDEDDMQCHNHCKSIKGYKGGYCAKGGFVCKCY**  
Length: 40  
Net charge: 1  
Hydrophobic residue%: 32%  
Boman Index: 1.4 kcal/mol  
3D Structure: Combine Helix and Beta structure  
Method: NMR  
SwissProt ID: PDB ID: 1ZFU [Go to PDB](#)  
Activity: anti-Gram+, antiviral, antifungal,

Plectasin : GFGCNGPWDEDDMQCHNHCKSIKGYKGGYCAKGGFVCKCY

Tick ( 蜱 ) : GYGCPFNQYQCHSHCSGIRGYKGGYCKGTFKQTCKCY

Mussel ( 蚌 ) : GFGCPNNYACHQHCKSIRGYCGGYCAGWPRLRCTCY

Dragonfly ( 蜻蜓 ) : GFGCPLDQMQRHCRHCRSIRRRGGYCSGPLKLTCTCY

Scorpion ( 蝎子 ) : GFGCPFNQGACHRHCRSIRRRGGYCAGLQTKTCTCY





| Species/Ab   | Group Name | * | * * * |   |   |   |   | * | * |   |   |   | * |   | * | * | * | * | * | * | * | * | * | * | * | * |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|--------------|------------|---|-------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Plectasin |            | G | F     | G | C | N | G | P | W | D | E | D | D | M | Q | C | H | N | H | C | K | S | I | K | G | Y | K | G | G | Y | C | A | K | G | - | G | F | V | C | K | C | Y |
| 2. Tick      |            | G | Y     | G | C | P | F | - | - | - | - | N | Q | Y | Q | C | H | S | H | C | S | G | I | R | G | Y | K | G | G | Y | C | K | G | T | F | K | Q | T | C | K | C | Y |
| 3. Mussel    |            | G | F     | G | C | P | - | - | - | - | - | N | N | Y | A | C | H | Q | H | C | K | S | I | R | G | Y | C | G | G | Y | C | A | G | W | P | R | L | R | C | T | C | Y |
| 4. Dragonfly |            | G | F     | G | C | P | L | - | - | - | - | D | Q | M | Q | C | H | R | H | C | Q | T | I | T | G | R | S | G | G | Y | C | S | G | P | L | K | L | T | C | T | C | Y |
| 5. Scorpion  |            | G | F     | G | C | P | F | - | - | - | - | N | Q | G | A | C | H | R | H | C | R | S | I | R | - | R | R | G | G | Y | C | A | G | L | F | K | Q | T | C | T | C | Y |

```
#=====
#
# Aligned_sequences: 2
# 1: Plectasin
# 2: Tick
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 42
# Identity:      24/42 (57.1%)
# Similarity:   30/42 (71.4%)
# Gaps:         7/42 (16.7%)
# Score: 129.5
#
#
#=====
```

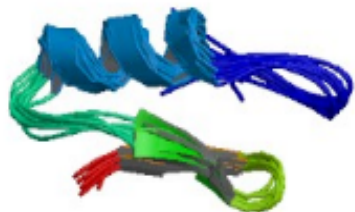
|           |   |   |   |   |   |    |   |   |   |   |    |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |    |   |   |   |    |   |    |  |
|-----------|---|---|---|---|---|----|---|---|---|---|----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|----|---|---|---|----|---|----|--|
| Plectasin | 1 | G | F | G | C | N  | G | P | W | D | E  | D | D | M | Q | C | H | N | H | C | K | S | I | K | G | Y | K | G | G | Y | C | A | K | G | G | F | -- | V | C | K | C  | Y | 40 |  |
|           |   |   | : |   |   |    |   |   |   |   | :  | : | : | : |   |   |   | : |   |   |   |   |   |   |   |   |   |   |   | : |   |   | : |   |   |   |    |   |   |   |    |   |    |  |
| Tick      | 1 | G | Y | G | C | -- | P | F | N | Q | -- | Y | Q | C | H | S | H | C | S | G | I | R | G | Y | K | G | G | Y | C | K | G | T | F | K | Q | T | C  | K | C | Y | 37 |   |    |  |

Plectasin与几种无脊椎动物（Tick, Mussel, Dragonfly, Spider, Scorpion）防御素氨基酸序列相似性均达60%-72%。



1ZFU

Download File View File

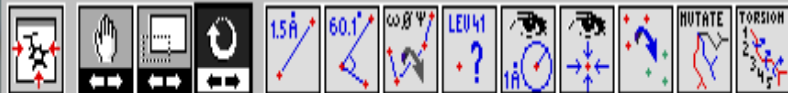


Plectasin: A peptide antibiotic with therapeutic potential from a saprophytic fungus

Mygind, P.H., Fischer, R.L., Schnorr, K.M., Hansen, M.T., Sonksen, C.P., Ludvigsen, S., Raventos, D., Buskov, S., Christensen, B., De Maria, L., Taboureau, O., Yaver, D., Elvig-Jorgensen, S.G., Sorensen, M.V., Christensen, B.E., Kjaerulf, S., Frimodt-Moller, N., Lehrer, R.I., Zaslloff, M., Kristensen, H.H.

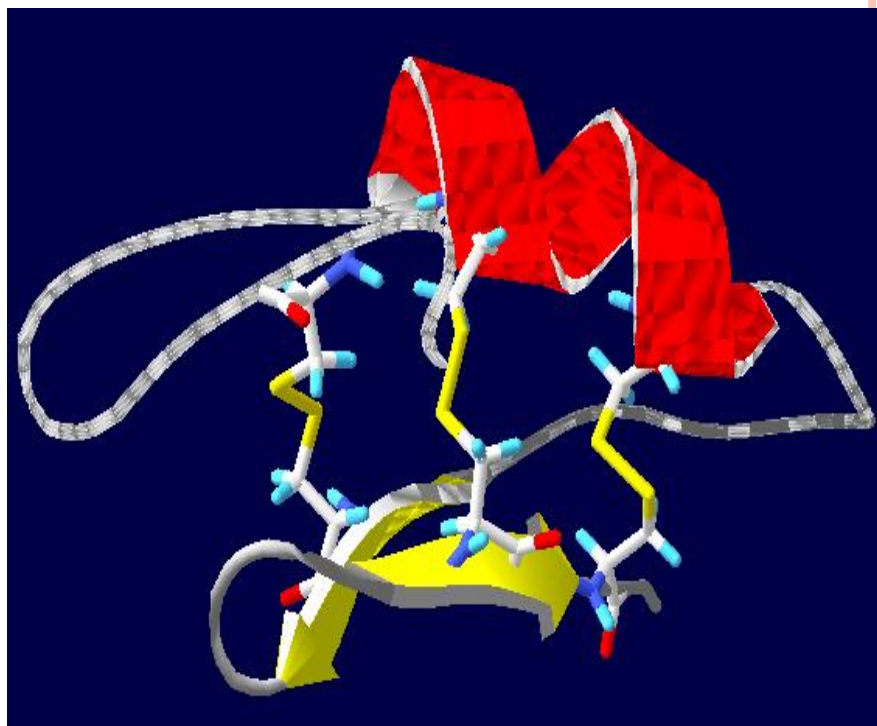
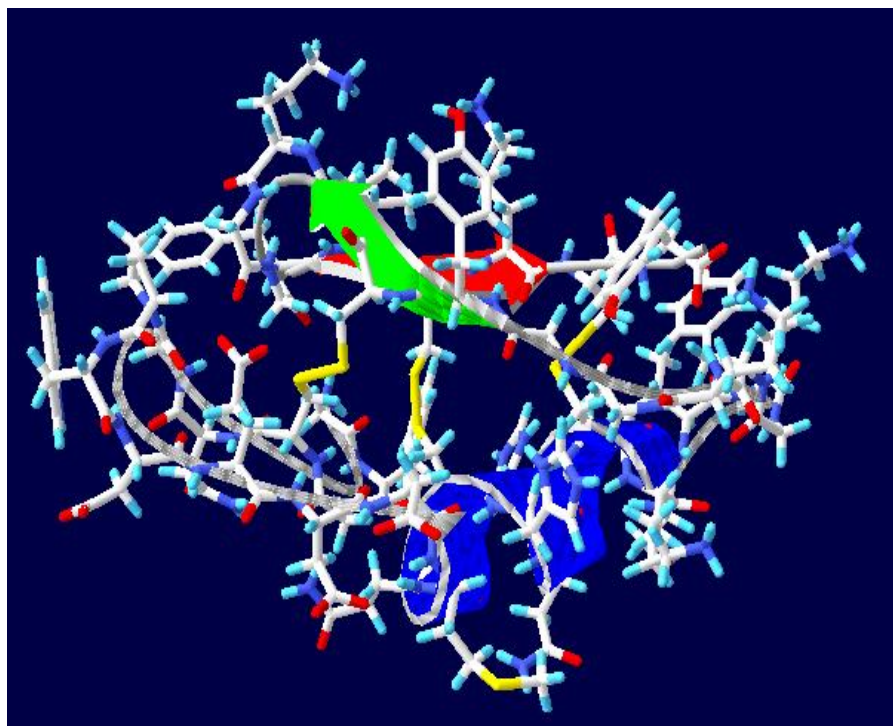
Swiss-PdbViewer 4.1.0

File Edit Select Build Tools Fit Display Color Prefs SwissModel Wind Help



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Plectasin三级结构由**3对二硫键**（Cys4-Cys30, Cys15-Cys37, Cys19-Cys39）、**N端1个 $\alpha$ -螺旋**和**C端2个反向平行的 $\beta$ -折叠结构**以及螺旋和折叠结构间的**Loop区**组成的 **CS $\alpha$  $\beta$** （半胱氨酸稳定的 $\alpha$ 螺旋/ $\beta$ 折叠基序）结构。



## 2

# 设计原则和改造方向

设计原则：

1. 改变的氨基酸个数1-3个；
2. 保留带电氨基酸(D,R,K)和芳香族氨基酸(F,Y,W)等潜在活性位点；
3. 选取和同源序列有差异的aa位点进行替换；
4. 不改变或尽量少改变CS $\alpha\beta$ 结构；
5. 净电荷变化+3~+8。

改造方向：

1. 改变二硫键；
2. 改变保守位点；
3. 增加电荷，改变疏水性；
4. 减少电荷，改变疏水性；
5. 电荷不变，改变疏水性。

NZ2114:Plectasin 第9, 13, 14位氨基酸Asp, Met, Gln突变为Asn, Leu, Arg  
MP2244:NZ2114第9, 13, 14分别突变为Gln, Val, Lys



### 3. 理化参数分析

1



#### ProtParam tool

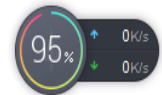
**ProtParam** ([References](#) / [Documentation](#)) is a tool which allows the computation of various physical and chemical parameters for a given protein stored in [Swiss-Prot](#) or [TrEMBL](#) or for a user entered protein sequence. The computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY) ([Disclaimer](#)).

Please note that you may only fill out **one** of the following fields at a time.

Enter a Swiss-Prot/TrEMBL accession number (AC) (for example **P05130**) or a sequence identifier (ID) (for example **KPC1\_DROME**):

Or you can paste your own amino acid sequence (in one-letter code) in the box below:

```
GFGCNCFWDEDDMQCHNHCKSIKGYKGGYCAKGGFVCK
```



ProtParam可以分析蛋白质的相对分子质量、氨基酸组成、PI、电荷数、消光系数、半衰期、不稳定系数、总亲水性指数（GRAVY）及脂肪族系数等。



MP2244:

GFGCNGPWQEDDVKCHNHCKSIKGYKGGYCAKGGFVCKCY

Number of amino acids: 40

Molecular weight: 4389.01

Theoretical pI: 8.61

Total number of negatively charged residues (Asp + Glu): 3

Total number of positively charged residues (Arg + Lys): 6

The instability index (II) is computed to be: 21.69

Aliphatic index: 26.75

Grand average of hydropathicity (GRAVY): -0.647



Plectasin:GFGCNGPWDEDDMQCHNHCKSIKGYKGGYCAKGGFVCKCY

NZ2114: GFGCNGPWNEDDLRCHNHCKSIKGYKGGYCAKGGFVCKCY

MP2244: GFGCNGPWQEDDVKCHNHCKSIKGYKGGYCAKGGFVCKCY

|           | 分子量     | 净电荷 | 等电点  | 不稳定系数 | 脂肪族系数 | 疏水力矩 | GRAVY  |
|-----------|---------|-----|------|-------|-------|------|--------|
| Plectasin | 4407.99 | 1   | 7.77 | 13.82 | 19.50 | 0.82 | -0.695 |
| NZ2114    | 4417.03 | 3   | 8.62 | 25.49 | 29.25 | 0.48 | -0.672 |
| MP2244    | 4389.01 | 3   | 8.61 | 21.69 | 26.75 | 0.56 | -0.647 |



3

通过helixturnhelix软件 (<http://emboss.bioinformatics.nl/>)，对Plectasin, NZ2114, MP2244的二级结构进行分析。

EMBOSS explorer

## helixturnhelix

Identify nucleic acid-binding motifs in protein sequences ([read the manual](#))

Only required fields are visible. ([show optional fields](#))

Input section

Select an input sequence. Use one of the following three fields:

1. To access a sequence from a database, enter the USA here:
2. To upload a sequence from your local computer, select it here:  未选择任何文件

3. To enter the sequence data manually, type here:





|           |       |  |
|-----------|-------|--|
|           |       | . 10 . 20 . 30 . 40                      |
|           |       | GFGCNGPWDEDDMQCHNHCKSIKGYKGGYCAKGGFVCKCY |
| Plectasin | helix | HHHHHHHHH                                |
|           | sheet | EE EEE                                   |
|           | turns | TTTT TT TTTTTTTTTT TTTT TT               |
|           | coil  | CC C                                     |
|           |       | . 10 . 20 . 30 . 40                      |
|           |       | GFGCNGPWNEDDLRCHNHCKSIKGYKGGYCAKGGFVCKCY |
| NZ2114    | helix | HHHH                                     |
|           | sheet | EE EEE                                   |
|           | turns | TTTT TT TTT TTTTTTTTTTTT TTTT TT         |
|           | coil  | CC C                                     |
|           |       | . 10 . 20 . 30 . 40                      |
|           |       | GFGCNGPWQEDDVKCHNHCKSIKGYKGGYCAKGGFVCKCY |
| Mp2244    | helix | HH HHHHHH                                |
|           | sheet | EE EEE                                   |
|           | turns | TTTT TT TT TTTTTTTTTT TTTT TT            |
|           | coil  | CC                                       |

结果显示， Plectasin突变为NZ2114后， $\alpha$ -螺旋（H）度由**37.5%**降到**16.7%**，而其它结果没有发生太多变化。MP2244 的 $\alpha$ -螺旋度相对于NZ2114提高至**33.3%**，C端转角（T）结构和无规卷曲（C）结构降低，有利于形成 $\beta$ 折叠刚性结构。



4

通过 [protscale](http://web.expasy.org/protscale/) 软件 (<http://web.expasy.org/protscale/>) 预测两亲性。



ProtScale

[Home](#) | [Contact](#)

## ProtScale

**ProtScale** [[Reference](#) / [Documentation](#)] allows you to compute and represent the profile produced by any amino acid scale on a selected protein.

An **amino acid scale** is defined by a numerical value assigned to each type of amino acid. The most frequently used scales are the hydrophobicity or hydrophilicity scales and the secondary structure conformational parameters scales, but many other scales exist which are based on different chemical and physical properties of the amino acids. This program provides 57 predefined scales entered from the literature.

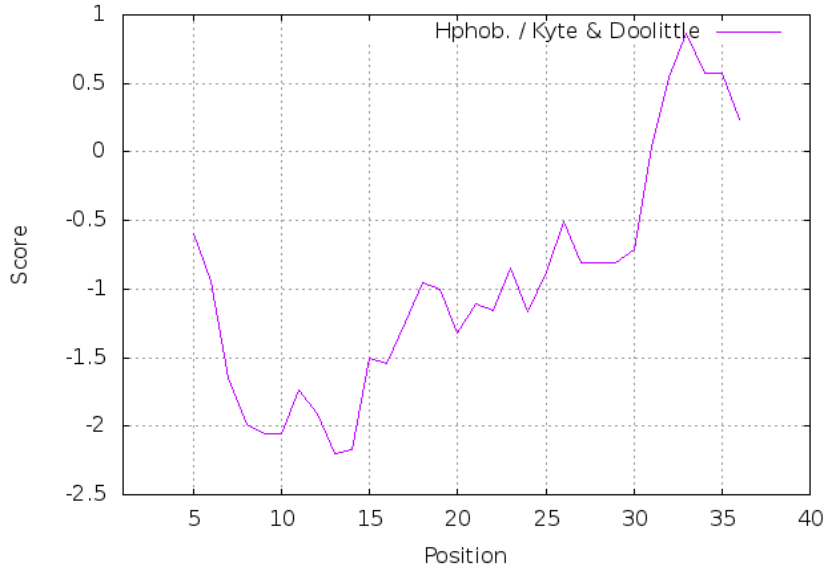
Enter a [UniProtKB/Swiss-Prot](#) or [UniProtKB/TrEMBL](#) accession number (AC) (e.g. **P05130**) or a sequence identifier (ID) (e.g. **KPC1\_DROME**):

Or you can paste your own sequence in the box below:

```
GFGCNGPWDEDDMQCHNHCKSIKGYKGGYCAKGGFVCKCY
```

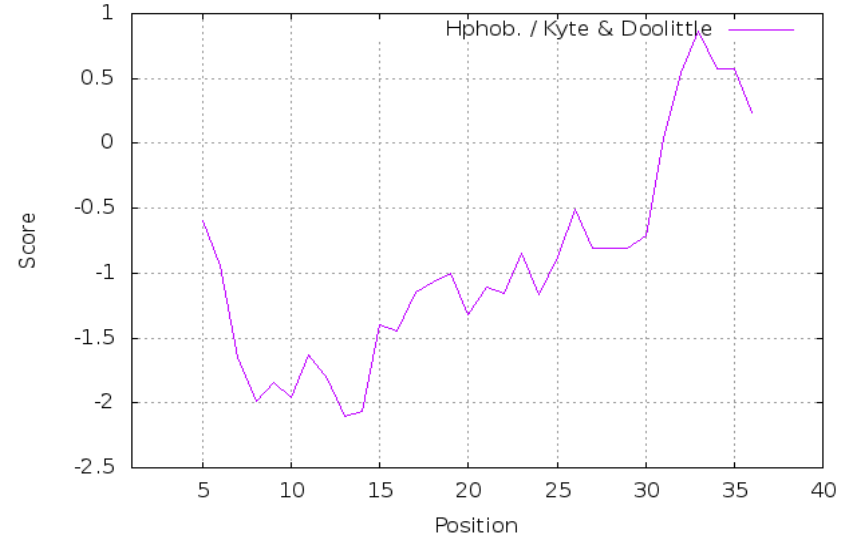


ProtScale output for user\_sequence



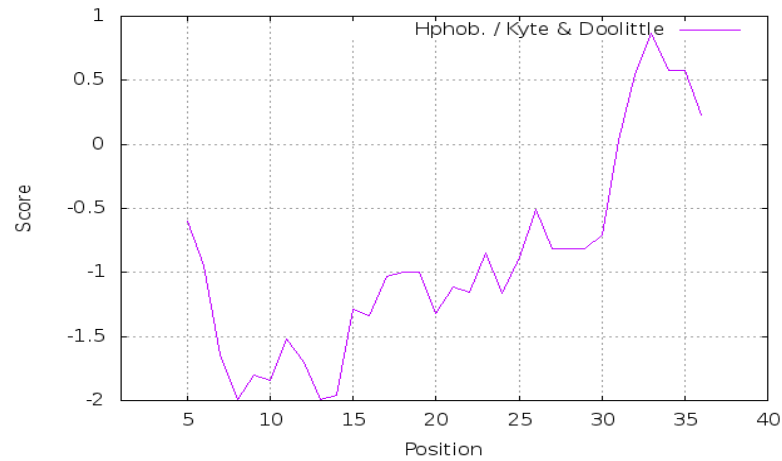
Plectasin

ProtScale output for user\_sequence



NZ2114

ProtScale output for user\_sequence



MP2244



## 4. 结构预测



**BIOZENTRUM**  
University of Basel  
The Center for Molecular Life Sciences

**SWISS-MODEL**

用SWISS-MODEL进行蛋白质三维结构预测：

SWISS-MODEL是一个有注解的基于同源建模的蛋白质结构服务器，它与专家蛋白质分析系统（Expert Protein Analysis System, ExPASy）网站是紧密相联系的，其中有的也是来自于DeepView（Swiss Pdb-Viewer）程序的，目前有30000个蛋白质三维结构模型。



5

通过 **protscale** 软件 (<http://web.expasy.org/protscale/>) 预测两亲性。



ProtScale

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## ProtScale

**ProtScale** [[Reference](#) / [Documentation](#)] allows you to compute and represent the profile produced by any amino acid scale on a selected protein.

An **amino acid scale** is defined by a numerical value assigned to each type of amino acid. The most frequently used scales are the hydrophobicity or hydrophilicity scales and the secondary structure conformational parameters scales, but many other scales exist which are based on different chemical and physical properties of the amino acids. This program provides 57 predefined scales entered from the literature.

Enter a [UniProtKB/Swiss-Prot](#) or [UniProtKB/TrEMBL](#) accession number (AC) (e.g. **P05130**) or a sequence identifier (ID) (e.g. **KPC1\_DROME**):

Or you can paste your own sequence in the box below:

```
GFGCNGPWDEDDMQCHNHCKSIRGYKGGYCAKGGFVCKCY
```



# SWISS-MODEL操作界面图



SWISS-MODEL

Modelling Repository Tools Documentation Log in Create Account

## Start a New Modelling Project

Target

Sequence(s):

*(Format must be  
FASTA, Clustal,  
plain string, or a valid  
UniProtKB AC)*

Paste your target sequence(s) or UniProtKB AC here

+ Upload Target Sequence File... Validate

Project Title:

Untitled Project

Email:

Optional

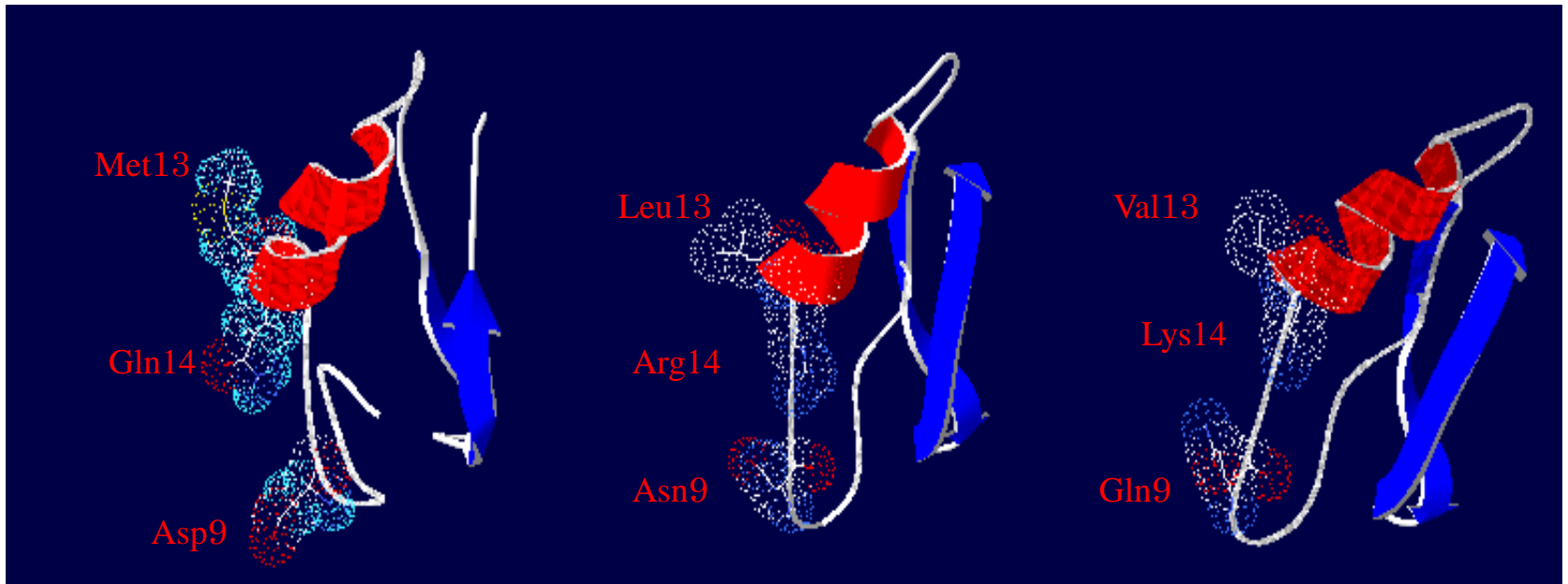
Search For Templates

Build Model

## Supported Inputs

- Sequence(s)
- Target-Template Alignment
- User Template
- DeepView Project





Plectasi  
n

NZ2114

MP2244

相同点：三者均由 $\alpha$ -螺旋，Loop环， $\beta$ -折叠组成的CS $\alpha\beta$ 结构。

不同点：

1. NZ12114和MP2244相对于Plectasin，其 $\beta$ 折叠结构变长。
2. MP2244相对于Plectasin和NZ2114， $\alpha$ -螺旋与N端无规卷曲发生位移。



# 致谢:

1. 首先感谢罗老师为我们讲授生物信息学这门课程，对我们今后的课题研究非常有帮助，感谢罗老师为我们提供这次学习交流的机会。
2. 然后感谢师兄师姐给予我的指导和帮助。
3. 最后感谢小组成员的积极配合，团结协作，希望今后继续交流。





谢谢!

Thank you!

