

PlifePred2: Peptide Half-Life Prediction and Design Suite

Version 2.0 – Standalone & Design Modules

1.0 Introduction

PlifePred2 is a machine learning–based software suite developed for:

- Prediction of peptide half-life
- Stability evaluation of modified peptides
- In silico mutational design
- Physicochemical characterization

The tool supports:

- Natural peptides
 - Modified peptides
 - Single mutation scanning
 - Physicochemical profiling
-

2.0 System Architecture

PlifePred2 consists of two major modules:

2.1 Prediction Module (`plifepred2.py`)

Performs half-life prediction using trained ML models.

2.2 Design Module (`plifepred2_design.py`)

Generates all possible single mutants and ranks them based on predicted stability.

3.0 Supported Models

Model 1 – Natural Peptides

- Feature type: QSO
- Input: Natural amino acid sequences
- Model: plifepred2_natural_model.sav
- Use case: Native peptide half-life estimation

Model 2 – Modified Peptides

- Feature type: 100 selected descriptors
- Includes modification flags
- Model: plifepred2_model.sav
- Use case: Modified peptide stability prediction

4.0 Input Requirements

4.1 FASTA Format

Input must be a multi-FASTA file:

```
>Sequence1  
ACDEFGHIKLMNPQRSTVWY  
>Sequence2
```

GAVLGKTRWQLMN

Accepted Residues:

A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y

Sequence Length:

12–100 amino acids

Invalid sequences are automatically filtered.

5.0 Modification Flags (Model 2 Only)

| Flag | Description |
|------|---------------------------------|
| 0 | D-amino acid |
| 1 | C-terminal modification |
| 2 | N-terminal modification |
| 3 | Cyclization |
| 4 | Post-translational modification |

Example:

-f 1,3

6.0 Physicochemical Properties

Optional properties can be computed using **-p**.

| Code | Property |
|------|------------------------|
| 1 | Hydrophobicity |
| 2 | Steric hindrance |
| 3 | Hydropathicity |
| 4 | Amphipathicity |
| 5 | Hydrophilicity |
| 6 | Net hydrogen |
| 7 | Charge |
| 8 | Isoelectric point (pl) |
| 9 | Molecular weight |

Example:

-p 1,7,8

7.0 Command Line Usage

7.1 Prediction Module

Natural Peptides

```
python plifepred2.py -i input.fasta -m 1 -p 5,4 -o output.csv
```

Modified Peptides

```
python plifepred2.py -i input.fasta -m 2 -f 1,2 -o output.csv
```

With Properties

```
python plifepred2.py -i input.fasta -m 2 -f 1 -p 8,9 -o output.csv
```

8.0 Output Format

Output CSV includes:

```
| ID | Sequence | HalfLife | [Optional Properties] |
```

Example:

```
ID,Sequence,HalfLife,pI,Mol wt  
Seq1,ACDEFGHIK,0.82,8.21,1024.4
```

9.0 Design Module

The design module performs exhaustive single mutation scanning.

9.1 Workflow

1. Reads input sequence
2. Generates all single mutants
3. Extracts full descriptor set
4. Applies trained ML model
5. Ranks mutants by predicted stability

9.2 Command

```
python design.py -i input.fasta -o design_output.tsv
```

For modified peptides:

```
python design.py -i input.fasta -f 1 -o design_output.tsv
```

10.0 Design Output

Output columns:

| Seq_ID | Mutant_ID | Sequence | Score | [Properties] |

Example:

| | | | |
|-------------|------|--------------|------|
| Original_ID | A15V | ACDEFGHIK... | 0.91 |
| Original_ID | L3F | ACFEFGHIK... | 0.88 |

Mutants are sorted by best predicted half-life.

11.0 Citation

If you use PlifePred2 in your research, please cite: