Prediction and Localization of Hotspots in Brain Derived Neurotrophic Factor (BDNF) Protein: An Insilco Approach

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Abstract

In this paper identification and location of hotspots in Brain derived neurotrophic factor (BDNF) protein is presented using an insilico method. Since the cellular processes are controlled by protein-protein interactions, it is essential to predict and study these interactions. Prediction of hotspots in BDNF protein is interest, because this molecule might be involved in controlling the type II diabetes mellitus (T2DM). The BDNF (1BND) protein structure in PDB format and its two chain identifiers (A &B) with the default van der Waals radii of two atoms value (0.5 A°) has given to the algorithm and predicted the interface residues as hotspots represented by 'H' symbol and the non interface residues as non hotspot represented with 'NH' symbol. The final output is tabulated mainly with these interface residues number, its name, total contact potential values. Also it provides an interactive Jmol based 3D visualization of the submitted protein-protein interface with the predicted hotspots in red in colour for observing their localization as shown in the fig(1). This study can be used for analysis of protein-protein interface of BDNF protein and its binding site characterizations that lead to estimating the functionality of this protein in type 2 diabetes problem.

Keywords: *Hotspots, BDNF, insilico, PDB, Jmol.*

1. Introduction

In systems biology the mechanisms like gene regulation, protein interaction networks, and complex metabolic networks have more importance in the contemporary research. In particular identification and localization of protein-protein interfaces and its 3D structure, as they are responsible for the functionality of the protein. In these biological processes, proteins undergo interactions, which are mediated by molecular mechanisms. During this interaction, a small set of residues (amino acids) play a vital role and the energy distribution among these protein-protein interfaces is not homogeneous. Such amino acids are called hotspots [1,2]. Understanding the structural basis of protein-protein interactions (PPIs) may shed light on the organization and functioning of signal transduction and metabolic networks and may assist in structure-based design of ligands (drugs) targeting protein-protein interfaces [3].

The amino acid chain forms in to a few secondary structures, based on the interactions of the peptide bond with nearby amino acids. Each secondary protein structure has one or more chain sequence segments determined by the primary protein structure i.e. there are several secondary protein structures for a protein sequence. Proteins generally interact with other molecules while functioning. During protein interactions, the energies are not uniformly distributed i.e they are not homogeneous and some critical residues comprising only a small fraction of interactions account for the majority of the binding energy. The amino acids present in the active site are called hot spots. Hot spots form tightly packed regions in protein interactions [4] and identification of hot spots is helpful in estimating the efficiency of functioning of a protein [5].

Hot spots form tightly packed regions in protein interfaces [6]. The presence of hot spots controls the malfunctioning associated with protein molecules and used for rational design of highly specific protein complexes [7,8]. Experimentally, a hot spot can be found by evaluating the change in binding free energy upon mutating it to an alanine (codon GCN). A biological experimental technique known as Alanine Scanning Mutagenesis (ASM) has been used to identify the hot spots, it uses the measure of the energy contribution of interface

amino acids by mutating each amino acid to alanine. The alanine scanning is considered as a good method of identification of hot spots and also it is widely accepted by many researchers [9,10,11]. The alanine is chosen because it eliminates its side chain easily without altering the main chain conformation, as the side chain does not directly involve in protein function. It also does not put any extreme electrostatic effects on the main chain conformation [12].

Brain-derived neurotrophic factor (BDNF) is a gene on chromosome 11, location 11p13, associated with many structural neuronal functions. BDNF modulates the secretion and actions of insulin, leptin, ghrelin, various neurotransmitters and peptides, and pro- inflammatory cytokines involved in energy homeostasis suggesting that it (BDNF) might be having a significant role in the pathobiology of type2 diabetes mellitus (T2DM)[13]. Alterations in brain-derived neurotrophic factor gene expression contribute to serious pathologies such as depression, epilepsy, cancer, Alzheimer's, Huntington and Parkinson's disease [13]. Therefore, exploring the mechanisms of BDNF regulation by predicting the hotspots represents a great clinical importance.

1BND is a heterodimer molecule of combinations of BDNF protein and neurotrophin 3 (NT3) protein. The neurotrophins (nerve growth factor, NGF; brain-derived neurotrophic factor, BDNF; neurotrophin 3, NT3; and neurotrophin 4, NT4) are dimeric molecules, which share approximately 50% sequence identity [14]. The NGF, BDNF, and NT3 protomers share the same topology and are structurally equivalent in regions which contribute to the dimer interface in line with the propensity of the neurotrophins to form heterodimers [14].

2. Method and Materials:

Algorithm:

Step1: Retrieve the BDNF protein structure from RCSB [15] in PDB format (1 BND)[16] along with two of its side chain identifiers A and B.

Step2: As per the Hotpoint server [17], keep the default distance between any two atoms belong to two residues, one from the each chain is kept at 0.5 A°. We can allow changing this value.

Step3: Then submit these details to the Hotpoint server and the result will be displayed by checking the condition given as, if the relative accessibility of an individual interface residue is ≤ 20% and its total contact potential is ≥18.0, it is labelled as hot spot [18]. Otherwise the residues treated as non-hotspot.

Step4: Save the result after finishing the result as interface file in PDB format and the Hotspot prediction file. The interactive 3D visualization model of the target protein and their hotspots can be visualized interactively using Jmol [19] molecular viewer.

3. Results discussion:

The Hotspots of BDNF protein are tabulated which consisting of the features of interface residues. The features are chain names, residue symbol, residue number, complex & monomer relative ASA, and total pair potential. Out of 238 amino acids of 1 BND (Chain A consist of 119 and Chain B Consist of 119[20]) 17 residues predicted as Hotspots (H) and 36 residues predicted as non-Hotspots (NH) and are shown in table (1).

Table:1- BDNF protein Hotspot residues and their features..

Residue Number	Residue Symbol	Chain	RelComp ASA	RelMonomer ASA	Potential	Predicted H/NH
9	Q	A	68.4	105.34	13.98	NH
10	L	A	44	71.44	16.33	NH
12	V	A	3.12	46.92	48.38	**H**
19	W	A	44.77	66.63	21.19	NH
31	M	A	48.52	58.96	21.09	NH
44	V	A	26.99	47.05	10.73	NH
49	L	A	22.48	54.73	23.47	NH
50	K	A	51.99	55.02	6.02	NH
52	Y	A	30.23	57.66	14.56	NH
54	Y	A	20.27	54.09	16.46	NH
69	R	A	17.47	25.69	18.46	**H**
70	G	A	13.9	102.56	15.67	NH
71	I	A	7.45	21.43	35.76	**H**
72	D	A	10.4	42.13	4.31	NH
74	R	A	59.8	79.15	6.03	NH
76	W	A	17.3	32.94	20.66	**H**
85	S	A	4.03	40.3	19.17	**H**
86	Y	A	15.43	41.24	19.03	**H**
88	R	A	21.35	57.81	21.36	NH
102	F	A	5.04	32.33	21.14	**H**
107	T	A	3.58	37.98	12.92	NH
108	S	A	4.75	33.58	16.03	NH
111	C	A	2.17	29.27	49.23	**H**
112	T	A	13.68	35.38	6.99	NH
113	L	A	0	42.91	45.84	**H**
114	T	A	35.21	56.44	3.86	NH
115	I	A	60.18	80.88	14.6	NH

9	G	B	39.91	85.54	5.31	NH
10	E	B	65.06	102.82	6.39	NH
11	V	B	25.52	69.01	19.74	NH
13	V	B	3.47	46.12	48.38	**H**
20	W	B	36.49	60.13	26.57	NH
30	I	B	43.58	53.7	16.97	NH
48	V	B	24.93	51.22	13.71	NH
49	K	B	60.31	61.98	0	NH
51	Y	B	31.15	53.83	14.77	NH
53	Y	B	18.55	53.48	21.57	**H**
68	R	B	23.28	30.15	16.53	NH
69	G	B	11.3	95.62	18.44	**H**
70	I	B	8.78	28.39	47.96	**H**
71	D	B	12.79	44.26	8.23	NH
73	K	B	73.46	87.89	2.11	NH
75	W	B	14.04	29.53	14.71	NH
84	T	B	3.19	37.78	19.13	**H**
85	Y	B	22.47	46.7	14.29	NH
87	R	B	24.38	57.84	13.87	NH
99	W	B	23.75	51.78	37.52	NH
101	W	B	9.4	40.88	20.61	**H**
106	T	B	3.83	37.3	13.02	NH
107	S	B	6.86	32.55	13.89	NH
110	C	B	2.13	24.33	43.7	**H**
111	A	B	11.62	44.72	11.29	NH
112	L	B	0.13	51.78	40.3	**H**

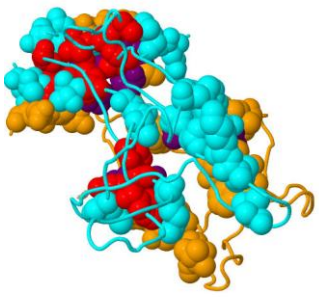

Fig:1-Interactive Jmol visualization of BDNF(PDB:1BND,Chain A and Chain B) residues[Red indicate Hotspot residue, Blue are Non Hotspot residues]

4. Conclusions:

The computational hotspots presented in BDNF protein are predicted along with their location using an insilico method called Hotpoint. A minimum number of amino acid residues in this molecule are predicted as hotspots. The 3D interactive visualization structure of the BDNF molecule is shown, where the red coloured residues are the hotspot locations. Finally we conclude that the predicted hotspots would be useful for computational biologist working on this protein and can be used these results to estimate its functionality and especially point out its role in type 2 diabetes related problems. These conclusions may useful in modelling and development of the drugs related to these problems.

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