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Identifying Active Site Cavities of Brain Derived Neurotrophic Factor Involved in Type II Diabetes

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Abstract: Active site prediction of BDNF is done to identify the locations of ligand binding sites and to predict functional similarities between cavities to estimate the locations of bound ligands. BDNF is a member of the neurotrophin family of growth factors, which are related to the canonical "Nerve Growth Factor", NGF. BDNF is a small dimeric protein which is abundantly and widely expressed in the adult mammalian brain. Various studies have shown possible links between BDNF and conditions, such as Diabetes, depression, schizophrenia, obsessive-compulsive disorder, Alzheimer's disease, Huntington's disease, Rett syndrome, and dementia, as well as anorexia nervosa and bulimia nervosa. Active site of BDNF is obtained by using active site prediction server. The cavity1 is identified as the cavity highest volume with the following amino acid sequence as the active site RTCEDFAIVKYSQGWLN the cavity points are 5.312A^o, 26.603A^o, 20.190A^o and the cavity volume is 1498 A^3.

Key words: Active site, BDNF, cavities, interaction sites, type II Diabetes

INTRODUCTION

The BDNF protein is coded by the gene that is also called BDNF. In humans this gene is located on chromosome 11 (Jones and Reichardt, 1990). BDNF acts on certain neurons of the central nervous system and the peripheral nervous system, helping to support the survival of existing neurons, and encourage the growth and differentiation of new neurons and synapses (Acheson *et al*., 1995). In the brain, it is active in the hippocampus, cortex, and basal forebrain areas vital to learning, memory and higher thinking (Yamada and Nabeshima, 2003). BDNF itself is important for long-term memory (Bekinschtein *et al*., 2008).

BDNF is actually found in a range of tissue and cell types, not just in the brain. BDNF is also found in human saliva (Mandel *et al*., 2009). BDNF is made in the endoplasmic reticulum and secreted from dense-core vesicles. It binds the sorting receptor carboxypeptidase E (CPE), and the disruption of this binding causes loss of sorting of BDNF into dense-core vesicles.

Exercise has been shown to increase the secretion of BDNF at the mRNA and protein levels in the rodent hippocampus, suggesting the potential increase of this neurotrophin after exercise in humans (Cotman and Berchtold, 2002). As of 2008, Val66Met is probably the most investigated SNP of the BDNF gene, but, besides this variant, other SNPs in the gene are C270T, rs7103411, rs2030324, rs2203877, rs2049045 and rs7124442. Variants close to the BDNF gene were found to be associated with obesity in two very large genome wide-association studies of body mass index (Thorleifsson *et al*., 2009).

Various studies have shown possible links between BDNF and conditions, such as depression (Dwivedi, 2009), schizophreniaossible links between obsessive-compulsive disorder (Maina *et al*., 2009), Alzheimer's disease (Zuccato and Cattaneo, 2009), Huntington's disease (Zajac *et al*., 2009), Rett syndrome (Zeev *et al*., 2009), and dementia (Arancio and Chao, 2007), as well as anorexia nervosa (Mercader *et al*., 2007) and bulimia nervosa (Kaplan *et al*., 2008).

Brain-derived neurotrophic factor has been shown to interact with TrkB (Haniu *et al*., 1997). BDNF has also been shown to interact with the reelin signaling chain (Fatemi, 2008). The expression of reelin by Cajal-Retzius cells goes down during development under the influence of BDNF (Ringstedt *et al*., 1998).

The active site of an enzyme contains the catalytic and binding sites. The structure and chemical properties of the active site allow the recognition and binding of the substrate. The active site is usually a small pocket at the

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surface of the enzyme that contains residues responsible for the substrate specificity and catalytic residues which often act as proton donors or acceptors or are responsible for binding a cofactor. The active site is also the site of inhibition of enzymes.

Regulation of protein function is vital for the control of cellular processes. Proteins are often regulated by allosteric mechanisms, in which effectors bind to regulatory sites distinct from the active sites and alter protein function. Intrasteric regulation, directed at the active site and thus the counterpart of allosteric control, is now emerging as an important regulatory mechanism (Kobe and Kemp, 1999).

Active sites in proteins are usually hydrophobic involving the side chains. It is non-trivial to quantify this rule of thumb. Goodford (1985), Miranker and Karplus (1991) used interaction energies between the receptor and different probes in an attempt to locate energetically favorable sites. While van der Waals energies indicate sterically available regions, the long-range nature of electrostatic potentials makes the interpretation of energy levels difficult. Alternatively, purely geometric methods seek to locate "pockets" without the use of energy models. This is advantageous since proton locations are not required. LigSite uses a grid representation of the molecular volume and computes exterior site scores by by projecting rays from the receptor exterior to the surface (Hendlich *et al*., 1997). The deeper and more surrounded a site is, the higher it scores (Del Carpio *et al*., 1993) using an analytical geometric algorithm to compute pocket sites (Goodford, 1985; Hendlich *et al*., 1997; Liang *et al*., 1998; Miranker and Karplus, 1991).

Active site prediction of BDNF is done to identify the locations of ligand binding sites and to predict functional similarities between cavities to estimate the locations of bound ligands and for estimating the volumetric extent of ligands (Kahraman *et al*., 2007). Identification and size characterization of surface pockets and occluded cavities are initial steps in protein structure-based ligand design (Liang *et al*., 1998). The largest cavity is identified as the active site of a protein. Auxiliary pockets near the active site have been suggested as additional binding surface for designed ligands (Mattos *et al*., 1994).

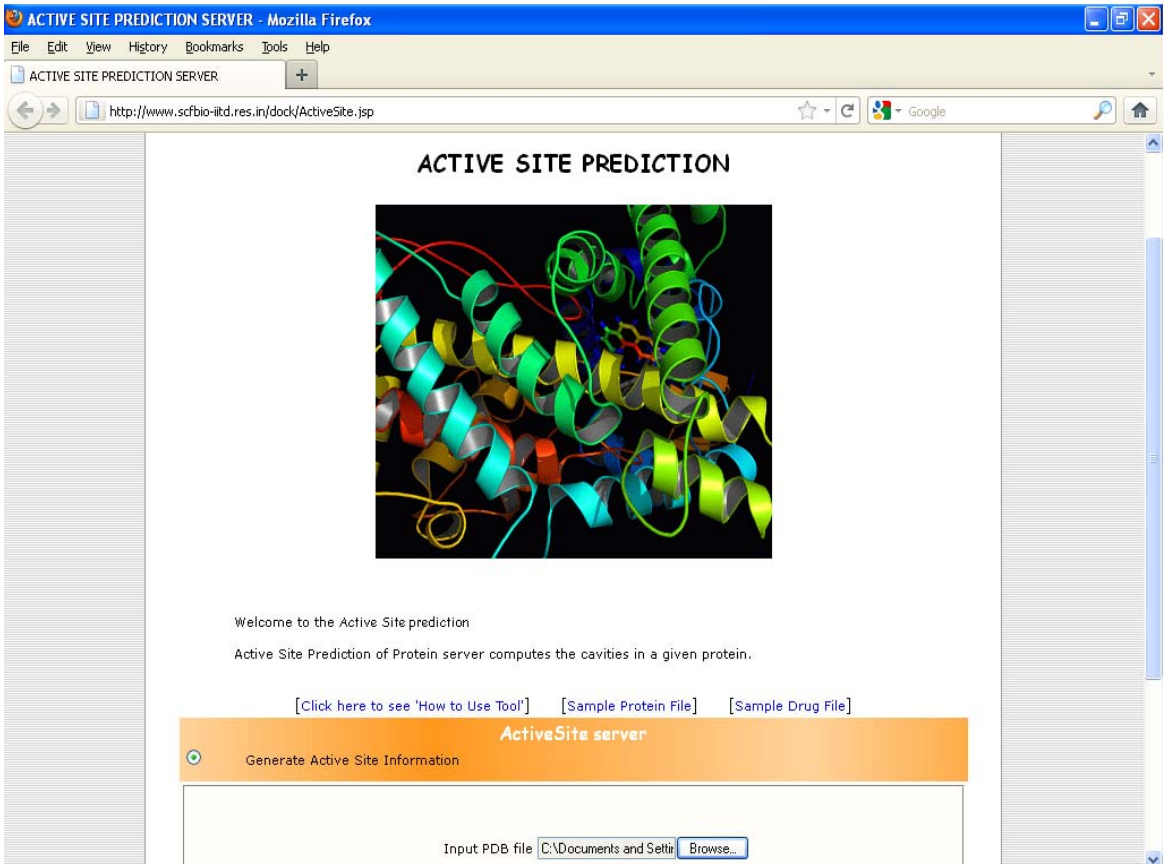

Fig. 1: Active sits prediction server

Table 1: Active sites obtained are as follows: Index of /dock/11862467ACTIVE

Name	Last Modified	Size	Description
Parent directory			
a2_11862467ACTIVEDRG.pdb	21-Mar-2011	08:34	224K
cavity_1_RTCEDFAIVKYSQGWLN	21-Mar-2011	08:34	7.1K
cavity_2_RDATWKVGSECFIQLY	21-Mar-2011	08:34	5.7K
cavity_3_EKQLAVRTSCWYHGDINM	21-Mar-2011	08:34	4.5K
cavity_4_KVSCARDETLYFGWQ	21-Mar-2011	08:34	5.4K
cavity_5_WVGSFAEMTRYIDQKC	21-Mar-2011	08:34	3.8K
cavity_6_RWDVKGPLAFYQET	21-Mar-2011	08:34	5.1K
cavity_7_YSRVGLTAQIFDKCEWP	21-Mar-2011	08:34	3.8K
cavity_8_RGCWVLSHTDEKNQAY	21-Mar-2011	08:34	6.2K
cavity_9_DIRASQVWKTLGFYECNPM	21-Mar-2011	08:34	5.7K
cavity_10_ARDCTKQVLEGSYWNIPM	21-Mar-2011	08:34	6.0K
cavity_11_KARCVDGTSIEQLYWN	21-Mar-2011	08:34	6.1K
cavity_12_AEVRTGQSCDKIP	21-Mar-2011	08:34	2.9K
cavity_13_SRPGQYLAWFVMKIDT	21-Mar-2011	08:34	4.4K
cavity_14_SPGREQLAYVFWIKMT	21-Mar-2011	08:34	5.2K
cavity_15_RESPGYQVLAFWDK	21-Mar-2011	08:34	5.5K
cavity_16_RTDWKVAGSCFEIQ	21-Mar-2011	08:34	3.0K
cavity_17_RTGEYQVLWFAMIPS	21-Mar-2011	08:34	4.5K
cavity_18_AVGWETIRCKLSNQY	21-Mar-2011	08:34	3.2K
cavity_19_KAGCRDLESWVT	21-Mar-2011	08:34	3.6K
cavity_20_ALWRGIPDKVSMTFQYE	21-Mar-2011	08:34	3.8K
cavity_21_TRWYQGVLFMSD	21-Mar-2011	08:34	2.7K
cavity_22_REFSTDMYAIVQWKC	21-Mar-2011	08:34	3.9K
cavity_23_RTDGWEYQVLFAI	21-Mar-2011	08:34	4.6K
cavity_24_RYWLQFMSGIDKVTA	21-Mar-2011	08:34	3.6K
cavity_25_RGCWVSHIDKTLNEY	21-Mar-2011	08:34	4.2K
cavity_26_RDETGYQVLPFAW	21-Mar-2011	08:34	3.6K
cavity_27_RWPVILAFQMYKTED	21-Mar-2011	08:34	4.5K
cavity_28_TDRESPGWYQVLFA	21-Mar-2011	08:34	3.0K
cavity_29_DRGLCEAWVSIHTK	21-Mar-2011	08:34	2.7K
cavity_30_SRDKIGVTMAL	21-Mar-2011	08:34	2.3K
cavity_31_GRECSWLVHD	21-Mar-2011	08:34	2.7K
cavity_32_ETVADRQGWLY	21-Mar-2011	08:34	3.0K
d2_11862467ACTIVEDRG.pdb	21-Mar-2011	08:34	239K
list	21-Mar-2011	08:34	815
v_11862467ACTIVEDRG.pdb	21-Mar-2011	08:35	130

Apache Server at www.scfbio-iitd.res.in Port 80

Table 2: Cavities obtained are as follows:

S.no	File Name	Cavity point in angstrom	Volume of the cavity in angstrom cube
1	cavity_1_RTCEDFAIVKYSQGWLN	5.312 26.603 20.190	1498
2	cavity_2_RDATWKVGSECFIQLY	- 4.196 26.533 23.153	1152
3	cavity_3_EKQLAVRTSCWYHGDINM	11.987 19.795 27.237	996
4	cavity_4_KVSCARDETLYFGWQ	1.991 11.444 16.220	989
5	cavity_5_WVGSFAEMTRYIDQKC	1.156 14.704 7.105	979
6	cavity_6_RWDVKGPLAFYQET	8.347 40.517 10.319	925
7	cavity_7_YSRVGLTAQIFDKCEWP	12.287 20.520 3.244	924
8	cavity_8_RGCWVLSHTDEKNQAY	12.633 9.377 29.981	875
9	cavity_9_DIRASQVWKTLGFYECNPM	11.250 29.536 15.028	856
10	cavity_10_ARDCTKQVLEGSYWNIPM	10.400 16.170 20.702	841
11	cavity_11_KARCVDGTSIEQLYWN	2.980 19.542 23.713	803
12	cavity_12_AEVRTGQSCDKIP	10.811 11.238 10.757	773
13	cavity_13_SRPGQYLAWFVMKIDT	- 6.235 29.461-5.554	753
14	cavity_14_SPGREQLAYVFWIKMT	- 1.361 37.119-4.220	699
15	cavity_15_RESPGYQVLAFWDK	- 5.461 42.016 3.535	666
16	cavity_16_RTDWKVAGSCFEIQ	- 8.552 25.295 20.177	595
17	cavity_17_RTGEYQVLWFAMIPS	- 4.219 32.628 6.203	581
18	cavity_18_AVGWETIRCKLSNQY	13.152 5.138 19.372	556
19	cavity_19_KAGCRDLESWVT	- 2.687 12.405 26.801	518
20	cavity_20_ALWRGIPDKVSMTFQYE	5.332 33.324-6.290	517
21	cavity_21_TRWYQGVLFMSD	- 8.739 24.319 1.306	514
22	cavity_22_REFSTDMYAIVQWKC	1.925 23.891 10.648	497
23	cavity_23_RTDGWEYQVLFAI	- 5.431 33.128 13.745	474
24	cavity_24_RYWLQFMSGIDKVTA	- 2.735 24.973-3.176	470

Table 2: (Continued)

25	cavity_25_RGCWVSHIDKTLNEY	9.131 5.120 29.517	466
26	cavity_26_RDETGYQVLPFAW	- 10.970 38.034 12.996	453
27	cavity_27_RWPVILAFQMYKTED	9.163 33.328 0.840	451
28	cavity_28_TDRESPGWYQVLFA	- 11.406 31.786 6.328	395
29	cavity_29_DRGLCEAWVSIHTK	0.095 4.220 28.941	394
30	cavity_30_SRDKIGVTMAL	6.380 24.298-7.138	365
31	cavity_31_GRECSWLVHD	1.428 7.115 34.605	318
32	cavity_32_ETVADRQGWLY	- 2.163 39.815 17.517	117

MATERIAL AND METHODS

Active Site Prediction of Protein server Fig. 1 computes the cavities in a given protein.

The RCSB PDB also provides a variety of tools and resources. The structure is of BDNF obtained through RCSB in a PDB format. Active site is predicted by submitting the PDF file to the active site prediction tool.

The results of active sites of BDNF are given in Table 1 and active site cavities, cavity volume are given in Table 2.

CONCLUSION AND DISCUSSION

The active site prediction is done to identify the cavities present in the BDNF protein. Thirty two cavities are given with the sequence, cavity point and volume of cavity to locate the active sites of BDNF for the ligands to bind with. It has predicted the shape of the site by extracting the cavity volume surfaces enclosing regions with highest probability. The different ligands can be chosen for interaction of the active sites of BDNF. In conclusion the active sites of BDNF are identified and these cavaties can be targeted for drug discovery.

Cavities on a proteins surface as well as specific amino acid positioning within it create the physicochemical properties needed for a protein to perform its function. Characterizing protein functions is an increasingly important challenging problem that has been approached from both the sequence and structure levels (Berman *et al*., 2000). The binding pocket shape analysis relies heavily on the correct localization of the ligand binding site (Glaser *et al*., 2006). Since the shape, size, and chemical composition of the protein surface dictate the type of interaction the protein can make with its cognate ligand or other interacting partner (Silberstein *et al*., 2003) many studies have concentrated on the analysis of those properties using a variety of computational methods (Goldsmith-Fischman and Honig, 2003). The overall objective is to predict as accurately as possible the ligand location, ligand can be decided from the binding site, and ultimately the protein function, based on the structure of that protein. In most cases the binding site pocket is considerably larger than the ligand itself. Furthermore, in many enzymes the binding pocket is occupied by more than one molecule (Pazos and Sternberg, 2004). The accurate identification of the ligand binding site and shape is very important for functional assignment and in different fields such as docking, de novo drug design (Laurie and Jackson, 2005). The cavity1 with the following amino acid sequence is identified as the active site RTCEDFAIVKYSQGWLN with the cavity point 5.312A˚, 26.603A˚, 20.190A˚ and the cavity volume is 1498 A^3.

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