

# PEPstrMOD Manual

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Considering the importance of modifications incorporated in the peptides for making them of therapeutic use, we have developed PEPstrMOD method for predicting the tertiary structure of peptide with different modifications. The PEPstrMOD method is available online and can be accessed at (<http://osddlinux.osdd.net/raghava/pepstrmod>). In this manual, we briefly cover the demonstrations on how to effectively use the PEPstrMOD and its different modules. If you have any queries or suggestions regarding this manual or the PEPstrMOD web service, kindly contact at [raghava@imtech.res.in](mailto:raghava@imtech.res.in).

The architecture of this manual is constructed as per the left menu of PEPstrMOD web service, which represents different modules of PEPstrMOD. Each module is covered with detailed information on how to use it for incorporating different modifications in the peptide sequence.

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## Home Page

The home page of PEPstrMOD can be assessed at (<http://osddlinux.osdd.net/raghava/pepstrmod>).

## 1. Natural Peptides Module

This module implements the original PEPstr (<http://imtech.res.in/raghava/pepstr/>) algorithm with some updates to predict the tertiary structure of peptide with natural amino acids. This module is further sub-divided in 4 sub modules.

### 1.1. Beginner sub module of Natural Peptides Module

It is a simple interface in which user needs to give an input sequence and his/her email address. User can also select different advance options, which include ‘Simulation Time’, ‘Peptide Environment’, ‘Download topology files’, ‘Cluster Analysis’, ‘Download whole trajectory’, ‘Energy and RMS graphs’. These advanced options assist the user in further analysis of their peptides. Figure 1A displays the graphical representation of how to use this module effectively, Figure 1B shows the result page obtained after the input is processed and Figure 1C displays the predicted tertiary structure of the example peptide (GSEKMSTAISVLLAQAVFLLLTSQR) using PEPstrMOD. The functions of the advanced options are briefly discussed below.

#### Simulation Time

PEPstrMOD performs molecular dynamics simulations of the structure to assist the correct folding of the peptide as well as to avoid any steric clashes occurring between the side-chains of different amino acids in the peptide. Simulation time refers to the total time for which a user wants to perform simulation of their peptide before obtaining the final predicted structure. Simulation time is expressed in terms of picoseconds (ps). By default the simulation time is set to 100ps. A user has the facility to select simulation time of 50ps or 100ps for simulating their peptides.

#### Peptide Environment

Peptides are dynamic entities and so is their tertiary structure. It is known that a peptide may adopt different structures in different environments like aqueous/hydrophobic. Therefore, PEPstrMOD assists a user in selecting the environment in which he/she wants to predict the tertiary structure of their peptide. By default, ‘vacuum’ is selected as an environment, but a user has an option to select ‘hydrophilic’ or ‘hydrophobic’ environments. For hydrophilic environment, the peptide is simulated inside a box of water using TIP3P and SPC water model in AMBER and GROMACS software respectively. For hydrophobic environment, a box of methane molecule is used.

#### Download topology files

Standard molecular dynamics packages like AMBER and GROMACS generate a topology file for the input peptide for performing simulations. These topology files can be used to further

simulate the structure using the same molecular dynamics software on any personal computer. PEPstrMOD provides an option to download these topology files so that the user can use these topology files to further simulate and analyze his/her peptide.

#### Download whole trajectory

During molecular dynamics simulation, the structures of the peptide are saved in a file after few timesteps. (10 structures saved in an interval of 1ps). This saved file containing the structures obtained after whole molecular dynamics is called as trajectory file. Therefore in a simulation time of 100ps, a total of 1000 peptide tertiary structures are generated in the trajectory file. PEPstrMOD provides an option to download this trajectory file, which is useful to analyze all the structures of the peptides. Using this trajectory file and the topology files, a user can further extend the simulation of that peptide or analyze all the structures of that peptide on his/her personal computer.

#### Cluster Analysis

PEPstrMOD performs the cluster analysis on all the structures present in the trajectory file to give the best representative structures from all clusters identified. For each of the structures present in the trajectory file, RMSD is calculated with every other structure. Structures having similar RMSD values are clustered together to form 1 cluster. Therefore, we may get 1 or more than 1 cluster(s). From each cluster, the representative structure is selected which is closest to the centroid of the cluster and given as an output structure. PEPstrMOD also gives an option to download all the representative structures of each cluster.

#### Energy and RMS graphs

To assist the user in the analysis of their peptide, PEPstrMOD gives the variation/fluctuation of energy during the simulation in the form of energy graph. This helps a user in analyzing the peptide conformations, which are energetically stable during the simulations. PEPstrMOD also provides the RMS fluctuations of the peptide structure during molecular dynamics simulations. From the trajectory file, the structure having lowest energy is used as a representative structure. Next, for each structure in the trajectory file, RMSD is calculated with the reference structure. Finally an RMS graph is sketched to show the RMS fluctuations of the peptide structures during the simulations. This helps in selecting the peptide conformation with least fluctuations in the structure.

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### Welcome to Natural Peptides Module for Beginners

This module is used to predict the tertiary structure of peptides having natural amino acids. A user needs to submit the peptide sequence in the text box along with the email address. An example sequence can be used by clicking on the 'Example Sequence' button. For further analysis, users can select the advanced options given below. For more information click [help](#)

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#### Peptide Sequence Submission Form

Peptide sequence in plain text format Example Sequence

GSEKMSTAISVLLAQAVLLLSQR Input sequence

Email Address: sandybioteck@gmail.com e-mail address

**Advanced Options:** CLICK

Simulation Time: 100 picoseconds Peptide Environment: Vacuum

Download topology files: Yes Cluster Analysis: Yes

Download whole trajectory: Yes Energy and RMS graphs: Yes

Reset or clear form Submit sequence for prediction Advanced options

Submit the sequence

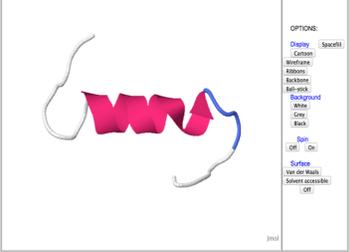
Figure 1A. Graphical representation showing how to use beginner sub module of natural peptide module of PEPstrMOD.

Welcome to Result Page of Natural Peptide Module for Beginners

Visualization | Download data | Energy Graph | RMS Graph | Animation | Cluster Analysis

**Structure visualization in PEPstrMOD**

This page display the structural view of peptide structure in Jmol applet. Please install the [JAVA](#) 230 for proper display and functioning of this page. For more information see [HELP](#) page.



Further modification

Welcome to Result Page of Natural Peptide Module for Beginners

Visualization | Download data | Energy Graph | RMS Graph | Animation | Cluster Analysis

This page allows user to download PDB file and other data for further simulation and analysis

**Download PDB**

**Topology and Coordinate files**

Download Topology (.top) file of your peptide: [topus4154.top](#)

Download Coordinate (.crd) file of your peptide: [crdus4154.crd](#)

**Trajectory file**

Download trajectory file of your peptide: [trajus4154.traj](#)

**Cluster Analysis (if Selected)**

A total of 1 cluster(s) are made

Best Structure from Cluster: [peptide1cluster.pdb](#)

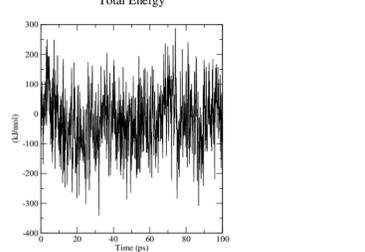
Download Best 5 Structures from all 1 clusters: [all5clusters.tgz](#)

Welcome to Result Page of Natural Peptide Module for Beginners

Visualization | Download data | Energy Graph | RMS Graph | Animation | Cluster Analysis

**Total Energy**

Total Energy graph of the complete simulation. The graph display the variation of the energy during the entire simulation time.

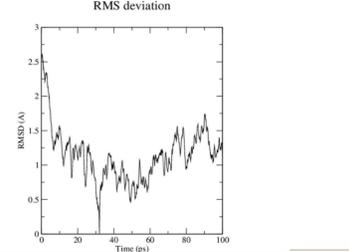


Welcome to Result Page of Natural Peptide Module for Beginners

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**RMS deviation**

The RMS graph of the complete simulation. First the structure with lowest energy is extracted and structures after every picosecond are also extracted. Second, RMSD is computed between the lowest energy structure and all other structure.

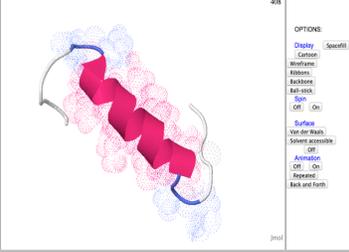


Welcome to Result Page of Natural Peptide Module for Beginners

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**Animation of the Simulation in PEPstrMOD**

This page display the animation of simulation in Jmol applet. The module take few minutes to load, depending upon the size of the peptide. Please install the [JAVA](#) 230 for proper display and functioning of this page. For more information see [HELP](#) page.



Welcome to Result Page of Natural Peptide Module for Beginners

Visualization | Download data | Energy Graph | RMS Graph | Animation | Cluster Analysis

**Cluster Analysis**

This page display the representative PDB from different clusters. During simulation the peptide adopt different conformations. Each cluster represent a conformation adopted by peptide during simulation. The overlay structure of representative cluster will help the user in understanding rigid and variable region of peptide. Green color shows the predicted peptide and representative cluster images are displayed in other colors (red etc). Please install the [JAVA](#) 230 for proper display and functioning of this page. For more information see [HELP](#) page.

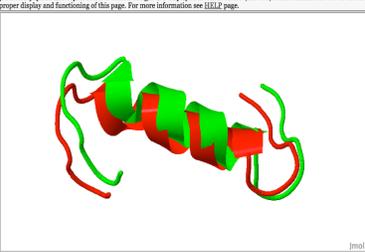


Figure 1B. Snapshot of the result page of PEPstrMOD which is divided into six tabs as follows: a) Visualization: The predicted peptide structure is displayed using Jmol Viewer. Residues falling in helix, strand and turns are displayed in pink, yellow and blue colors respectively. Modified residues are displayed in green color. b) Download Data: Links to download the predicted peptide structure along with other files. c) Energy Graph: Graphical representation of variation in energy of the system during simulation. d) RMS Graph: Graphical representation of the deviation in RMS during simulation. e) Animation: All the structures present in the trajectory of the simulation are displayed in an animated form. f) Cluster Analysis: The predicted structure is aligned with the representative structures obtained by cluster analysis.

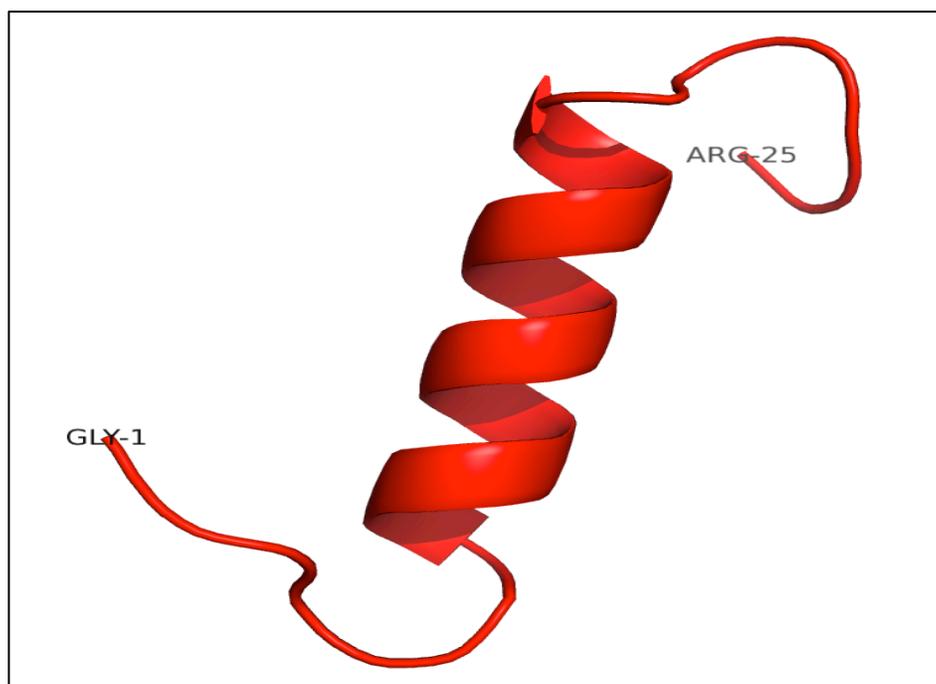


Figure 1C. Tertiary structure of the peptide (sequence GSEKMSTAISVLLAQAVFLLLTSQR) predicted using beginner sub module of natural peptide module of PEPstrMOD.

## 1.2. Expert sub module of Natural Peptides module

This facility allows a user to design his/her peptide using defined secondary structure (helix/sheet) or backbone torsion angles phi and psi. A user can select the region of a peptide to adopt a particular secondary structure or any shape by input of phi and psi torsion angles. The interface is designed in a very user-friendly manner, which makes very easy to design a peptide. Figure 2A and 2B shows the snapshot of this sub module and how to use it effectively. Figure 2C shows the predicted tertiary structure of the peptide (sequence FATMRYPSDSDE) using PEPstrMOD with user assisted modeling of the peptide with secondary structural restraints. Figure 2D shows the snapshot of the same peptide tertiary as Figure 2C but without any user aided modeling. There is a clear-cut adoption of helical structure by the peptide after application of user-aided helical restraints (Figure 2C) in an otherwise loop structure (Figure 2D).

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### Welcome to Natural Peptides Module for Experts

This module predicts the tertiary structure of peptides having natural amino acids. It also gives the facility to the users to design the peptides with desirable restraints like specific secondary structure in specific region of the peptide. In the next step, a user can specify the region of the peptide to adopt specific secondary structure or dihedral angles. For more information click [help](#)

#### Peptide Sequence Submission Form

Peptide sequence in plain text format

Example Sequence

FATMRYPSDSDE

Reset or clear form Submit and Go to Next Step

Click here for example sequence

Input sequence

Click here to go to next step

Figure 2A. Snapshot of expert sub module of natural peptide module of PEPstrMOD and labels to assist users to use the service effectively. At this step a user only needs to input the sequence and click on the link to go to the next step.

No	Residue	Secondary Structure	Phi	Psi
1	F	Coil	180.0	180.0
2	A	Coil	180.0	180.0
3	T	Helix	-60	-40
4	M	Helix	-60	-40
5	R	Helix	-60	-40
6	Y	Helix	-60	-40
7	P	Helix	-60	-40
8	S	Helix	-60	-40
9	D	Helix	-60	-40
10	S	Helix	-60	-40
11	D	Coil	180.0	180.0
12	E	Coil	180.0	180.0

Email Address:

**Advanced Options:** CLICK

Simulation Time:  Peptide Environment:

Download topology files:  Cluster Analysis:

Download whole trajectory:  Energy and RMS graphs:

Figure 2B. This snapshot is the extension of the Expert sub module of “Natural Peptide” module of PEPstrMOD. The peptide sequence submitted in the previous step is displayed here in the tabular format and a user can apply secondary structural or torsion angle restraints to model his/her peptide. In this example peptide shown in this figure, secondary structural (helical) restraints are applied to the region from 3<sup>rd</sup> to 10<sup>th</sup> residue of the peptide.

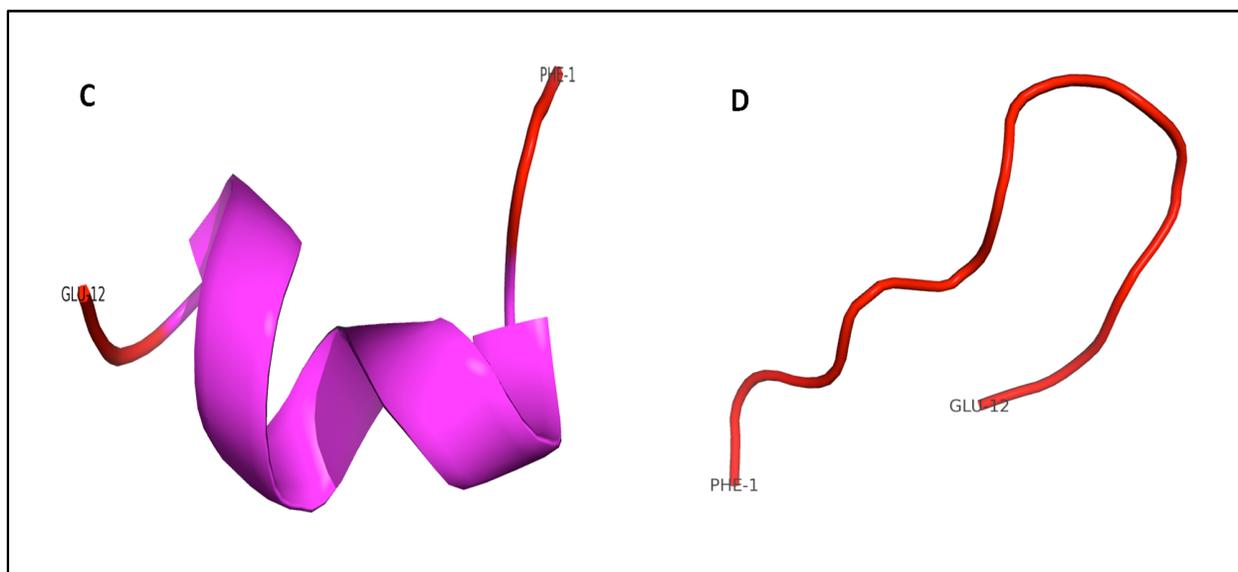


Figure 2. Snapshot of the predicted tertiary structure of peptide (sequence FATMRYPSDSDE) C) using expert sub module of “Natural Peptide” module of PEPstrMOD. The peptide region from 3-10 residues was modeled and restrained as helix (as shown in previous Figure 2B), which is shown in magenta color in the predicted tertiary structure. D) Predicted tertiary structure of same peptide without applying any modeling of secondary structure or torsion angle restraints.

### 1.3. Structure Modification sub module of Natural Peptides Module

If a user already has a peptide tertiary structure in PDB format and want to further design the region of the peptide with his/her intuition of backbone torsion angles or specific secondary structure or a mutation at a specific position, then this option allows him/her to do this task in a very easy manner. This option will be very helpful in user aided designing of the peptide tertiary structure. Figure 3 shows the graphical representation of this feature showing how to input the peptide sequence and other advanced options effectively.

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### Welcome to Natural Peptides Module for Structure Modification

This module allows users to modify a peptide tertiary structure in PDB file format or PDB file. The peptide structure can be modeled/designed with user defined secondary structure or dihedral angle restraints. User needs to provide the peptide structure in PDB format either in text area or upload the PDB file of his/her peptide. Existing structure in the PDB can also be modified if the PDB code is provided. For more information click [help](#)

#### Peptide Structure Submission Form

**Peptide structure in PDB format**

```

HEADER ACETYLCHOLINE RECEPTOR 19-DEC-97 1A11
ATOM 1 N GLY A 1 15.933 2.572 -9.465 1.00 0.00 N
ATOM 2 CA GLY A 1 15.248 3.793 -8.849 1.00 0.00 C
ATOM 3 C GLY A 1 14.402 4.670 -9.708 1.00 0.00 C
ATOM 4 O GLY A 1 13.527 4.207 -10.413 1.00 0.00 O
ATOM 5 H1 GLY A 1 16.550 2.876 -10.244 1.00 0.00 H

```

**OR**

Upload a peptide structure in PDB format  No file chosen

**OR**

Enter PDB ID

Click here for example structure

Input structure

Click here to go to next step

Figure 3. Snapshot of the “Structure Modification” sub module of the “Natural Peptide” module of the PEPstrMOD showing how to use the features and options effectively.

#### 1.4. Multiple Sequences sub module of Natural Peptides Module

If a user has multiple peptides, he/she can process such peptides with this sub module. A user needs to give the multiple peptide sequences in a single line format (i.e. each peptide sequence in one line) and his/her email address (Figure 4). Due to the limited computational power and to avoid heavy load on our server, we do not perform the molecular dynamics simulation of these peptides, which is the last step of PEPstrMOD. Instead we only perform energy minimization step on the initial predicted structure. The results (PDB structure file) for all the peptides is compressed and provided in a .tgz format. This can be extracted using following command in linux “tar -xzf file.tgz” or using the WinRAR utility in Window based operating system. The result is also provided in the tabular format in which the user can download individual peptide structure (Figure 4).

**Welcome to Natural Peptide Module for Multiple Sequences**

This module is used to input multiple peptide sequences at once. The predicted tertiary structures of all the input peptides are provided to the user. This module however has one limitation that the final step of PEPstrMOD algorithm (involving molecular dynamics of the peptide structure) is not done and the energy minimized peptide structure is given as final output. To limit the load on the PEPstrMOD web service and to provide opportunities to every user, this module is devoid of molecular dynamics step. For more information click [help](#)

**Peptide Sequence Submission Form**

Peptide sequences in single line format [Example Sequences](#)

GSEKMTAISVLLAQAVFLLLSQSR  
STAIISVLLAQAVFLLLEKM  
SEQACRTYEAFSRCEPQLLA  
GCCSDPRCAWRC

Email Address:

**Welcome to Result Page of Multiple Sequences Module**

All Minimized Structures of your Sequences: [output73273.tgz](#)

No.	Sequence	PDB Download link	View Structure in Jmol
1	GSEKMTAISVLLAQAVFLLLSQSR	<a href="#">PDB file</a>	<a href="#">View in Jmol</a>
2	STAIISVLLAQAVFLLLEKM	<a href="#">PDB file</a>	<a href="#">View in Jmol</a>
3	SEQACRTYEAFSRCEPQLLA	<a href="#">PDB file</a>	<a href="#">View in Jmol</a>
4	GCCSDPRCAWRC	<a href="#">PDB file</a>	<a href="#">View in Jmol</a>

Figure 4. Snapshot of the Multiple Sequences sub module of the Natural Peptides module of PEPstrMOD.

## 2. D Amino Acids Module

This module helps a user to swap or flip the stereo-chemical arrangement of an amino acid from L-form to D- form (i.e. incorporation of D-amino acid). This module is further divided into three sub modules.

### 2.1. Beginner sub module of D Amino Acids Module

A user needs to input the peptide sequence and after submitting the sequence, a user friendly tabular interface is displayed with amino acid and its corresponding stereo-chemistry in drop down menu (Figure 5A and 5B). A user can select either the Laevo (L) or the Dextro (D) stereo-chemistry of any amino acid in the peptide sequence and proceed further for the prediction of its tertiary structure.

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### Welcome to D-amino acids Module for Beginners

This module predicts the tertiary structure of peptides having D-amino acids. A user needs to provide peptide sequence as input. In next step, a simple tabular representation allows to select the amino acids, which needs to be changed from L- to D- amino acids. For more information click [help](#)

#### Peptide Sequence Submission Form

Peptide sequence in plain text format

Click here for example sequence

Input sequence

Click here to go to next step

Figure 5A. Snapshot of Beginner sub module of “D-amino acid” module of PEPstrMOD labeling the inputs and options required for the peptide submission. After submission, the next step involves selection of residues and its L/D stereo-chemistry.

No	Residue	Stereochemistry
1	G	Laevo (L)
2	S	Laevo (L)
3	E	Laevo (L)
4	K	Laevo (L)
5	M	Laevo (L)
6	S	Laevo (L)
7	T	Dextro (D)
8	A	Dextro (D)
9	I	Dextro (D)
10	S	Dextro (D)
11	V	Laevo (L)
12	L	Laevo (L)
13	L	Laevo (L) Dextro (D)
14	A	Laevo (L)
15	G	Laevo (L)

Figure 5B. This snapshot is the extension of the Beginner sub module of “D-amino acid” module of PEPstrMOD. Using the dropdown menu in front of each amino acid of the peptide sequence (submitted in the previous step), a user can select the amino acid to be either Laevo or Dextro. Residues selected with Dextro stereo-chemistry are labeled with red circle.

## 2.2. Expert sub module of D Amino Acids Module

This is a simple interface which allows a user to enter a peptide sequence with L-amino acids in capital letter and D-amino acids in small letter representation as shown in Figure 6A. Alternatively, a user can enter the peptide sequence with all amino acids in capital letter and specify the positions of amino acids in a text box which needs to be converted in D-amino acids as shown in Figure 6B. Finally, Figure 6C represents the predicted tertiary structure of the example peptide using this module where D-amino acids are represented in magenta color.

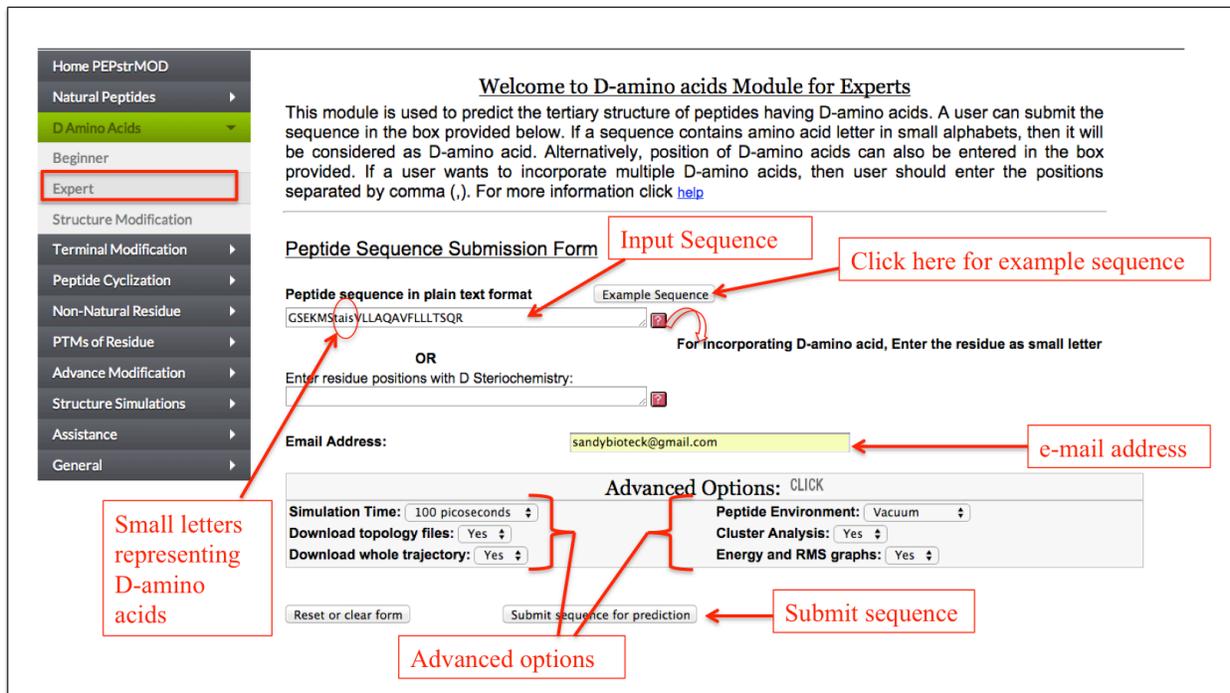


Figure 6A. Graphical representation of “Expert” sub module of “D Amino acid” module of PEPstrMOD. Residues “tais” are written in small letter representing D-amino acids and therefore are treated as such in prediction of the tertiary structure of the peptide.

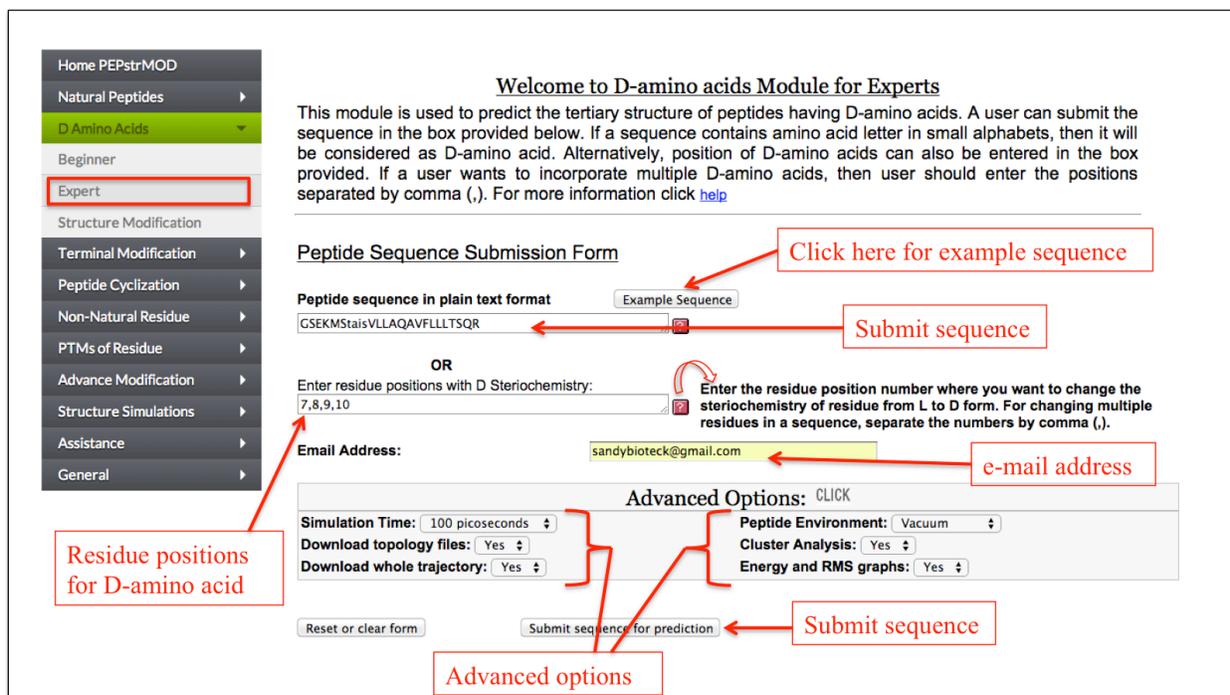


Figure 6B. This figure also represents “Expert” sub module of “D Amino acid” module of PEPstrMOD but rather than representing D-amino acids as small letter, their respective positions in the peptide sequence are given as input in the box provided.

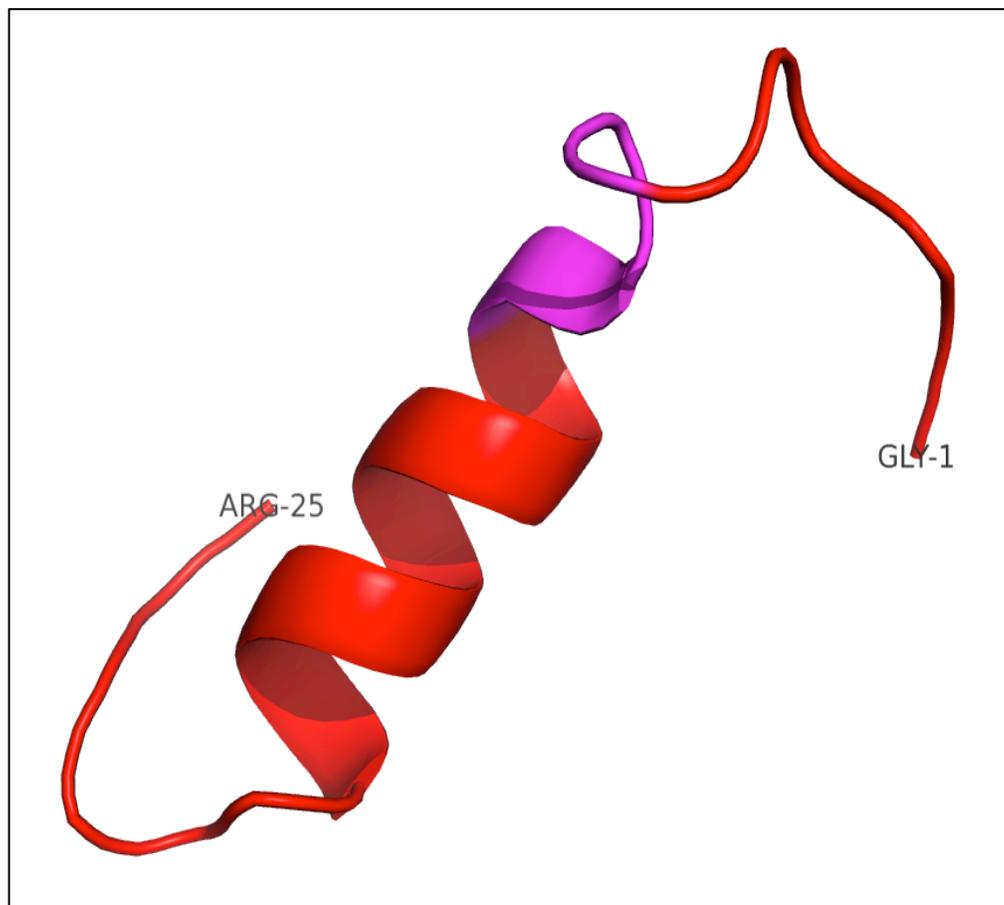


Figure 6C. Predicted tertiary structure of example peptide (GSEKMSTAISVLLAQAVFLLLSQR) using “Beginner/Expert” sub module of the “D-amino acid” module of PEPstrMOD. The D-amino acids (7-10 residues) are represented using magenta color.

### 2.3. Structure Modification sub module of D Amino Acids Module

This facility is same as described above with the additional option of incorporating D-amino acids at any position in the tertiary structure of the input peptide.

### 3. Terminal Modification Module

#### 3.1. Sequence Modification sub module of Terminal Modification Module

If a user wants to block the N and C terminus of the peptide by capping these terminals with some chemical groups, he/she can perform the capping using this module of PEPstrMOD (Figure 7A). A user can add acetylation at the N-terminus and amidation or n-methylamide groups at the C-terminus residue of the peptide respectively. Figure 7B shows the predicted tertiary structure after applying the terminal modifications using this module.

The screenshot displays the 'Sequence Modification' sub-module of the PEPstrMOD software. On the left is a navigation menu with 'Terminal Modification' selected and 'Sequence Modification' highlighted. The main content area features a 'Welcome to Terminal Modification Module' message and a 'Peptide Sequence Submission Form'. The form includes an 'Input Sequence' field containing 'GSEKMSTAIIVLLAQAVFLLLSQR', an 'Example Sequence' button, and dropdown menus for 'N-terminal Modification' (set to 'Acetylation') and 'C-terminal Modification' (set to 'Amidation'). Below these are 'Advanced Options' for simulation time (100 picoseconds), topology files, whole trajectory, peptide environment (Vacuum), cluster analysis, and energy/RMS graphs. A 'Submit Sequence' button is at the bottom left, and a 'Submit sequence for prediction' button is at the bottom right. Red annotations with arrows point to these key features.

Figure 7A. Graphical representation of the “Sequence Modification” sub module of “Terminal Modification” module of PEPstrMOD and labels showing how to input the peptide sequence along with selecting other options.

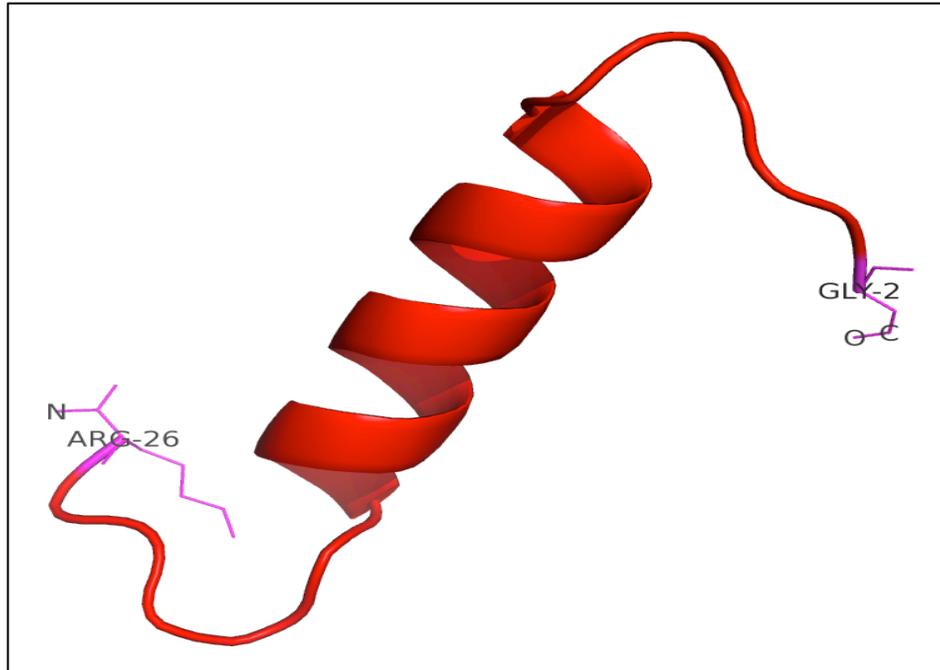


Figure 7B. Predicted tertiary structure of the example peptide (GSEKMSTAISVLLAQAVFLLLSQR) with its modified N and C terminal residues. N-terminal is modified using acetylation while C-terminal using amidation and shown in magenta color.

### 3.2. Structural Modification sub module of Terminal Modification Module

This facility is same as described in the “Natural Peptides” module of PEPstrMOD with additional option of incorporating N and C terminal modifications in the tertiary structure of the input peptide as shown in Figure 8.

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**Welcome to Terminal Modification Module for Structure Modification**

This module is used to input an already available peptide tertiary structure in PDB file format and subsequently if a structure contains any N-terminal and C-terminal Modifications user can choose the appropriate options which is given below to modify its structure. For more information click [help](#)

**Peptide Structure Submission Form**

**Peptide structure in PDB format**

TARGET	34855
MODEL	1 REFINED
PARENT	N/A
ATOM	1 N GLY 1 -2.298 2.638 0.297 1.00 0.00
ATOM	2 CA GLY 1 -0.970 2.367 0.840 1.00 0.00
ATOM	3 C GLY 1 -0.386 1.025 0.380 1.00 0.00
ATOM	4 O GLY 1 -1.080 0.011 0.328 1.00 0.00
ATOM	5 N SER 2 0.912 0.994 0.049 1.00 0.00

**OR**

Upload a peptide structure in PDB format  No file chosen

**OR**

Enter PDB ID

Email Address:

N-terminal Modification:  C-terminal Modification:

**Advanced Options:**

Simulation Time:  Peptide Environment:

Download topology files:  Cluster Analysis:

Download whole trajectory:  Energy and RMS graphs:

Figure 8. The snapshot of “Structure Modification” sub module of “Terminal Modification” module of PEPstrMOD showing the labels on how to use the module effectively.

## 4. Peptide Cyclization Module

A user can cyclize a peptide either from N to C linkage by forming a bond between N-terminal and C-terminal residue or with the help of disulfide bridges between pair of cysteine residues. This module has 3 sub modules, which are as follows:

### 4.1. N-C Cyclization sub module of Peptide Cyclization Module

This facility provides a user to cyclize his/her peptide using N-to-C cyclization (Figure 9A). In this type of cyclization, the N-terminal residue is linked to C-terminal residue by making a bond between them (Figure 9B).

The screenshot shows the 'Peptide Sequence Submission Form' for N-C cyclization. The interface includes a sidebar menu on the left with 'N-C Cyclization' highlighted. The main content area has a title 'Welcome to Peptide Cyclization Module for N-C cyclization' and a brief description. Below this is the submission form with fields for 'Peptide sequence in plain text format' (containing 'GSEKMSTAISVLLAQAVFLLTSQR'), 'Email Address' (containing 'sandybiotech@gmail.com'), and 'Advanced Options' (including Simulation Time, Download topology files, Download whole trajectory, Peptide Environment, Cluster Analysis, and Energy and RMS graphs). A 'Submit sequence for prediction' button is at the bottom. Red arrows and boxes point to various elements: 'Click here for example sequence' points to the 'Example Sequence' button; 'Input Sequence' points to the peptide sequence text box; 'e-mail address' points to the email text box; 'Advanced options' points to the 'Advanced Options' section; and 'Submit sequence' points to the 'Submit sequence for prediction' button.

Figure 9A. Labels showing the use of “N-C Cyclization” sub module of “Peptide Cyclization” module of PEPstrMOD. The structure predicted by this feature is shown in the next figure.

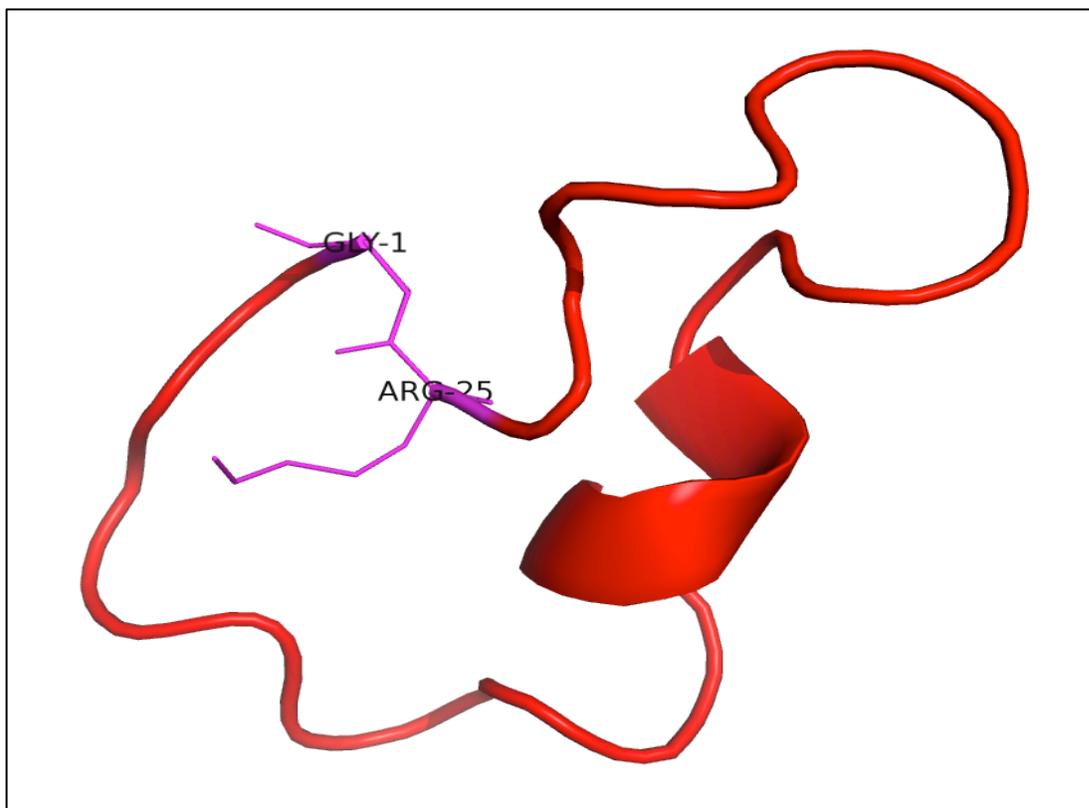


Figure 9B. The tertiary structure of example peptide predicted using “N-C Cyclization” sub module of “Peptide Cyclization” module of PEPstrMOD. The N to C cyclization is achieved by making a bond between N and C atoms of the terminal residues and is shown in magenta color.

## 4.2. Disulfide Bridge (S-S) sub module of Peptide Cyclization Module

This facility provides a user to cyclize his/her peptide by making a disulfide bridge between pair of cysteine molecules. A user needs to enter the position of cysteine residues, which needs to be linked using a disulfide bond. If a disulfide bond needs to be incorporated between CYS2 and CYS8, a user needs to submit residue position “2-8” in the text box. For more than 1 disulfide bridges, (e.g. CYS2 and CYS8, CYS3 and CYS12), a user needs to submit residue positions “2-8, 3-12” in the text box as shown in Figure 10A. Figure 10B shows the predicted structure of the example peptide (GCCSDPRCAWRC) with disulfide bonds between 2<sup>nd</sup> and 8<sup>th</sup> cysteine and 3<sup>rd</sup> and 12<sup>th</sup> cysteine respectively.

The screenshot shows the web interface for the Disulfide Bridge (S-S) sub-module. On the left is a navigation menu with 'Disulfide Bridge (S-S)' highlighted. The main content area has a 'Welcome to Peptide Cyclization Module for Disulfide Bridge (S-S)' message. Below this is the 'Peptide Sequence Submission Form' with an 'Example Sequence' button. The 'Peptide sequence in plain text format' field contains 'GCCSDPRCAWRC'. The 'Cysteine positions for di-sulfide bridge (S-S Bond):' field contains '2-8,3-12'. The 'Email Address:' field contains 'sandybiotech@gmail.com'. An 'Advanced Options' section includes 'Simulation Time: 100 picoseconds', 'Download topology files: Yes', 'Download whole trajectory: Yes', 'Peptide Environment: Vacuum', 'Cluster Analysis: Yes', and 'Energy and RMS graphs: Yes'. A 'Submit sequence for prediction' button is at the bottom right. Red annotations include a box around 'Disulfide Bridge (S-S)', arrows pointing to the sequence and cysteine position fields, a box around the email field, and boxes around the 'Submit sequence for prediction' button and the 'Advanced Options' section.

Figure 10A. Graphical representation of the “Disulfide Bridge” sub module of the “Peptide Cyclization” module of the PEPstrMOD. The different labels show how to input the peptide sequence along with the cysteine positions, which needs to be joined by a disulfide bridge.



Figure 10B. Predicted tertiary structure of the example peptide (GCCSDPRCAWRC) showing disulfide bridge between 2<sup>nd</sup> and 8<sup>th</sup> cysteine and 3<sup>rd</sup> and 12<sup>th</sup> cysteine respectively in magenta color.

#### 4.3. Structure Modification sub module of Peptide Cyclization Module

This facility is same as described in the “Natural Peptides” module of PEPstrMOD with additional option of incorporating disulfide bridges between cysteine residues in the tertiary structure of the input peptide.

### 5. Non-Natural Residue Module

This module implements the incorporation of any non-natural amino acid in the peptide at a desired position. For the incorporation of non-natural residues, we have integrated two special force field libraries named Forcefield\_NCAA and SwissSideChain. Forcefield\_NCAA and SwissSideChain library contains force field parameters for 147 and 210 non-natural amino acids

respectively. The force field parameter for each of the non-natural amino acid is derived from the closest structurally similar natural amino acid. These libraries allow successful incorporation and subsequent simulation of a non-natural amino acid in the peptide. It is divided into 3 sub modules.

### 5.1. Beginner sub module of Non-Natural Residue Module

This sub module provides an easy alternative to incorporate non-natural modifications in the peptide. At first step, a user only needs to enter the peptide sequence, and select either Forcefield\_NCAA or SwissSideChain library of non-natural amino acids (Figure 11A). After submission, a user friendly interface in the tabular form is displayed in which each amino acid of the input peptide sequence is present in one column and a drop down menu of all possible non-natural amino acids occur in the next column. A user can easily replace any amino acid of the peptide with any non-natural modification from the drop down menu (Figure 11B).

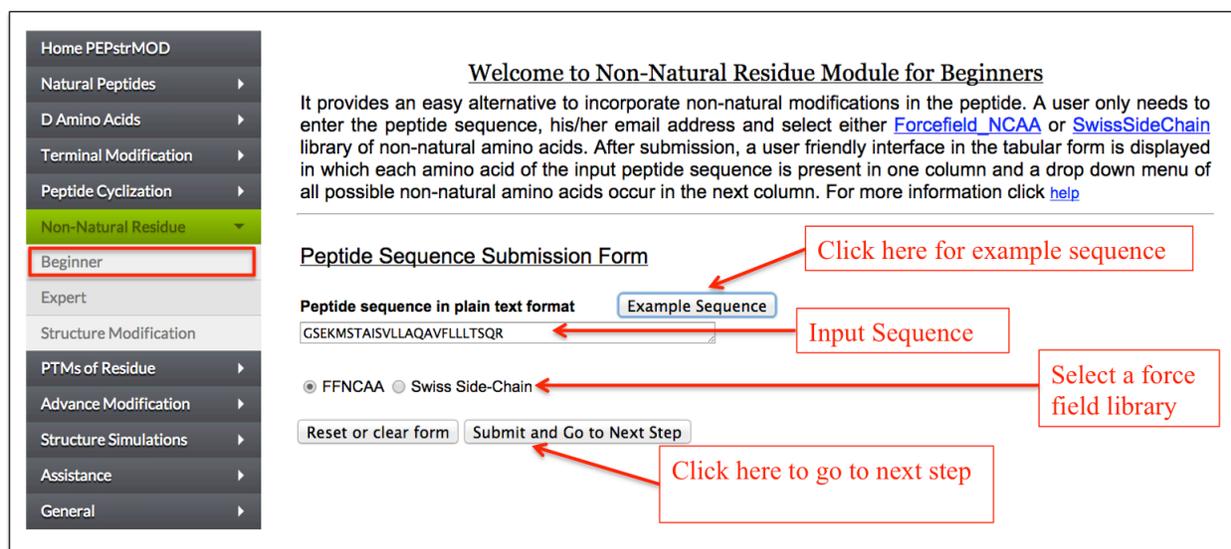


Figure 11A. Snapshot of “Beginner” sub module of the “Non-Natural Residue” module of PEPstrMOD with labels showing how to use its options effectively. The option to incorporate non-natural modification is provided in the next page as shown in the next figure.

No	Residue	Modification Type
1	G	No Modification
2	S	No Modification
3	E	No Modification
4	K	diethylalanine
5	M	No Modification
6	S	No Modification
7	T	No Modification
8	A	nmethylvaline
9	I	phenylserine homoserine nmethylserine betaserine
10	S	<b>Threonine Based</b> nmethylthreonine omethylthreonine betathreonine
11	V	<b>Valine Based</b> rpluslpmethylvaline norvaline hydroxynorvaline
12	L	nmethylvaline betavaline
13	L	<b>Tryptophan Based</b> 5methyltryptophan 1methyltryptophan nmethyltryptophan 2hydroxytryptophan 4aminoltryptophan
14	A	No Modification
15	Q	No Modification
16	A	No Modification
17	V	No Modification
18	F	No Modification
19	L	No Modification

Figure 11B. This snapshot is the extension of the previous figure 11A in which peptide sequence was taken as input. In this step, non-natural modifications can be incorporated by using dropdown menu in front of amino acids of the input peptide sequence. In this figure the incorporated non-natural residues are labeled with red ellipse.

## 5.2. Expert sub module of Non-Natural Residue Module

It allows users to incorporate any non-natural amino acid at any position in the peptide in a single step. A user needs to input the sequence of his/her peptide and needs to select either Forcefield\_NCAA or SwissSideChain library. After selecting a particular force field library, the respective non-natural amino acids are displayed in a drop down menu along with the natural amino acids from which their parameters are derived. For example, diethylalanine non-natural amino acid in the ForceField\_NCAA library is similar to alanine and therefore all the non-natural amino acids (like diethylalanine, N-methylalanine etc.) similar to alanine are displayed in a drop down in front of alanine. In this way, a user can incorporate multiple non-natural amino acids at different positions in the peptide (Figure 12A). Figure 12B shows the predicted tertiary structure after incorporation of non-natural modification.

**Welcome to Non-Natural Residue Module for Experts**

This module is used to predict the tertiary structure of peptides having non-natural modified residues. Two libraries of non-natural residues are implemented which are [Forcefield\\_NCAA](#) and [SwissSideChain](#) covering 147 and 210 non-natural residues respectively. A user needs to select either of the force field libraries and needs to enter the position of residue in the box in front of the selected modified residue. For more information click [help](#)

---

**Peptide Sequence Submission Form**

Peptide sequence in plain text format

Email Address:

FFNCAA  Swiss Side-Chain

**SELECT THE MODIFIED RESIDUES AND SUBMIT POSITION OF MODIFICATION**

ALA:	diethylalanine	4, 15	ARG:	N-methylarginine	
GLY:	vinylglycine		PHE:	biphenylalanine	
TYR:	o-tyrosine		TRP:	beta-tryptophan	
SER:	homoserine		LEU:	homoleucine	
CYS:	benzylcysteine		GLN:	3-methyl glutamine	
ILE:	beta-isoleucine		LYS:	beta-lysine	
MET:	(R)-(+)-alpha-methylvaline		ASP:	beta-aspartic acid	
VAL:	N-methyl-valine	8	GLU:	N-methylglutamic acid	
ASN:	hydroxynorvaline		THR:	beta-threonine	
HIS:	N-methyl-histidine		Others:	Ornithine	

**Advanced Options:**

Simulation Time:

Download topology files:

Download whole trajectory:

Peptide Environment:

Cluster Analysis:

Energy and RMS graphs:

Figure 12A. Labels showing how to use the “Expert” sub module of the “Non-Natural Residue” module of PEPstrMOD.

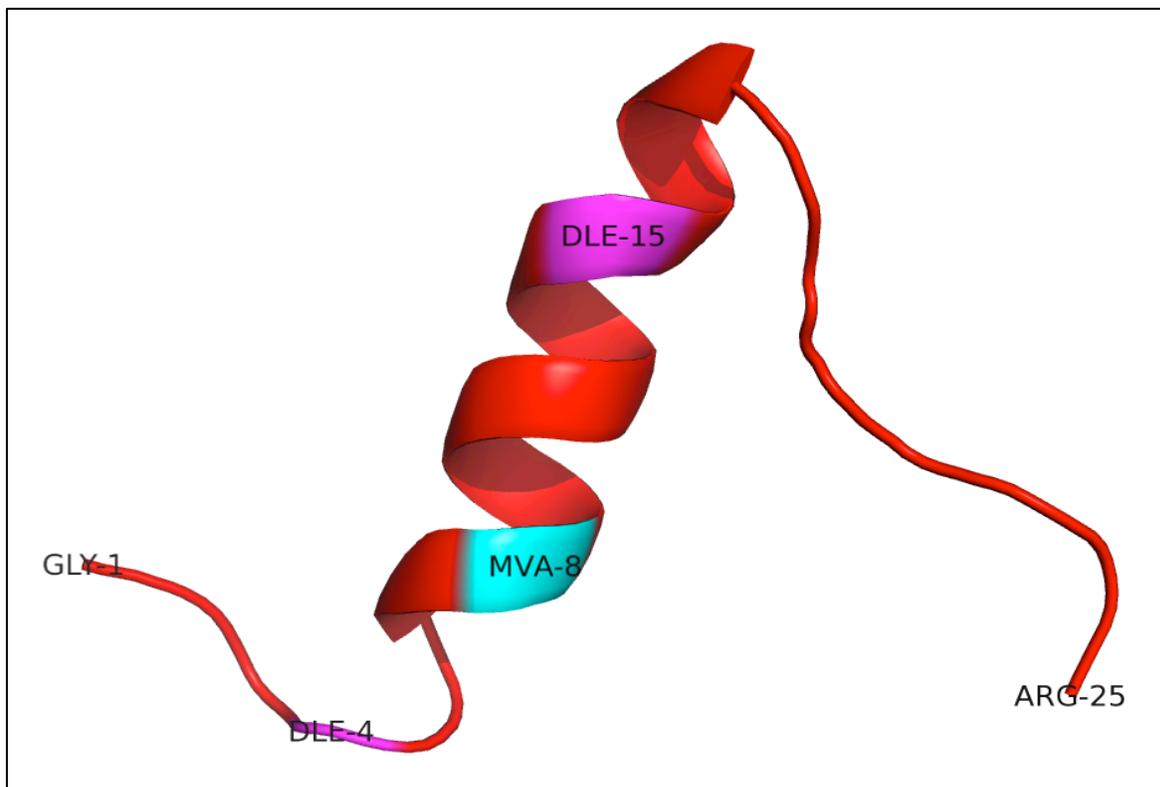


Figure 12B. Snapshot of the predicted tertiary structure of the peptide using “Expert” sub module of the “Non-natural Residue” module of PEPstrMOD. In this figure, the non-natural residue “diethylalanine” occupies position 4<sup>th</sup> and 15<sup>th</sup> (magenta color) and “methylvaline” occupies position 8<sup>th</sup> (cyan color) in the peptide sequence.

### 5.3. Structure Modification sub module of Non-Natural Residue Module

This facility is same as described in the “Natural Peptides” module of PEPstrMOD with additional option of incorporating non-natural modifications as described above in “Expert” sub module. Therefore, using this sub module, a user can incorporate non-natural modifications in any peptide with already available tertiary structure as shown in Figure 13.

**Peptide Structure Submission Form**

Peptide structure in PDB format Example Structure

```

HEADER ACETYLCHOLINE RECEPTOR 19-DEC-97 1A11
ATOM 1 N GLY A 1 15.933 2.572 -9.465 1.00 0.00 N
ATOM 2 CA GLY A 1 15.248 3.793 -8.849 1.00 0.00 C
ATOM 3 C GLY A 1 14.402 4.670 -9.708 1.00 0.00 C
ATOM 4 O GLY A 1 13.527 4.207 -10.413 1.00 0.00 O
  
```

**OR**

Upload a peptide structure in PDB format  No file selected.

**OR**

Enter PDB ID  Example ID

Email Address:

FFNCAA  Swiss Side-Chain

**SELECT THE MODIFIED RESIDUES AND SUBMIT POSITION OF MODIFICATION**

ALA: diethylalanine	4,15	ARG: N-methylarginine	
GLY: vinylglycine		PHE: biphenylalanine	
TYR: o-tyrosine		TRP: beta-tryptophan	
SER: homoserine		LEU: homoleucine	
CYS: benzylcysteine		GLN: 3-methyl glutamine	
ILE: beta-isoleucine		LYS: beta-lysine	
MET: ethionine		ASP: beta-aspartic acid	
VAL: N-methyl-valine	8	GLU: N-methylglutamic acid	
ASX: (R)-(+)-alpha-methylvaline		THR: beta-threonine	
Other: N-methyl-valine			
hydroxynorvaline			
beta-valine			

Figure 13. The snapshot of “Structure Modification” sub module of the “Non-Natural Residue” module of PEPstrMOD. The labels show how to use it effectively.

## 6. PTMs of Residue Module

This module allows a user to incorporate different types of frequently occurring post-translational modifications (PTM) in the peptide and subsequent prediction of their tertiary structure. For incorporating PTMs we integrated Forcefield\_PTMs library covering 32 frequently occurring PTMs in proteins and peptides. This module is divided into 3 sub modules.

## 6.1. Beginner sub module of PTMs of Residue Module

This sub module provides an easy alternative to post-translationally modify residues in the peptide. At first step, a user only needs to enter the peptide sequence (Figure 14A). After submission, a user-friendly interface in the tabular form is displayed in which each amino acid of the input peptide sequence is present in one column and a drop down menu of all possible PTMs of that residue in the next column. A user can easily do post-translational modification of any residue using this facility (Figure 14B).

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- PTMs of Residue**
  - Beginner**
  - Expert
- Structure Modification
- Advance Modification
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**Welcome to PTMs of Residue Module for Beginners**

It provides an easy alternative to incorporate PTMs in the peptide. A user only needs to enter the peptide sequence and his/her email address. After submission, a user friendly interface in the tabular form is displayed in which each amino acid of the input peptide sequence is present in one column and a drop down menu of all possible PTMs occur in the next column. For more information click [help](#)

**Peptide Sequence Submission Form**

Click here for example sequence

Peptide sequence in plain text format  Example Sequence

Input Sequence

Reset or clear form Submit and Go to Next Step

Click here to go to next step

Figure 14A. Snapshot of “Beginner” sub module of the “PTM” module of PEPstrMOD with labels showing how to input the peptide sequence. The option to incorporate PTMs is provided in the next page as shown in the next figure.

No	Residue	Modification Type
1	G	Not Applicable
2	S	phosphoserine
3	E	No Modification
4	K	No Modification
5	M	Not Applicable
6	S	phosphoserine
7	T	phosphothreonine
8	A	No Modification phosphothreonine phosphothreonine(neg)
9	I	Not Applicable
10	S	No Modification
11	V	Not Applicable
12	L	Not Applicable
13	L	Not Applicable
14	A	Not Applicable

Figure 14B. This snapshot is the extension of the previous figure 14A in which peptide sequence and e-mail address was taken as input. In this step, PTMs can be incorporated by using dropdown menu in front of amino acids of the input peptide sequence. In this figure the incorporated PTMs are labeled with red ellipse.

## 6.2. Expert sub module of PTMs of Residue Module

It allows a user to do post-translational modification of any residue in the peptide in a single step. For each residue a limited set of PTMs are possible. Only the possible modifications are displayed in front of the amino acids in the drop down menu followed by a text box. For incorporating a PTM, a user needs to enter the position of the amino acid in the respective text box (Figure 15A). Figure 15B shows the predicted tertiary structure obtained using this sub module.

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- General

### Welcome to PTMs of Residue Module for Experts

This module is used to predict the tertiary structure of peptides having post-translational modifications (PTMs). [Forcefield\\_PT](#) library is implemented covering 32 frequently occurring PTMs. A user needs to enter the position of residue in the box in front of the selected modified residue. For more information click [help](#)

#### Peptide Sequence Submission Form

[Click here for example sequence](#)

**Peptide sequence in plain text format**  [Example Sequence](#)

**Email Address:**  **e-mail address**

**SELECT THE PTM AND SUBMIT POSITION OF PTM**

ARG: (omega)-methylarginine	<input type="checkbox"/>	PHE: Dihydroxyphenylalanine	<input type="checkbox"/>
TYR: Phosphotyrosine (neutral)	<input type="checkbox"/>	TRP: 7-hydroxytryptophan	<input type="checkbox"/>
SER: Phosphoserine (neutral)	<input type="checkbox"/>	LEU: N-methyl-leucine	<input type="checkbox"/>
CYS: Cysteinepersulfide	<input type="checkbox"/>	PRO: 3,4-hydroxyproline	<input type="checkbox"/>
LYS: 5-hydroxylysine	<input type="checkbox"/>	MET: hydroxyl-methionine	<input type="checkbox"/>
GLU: 1-Carboxyglutamic Acid	<input type="checkbox"/>	THR: <b>Phosphothreonine (neutral)</b>	<input type="checkbox"/>
Others: Ornithine	<input type="checkbox"/>	Phosphothreonine (-2 charge)	<input type="checkbox"/>

**Position of residues to modify**

**Possible PTMs for the respective amino acid**

**Advanced Options:** CLICK

Simulation Time:  Peptide Environment:

Download topology files:  Yes Cluster Analysis:  Yes

Download whole trajectory:  Yes Energy and RMS graphs:  Yes

**Submit sequence**

**Advanced options**

Figure 15A. Snapshot of the “Expert” sub module of the “PTM” module of PEPstrMOD with labels showing how to input peptide sequence and PTMs with their positions.

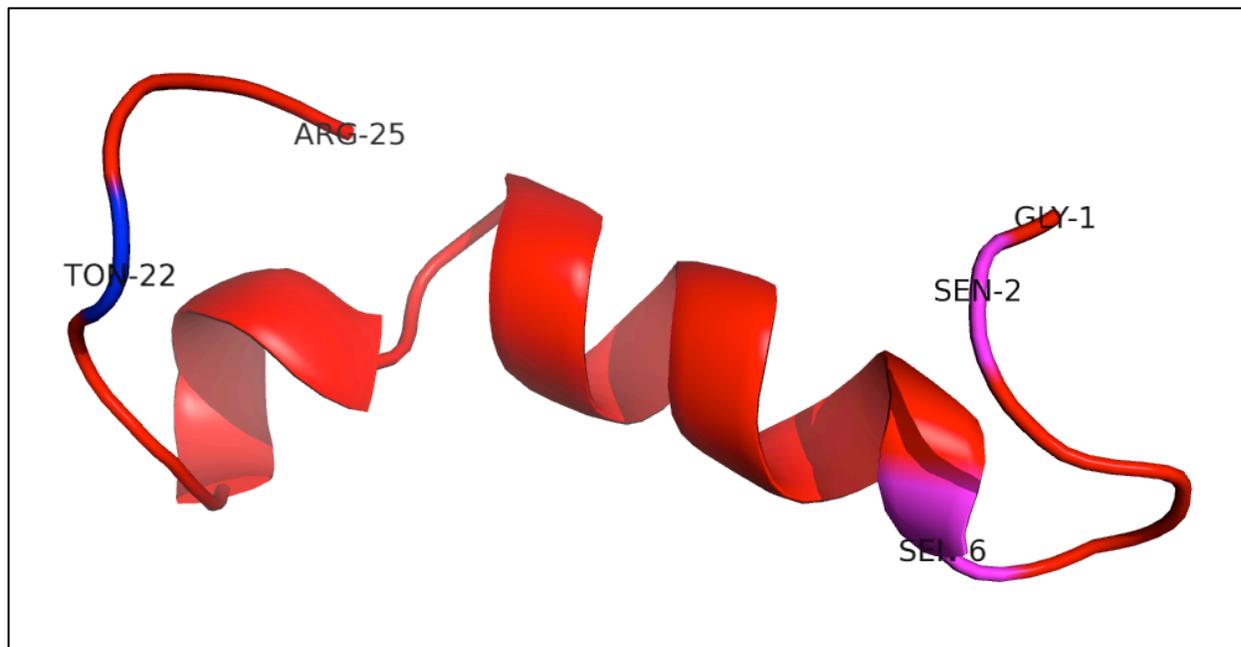


Figure 15B. Snapshot showing the predicted tertiary structure of the example peptide with PTMs phosphoserine at positions 2, 6 (magenta color) and phosphothreonine at 22 (blue color).

### 6.3. Structure Modification sub module of PTMs of Residue Module

This facility is same as described in the “Natural Peptides” module of PEPstrMOD with additional option of incorporating post-translational modifications as described above in “Expert” sub module. Therefore, using this sub module, a user can incorporate PTMs in any peptide with already available tertiary structure (Figure 16).

**Peptide Structure Submission Form**

Peptide structure in PDB format Example Structure

HEADER	ACETYLCHOLINE	RECEPTOR				19-DEC-97	1A11		
ATOM	1	N	GLY	A	1	15.933	2.572	-9.465	1.00 0.00 N
ATOM	2	CA	GLY	A	1	15.248	3.793	-8.849	1.00 0.00 C
ATOM	3	C	GLY	A	1	14.402	4.670	-9.708	1.00 0.00 C
ATOM	4	O	GLY	A	1	13.527	4.207	-10.413	1.00 0.00 O

**OR**

Upload a peptide structure in PDB format  No file selected.

**OR**

Enter PDB ID   Input structure

Email Address:  e-mail address

**SELECT THE MODIFIED RESIDUES AND SUBMIT POSITION OF MODIFICATION**

ARG: (omega)-methylarginine	<input type="text"/>	PHE: Dihydroxyphenylalanine	<input type="text"/>
TYR: Phosphotyrosine (neutral)	<input type="text"/>	TRP: 7-hydroxytryptophan	<input type="text"/>
SER: Phosphoserine (neutral)	2,6	LEU: N-methyl-leucine	<input type="text"/>
CYS: Cysteinepersulfide	<input type="text"/>	PRO: 3,4-hydroxyproline	<input type="text"/>
LYS: 5-hydroxylysine	<input type="text"/>	MET: hydroxyl-methionine	<input type="text"/>
GLU: 1-Carboxyglutamic Acid (r	<input type="text"/>	THR: Phosphothreonine (neutral)	22
Others: Ornithine	<input type="text"/>	Phosphothreonine (neutral)	
		Phosphothreonine (-2 charge)	

Possible PTMs for the respective amino acid

Submit sequence

Position of residues to modify

Figure 16. Snapshot of the “Structure Modification” sub module of the “PTM” module with labels showing how to input the peptide tertiary structure in PDB format and other options effectively.

## 7. Advance Modification Module

This is the most useful module of PEPstrMOD. A user can encounter a situation in which he/she has a peptide with non-natural amino acid at one residue, PTM at other residue along with few residues as D-amino acids and capping of terminal residues. This module provides facility to incorporate multiple modifications (non-natural/PTMs/cyclization/D-amino acids etc) in the peptide sequence at different positions. It is divided into 3 sub modules.

### 7.1. Beginner sub module of Advance Modification Module

This sub module provides an easy alternative to incorporate multiple modifications at different positions in a peptide. It is an amalgamation of the features of all the above modules giving an option to perform multiple modifications at different positions in a peptide. At first step, a user only needs to enter the peptide sequence (Figure 17A). After submission, a user friendly interface in the tabular form is displayed in which each amino acid of the input peptide sequence is present in one column and different modifications corresponding to that amino acid are displayed in the drop down menu in subsequent columns. For disulfide bridge, a text box appears in front of cysteine residue. If a user wants to make disulfide bridge between 3<sup>rd</sup> and 12<sup>th</sup> cysteine, he/she needs to enter 1 in the text box in front of 3<sup>rd</sup> cysteine and enter 2 in front of 12<sup>th</sup> cysteine (Figure 17B). Figure 17C shows the predicted tertiary structure after incorporation of different modifications using this module.

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**Welcome to Advance Modification Module for Beginners**

It provides an easy alternative to incorporate multiple modifications (non-natural, PTM, D-amino acids, cyclization etc.) at different positions in the peptide. A user only needs to enter the peptide sequence and his/her email address. After submission, a user friendly interface in the tabular form is displayed in which amino acids of peptide sequence are present in one column and the other columns contain dropdown menu for incorporating different modifications. For more information click [help](#)

**Peptide Sequence Submission Form**

Peptide sequence in plain text format

Click here for example sequence

Input Sequence

Click here to go to next step

Figure 17A. Snapshot of the “Beginner” sub module of the “Advance Modification” module of PEPstrMOD with labels showing how to input peptide sequence and other options. The option to incorporate different modification is provided in the next page as shown in the next figure

No	Residue	Disulfide Bridges	Stereochemistry	Non-Natural Modification	PTMs
1	G	Not Applicable	Laevo (L)	No Modification	Not Applicable
2	S	Not Applicable	Laevo (L)	No Modification	phosphoserine
3	C	1	Laevo (L)	No Modification	No Modification
4	K	Not Applicable	Laevo (L)	No Modification	5hydroxylysine
5	M	Not Applicable	Laevo (L)	No Modification	No Modification
6	S	Not Applicable	Laevo (L)	nmethylvaline	5hydroxylysine
7	T	Not Applicable	Dextro (D)	No Modification	No Modification
8	A	Not Applicable	Dextro (D)	No Modification	No Modification
9	I	Not Applicable	Dextro (D)	No Modification	No Modification
10	S	Not Applicable	Dextro (D)	No Modification	No Modification
11	V	Not Applicable	Laevo (L)	No Modification	No Modification
12	C	2	Laevo (L)	No Modification	No Modification
13	L	Not Applicable	Laevo (L)	No Modification	No Modification
14	A	Not Applicable	Laevo (L)	No Modification	No Modification
15	Q	Not Applicable	Laevo (L)	No Modification	No Modification
16	A	Not Applicable	Laevo (L)	No Modification	No Modification

Figure 17B. This snapshot is the extension of the previous figure 17A in which peptide sequence was taken as input. In this step, different modifications can be incorporated by using dropdown menu in front of amino acids of the input peptide sequence. For incorporating disulfide bridge, a text box appears in front of cysteine residue. If a user wants to make disulfide bridge between 3<sup>rd</sup> and 12<sup>th</sup> cysteine, he/she needs to enter 1 in the text box in front of 3<sup>rd</sup> cysteine and enter 2 in front of 12<sup>th</sup> cysteine. This means that cysteine number 1 (given by user) is connected to cysteine number 2. For multiple disulfide bonds, number 3, 4 and so on can be given.

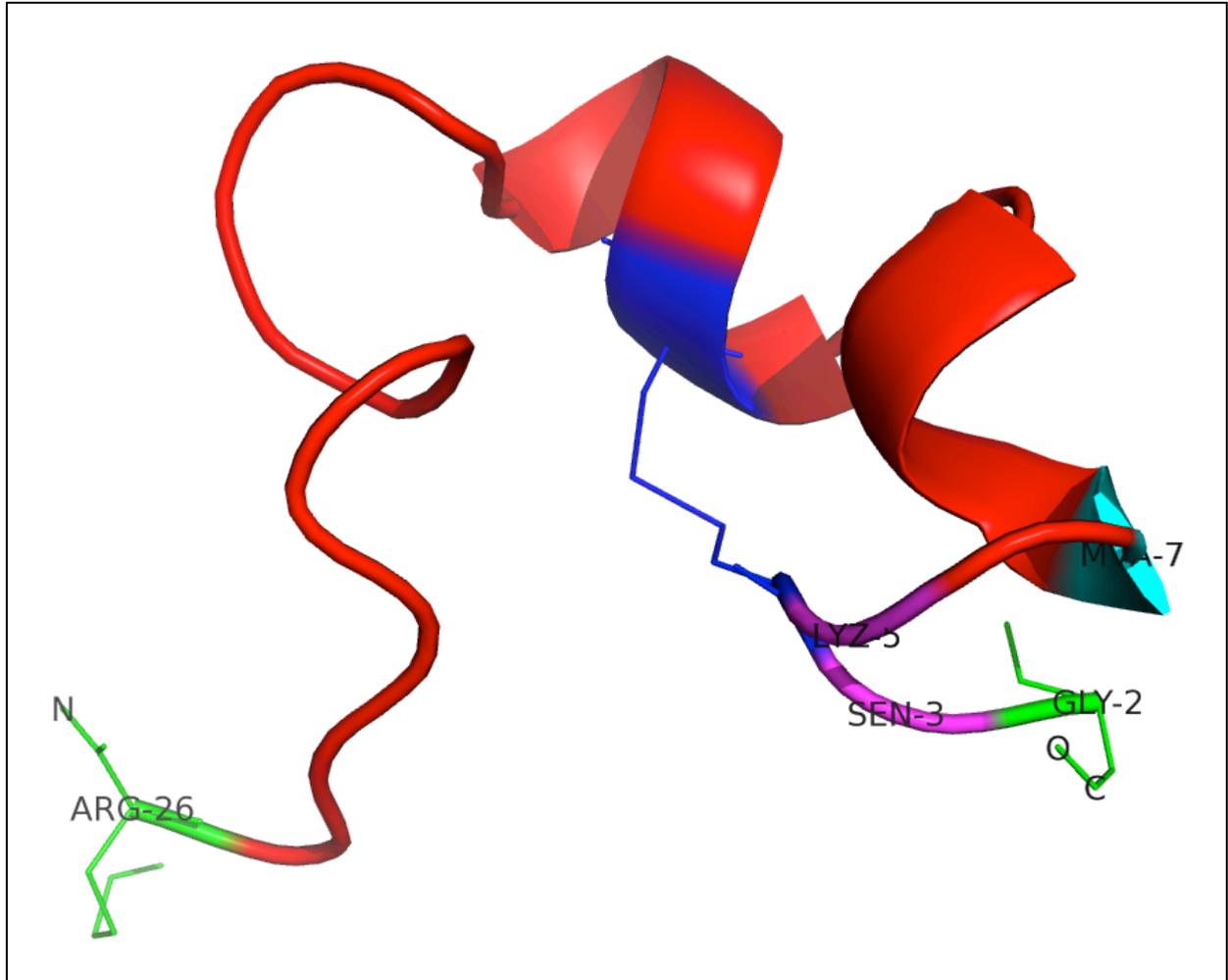


Figure 17C. Snapshot of the predicted tertiary structure of the example peptide using “Expert” sub module of the “Advance Modification” module of PEPstrMOD. The modifications incorporated in this figure are “Terminal modifications” (N-acetylation and C-amidation) labeled with green color, PTMs “phosphoserine” and “5hydroxylysine” at positions 2<sup>nd</sup> and 4<sup>th</sup> respectively labeled with magenta color, non-natural modification of “methylvaline” at position 6 labeled with cyan color and disulfide bond between CYS3 and CYS12 labeled with blue color. **NOTE:** Due to the N-terminal acetylation being displayed as first residue, the original first residue of the peptide sequence (GLY) is now being displayed as 2<sup>nd</sup> residue. Therefore although the PTM (phosphoserine SEN) was done at 2<sup>nd</sup> position yet in the above figure the same position is being displayed as SEN-3.

## 7.2. Expert sub module of Advance Modification Module

A user needs to enter the sequence and his/her email address along with desired modifications. If a user wants to perform N-C cyclization, he can select 'yes' or 'no'. For incorporation of disulfide bridges, he/she can enter the residue positions in the box in the same manner as described in the “Disulfide Bridge (S-S)” sub module of “Peptide Cyclization” module. D-amino acids can be incorporated by making the letters small in the peptide sequence as described in “D-amino acids” module. Further, a user can incorporate non-natural amino acids and PTMs by entering residue positions at respective text boxes (Figure 18A).

**Peptide Sequence Submission Form**

Peptide sequence (D-amino acids in small letter)  Example Sequence

Email Address:  e-mail address

N-terminal Modification:  C-terminal Modification:  Terminal modifications

N-C Cyclization:  Yes  No Peptide Cyclization

Enter Disulfide Bridge(S-S Bonds):

**SELECT THE MODIFIED RESIDUES AND SUBMIT POSITION OF MODIFICATION**

ALA: diethylalanine	<input type="text" value="4"/>	ARG: N-methylarginine	<input type="text"/>
GLY: vinylglycine	<input type="text"/>	PHE: biphenylalanine	<input type="text"/>
TYR: o-tyrosine	<input type="text"/>	TRP: beta-tryptophan	<input type="text"/>
SER: homoserine	<input type="text"/>	LEU: homoleucine	<input type="text"/>
CYS: benzylcysteine	<input type="text"/>	GLN: 3-methyl glutamine	<input type="text"/>
ILE: beta-isoleucine	<input type="text"/>	LYS: beta-lysine	<input type="text"/>
MET: methionine	<input type="text"/>	ASP: beta-aspartic acid	<input type="text"/>
VAL: N-methyl-valine	<input type="text"/>	GLU: N-methylglutamic acid	<input type="text"/>
ASN: beta-asparagine	<input type="text"/>	THR: beta-threonine	<input type="text"/>
Others: Ornithine	<input type="text"/>		

**SELECT THE PTM AND SUBMIT POSITION OF PTM**

ARG: (omega)-methylarginine	<input type="text"/>	PHE: 5-hydroxyphenylalanine	<input type="text"/>
TYR: Phosphotyrosine (neutral)	<input type="text"/>	TRP: 7-hydroxytryptophan	<input type="text"/>
SER: Phosphoserine (neutral)	<input type="text" value="6"/>	LEU: N-methyl-leucine	<input type="text"/>
CYS: Cysteinepersulfide	<input type="text"/>	PRO: 3,4-hydroxyproline	<input type="text"/>
LYS: 5-hydroxylysine	<input type="text"/>	MET: hydroxyl-methionine	<input type="text"/>
GLU: 1-Carboxyglutamic Acid	<input type="text"/>	THR: Phosphothreonine (neutr: <input type="text" value="22"/>	<input type="text"/>
Others: Ornithine	<input type="text"/>	Phosphothreonine (neutral)	
		Phosphothreonine (-2 charge)	

Submit Sequence

Figure 18A. Graphical representation of the “Expert” sub module of the “Advance Modification” module of PEPstrMOD with labels showing how to incorporate different modifications in the peptide.

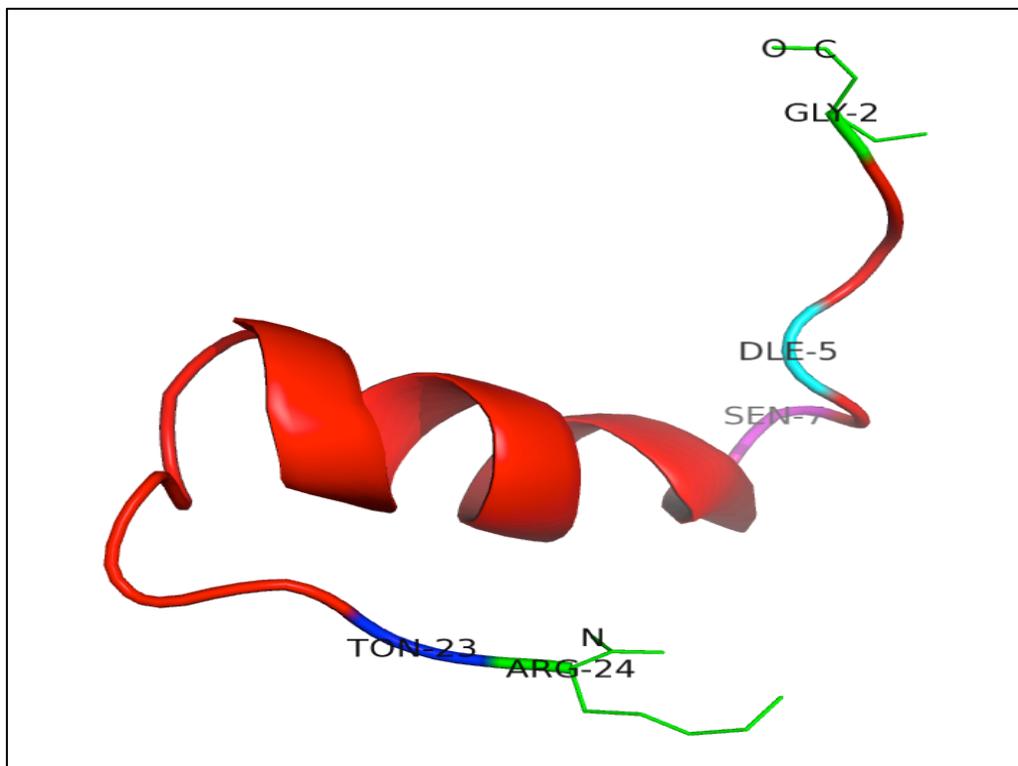


Figure 18B. Snapshot of the predicted tertiary structure of the example peptide using “Expert” sub module of the “Advance Modification” module of PEPstrMOD. The modifications incorporated in this figure are “Terminal modifications” (N-acetylation and C-amidation) labeled with green color, PTMs “phosphoserine” (magenta color) and “phosphothreonine” (blue color) at positions 6<sup>th</sup> and 22<sup>nd</sup> respectively, non-natural modification of “diethylalanine” at position 4<sup>th</sup> labeled with cyan color. **NOTE:** Due to the N-terminal acetylation being displayed as first residue, the original first residue of the peptide sequence (GLY) is now being displayed as 2<sup>nd</sup> residue. Therefore although the non-natural modification of diethylalanine residue was done at 4<sup>th</sup> position yet in the above figure the same position is being displayed as DLE-5.

### 7.3. Structure Modification sub module of Advance Modification Module

This sub module integrates the “Structure Modification” sub module of “Natural Peptides” module and “Expert” sub module of “Advance Modification” module. Therefore, it provides facility to the user to incorporate multiple modifications in the tertiary structure of the peptide given as an input.

### 7.4 Manual Modification sub module of Advance Modification Module

This module provides an option to open any PDB structure in a web based chemical drawing software (Marvin Applet) and user can add any modification in any particular position on the

structure and submit it. The submitted initial structure will be subjected to energy minimization by open babel software and minimized structure will be given as output. This service will be useful for incorporating modifications whose force field parameters are not present in the standard force field libraries used in this study. **NOTE:** If a user wants to edit any structure in his/her own favorite editor/software (like Pymol etc), he/she can do it and open that structure here in marvin sketch and submit the structure here.

## **8. Structure Simulations Module**

If a user has a peptide tertiary structure in PDB format and he/she is only interested in performing the simulation of that peptide without predicting its tertiary structure, he/she can successfully perform the simulation of that peptide with this module. This module is also helpful in extending the simulations of the predicted structure obtained by any of the PEPstrMOD modules. A user needs to request a token for accessing the facility of this module.

### **8.1. Request Token**

A user needs to request for a token before using the facility of this module. A user needs to enter his/her name, email address and institute name and finally click on 'Submit'. A token will be granted to the user, which will be mailed to his/her provided email address.

### **8.2. Simulate a PDB File**

This facility allows user to perform the simulation of the peptide by inputting a PDB file of that peptide. This facility is also helpful in extending the simulations of the predicted peptide structure obtained by any of the PEPstrMOD modules. User can also give different options like "Simulation Time", "Peptide Environment", "Cluster Analysis" etc. as per his/her needs. A user first needs to request a token. The same token can be used for 3 times.