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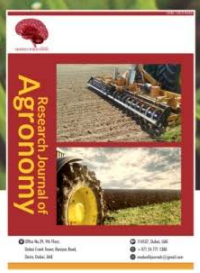

In Silico Engineering of Brain Derived Neurotrophic Factor (BDNF) to Improve its Neurogenerative Bioactivity

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Abstract: Brain Derived Neurotrophic Factor (BDNF), Neurotrophin-3(NT-3), Neurotrophin-4/5 (NT-4/5) and Neurotrophin-6 (NT-6) are known as neurotrophins. BDNF secreted to extracellular and binds to the soluble tropomyosin-related kinase B (trkB). Interaction of BDNF to trkB is linked to several intracellular signal transduction pathways such as Ras-MAP kinase cascade. These cascades regulate neuronal development, plasticity, long-term potentiation and synapses. BDNF is known to exist in solution as homodimeric proteins. Also, this protein forms heterodimer structures with Neurotrophin 4 (NT4) and Neurotrophin-3 (NT-3). The biological activity of BDNF heterodimers was reported to be less active in neuronal survival assays than a BDNF homodimers structures. In this research, new structures of BDNF that have better interaction with each other to form homodimer was modelled. Therefore, the amino acids that participate in interaction with BDNF were identified by search in neurochemical evidences. The amino acids are Tyr112, Tyr114 and Lys97. This amino acids were mutated by swiss pdb viewer software. The predicted model was submitted to 3Drefine server for optimization of hydrogen bonding network and energy minimization. To analyzing the predicted models, RAMPAGE and Qmean server were used. Finally, binding interaction between new structures of BDNF was done by using Hex.8.0.0 software. The results showed that interaction between these structures is better than the two native BDNF. Recently, researchers found that BDNF played an important therapeutic role in many human neurodegenerative diseases and this protein has potential for the treatment of the diseases.

INTRODUCTION

The neurotrophins are Brain Derived Neurotrophic Factor (BDNF), Neurotrophin-3(NT-3), Neurotrophin-4/5 (NT-4/5) and Neurotrophin-6 (NT-6) that are responsible for the development, maintenance, differentiation and function of the vertebrate nervous system (Binder and Scharfman, 2004).

In 1982 Brain-Derived Neurotrophic Factor (BDNF) purified from pig brain (Barde *et al*., 1982). The immature form of BDNF consists of 247 amino acids in comparison with the mature form of this that has 119 amino acids (Cardenas-Aguayo *et al*., 2013). The structure of the BDNF homodimer was determined using X-ray crystallography. The overall structure of BDNF resembles that of the other neurotrophins, NT3 and NT4; each protomer forms a twisted four-stranded β sheet. Robinson *et al*. (1999) indicated that lysin116, Tyrosin112 and 114 participate in interaction between two BDNF. The biological activity of BDNF heterodimers is 10- fold less active in neuronal survival assays than BDNF homodimers (Robinson *et al*., 1999). BDNF binds to the tropomyosin-related kinase B (trkB) receptors, members of the family of receptor tyrosine kinases. TrkB activation by BDNF, possibly mediated by dimerization of the receptor. Activation of this receptor is linked to several intracellular signal transduction pathways Mitogen-Activated Protein Kinase (MAPK) cascade and phosphorylation of Cyclic AMP-Response Element Binding protein (CREB) (Cunha *et al*., 2010). BDNF plays important roles in the plasticity of several regions of the Central Nervous System (CNS) during development, adulthood ageing, neurogenesis, learning, memory, encourage the growth, differentiation of neurons and synapses. Also BDNF Participates in axonal growth and in the modulation of dendritic growth (Nieto *et al*., 2013). The versatility of BDNF is emphasized by its contribution to a range of adaptive neuronal responses including Long-Term Potentiation (LTP), Long-Term Depression (LTD) short-term synaptic plasticity and homeostatic regulation of intrinsic neuronal excitability (Alonso *et al*., 2003). The BDNF is expressed, at high levels in hippocampal cerebellum and brain neurons. Also this protein is expressed in striatum, septum and thalamus (Cattaneo *et al*., 2010). Degenerative diseases of the nervous system may result from insufficient supply BDNF. Many reports have documented evidence of decreased expression of BDNF in Huntington (Ma *et al*., 2010), Parkinson (Peterson and Nutt, 2008), Alzheimer (Ferrer *et al*., 1999) and multiple sclerosis (Tjalf *et al*., 2002). Therefore, it can be concluded that BDNF is a good choice for treatment of nervous system diseases. In 1990 cDNA of this protein was cloned in *E. coli* (Jones and Reichardt, 1990) and in 2013 the functional BDNF coding sequence was cloned in a prokaryotic vector to produce BDNF protein in *E. coli* (BL21) (Hajar *et al*., 2004). The purpose of this study is designing the new structures of BDNF that have better interaction with each other to form homodimer and the interaction was compared with the native form. Therefore, bioinformatic tools were used. The swiss pdb viewer software is an application that analyzes structural proteins and models the 3D structure of a protein. Amino acid mutations, H-bonds, angles and distances between atoms are obtain by the intuitive graphic and menu interface. The output of this software is a file in pdb format (Johansson *et al*., 2012). The 3Drefine server (http://sysbio.rnet.missouri.edu/ 3Drefine) refines protein structure by optimizing hydrogen bonding network and atomic-level energy minimization (Bhattacharya and Cheng, 2013). To analyze and select best structure the RAMPAGE server (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) and the Qmean server (http://swissmodel.expasy.org/qmean/cgi/index.cgi) can be used. The stereochemical properties of obtained structural protein models check using RAMPAGE server and Ramachandran plot that generated by this server. This plot provides the amino acids position in particular segments based on φ and Ψ angles between N-C_α and C_α-C atoms of amino acids. This plot was divided into three regions: favored, allowed and outlier (Abdelmonaem *et al*., 2011). The overall stereo chemical properties of the new structure of proteins were analyzed using QMEAN server. QMEAN which stands for Qualitative Model Energy Analysis is a composite scoring function that describing the major geometrical aspects of structural protein models. The local geometry is analyzed by a new kind of torsion angle potential over three consecutive residues. Structure-specific distance-dependent pairwise residue-level potential is used to assess long-range interactions. Solvation potential describes the burial status of the amino acids. Also, describing the agreement of predicted and calculated secondary structure and solvent accessibility are included (Benkert *et al*., 2009). Hex.8.0.0 software is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of proteins (Ghoorah *et al*., 2013).

MATERIALS AND METHODS

New structures of BDNF modelling: Three-dimensional structure of BDNF was downloaded from PDB server (www.rcsb.org).

Then lysin116, Tyrosin112 and 114 (that participate in interaction between two BDNF) mutated by Swiss pdb viewer Version 4.0.1. The output of this software was a file in pdb format. The mutations were depicted in below:

- Structure 1: lysin116→Leu116
  Structure 2: Tyrosin112→Leu112 Tyrosin114→ Leu114
- Structure 3: Tyrosin112→Val112 Tyrosin114→ Val114

The modeled structures of the protein were submitted to the 3Drefine server. Then each models were tested for quality by using QMEAN server and assessed by using Ramachandran plots.

The calculation of the interaction energy: Docking was performed by using Hex.8.0.0 software. Firstly were calculated interaction energy between two native BDNF and then between new structures of BDNF (mutated). And the next time was calculated interaction energy of these two kinds of BDNF (native and mutated) by the means of mentioned.

RESULTS AND DISCUSSION

Modelling of the mutated BDNF: Swiss pdb viewer software modelled new structures of BDNF in pdb format and 3Drefine server refined these structures and formed refined models files in pdb format. In RAMPAGE server the Ramachandran plots was divided into three regions: favored (98%), allowed (2%) and outlier (0%) for all of the new structures of BDNF (Fig. 1). Also, Qmean server generated average Z score and Qmean score that were depicted in Table 1.

The calculation of the interaction energy: Hex.8.0.0 software calculated Binding energy. The results of this software were displayed in Table 2, 3 and Fig. 2-4.

Brain Derived Neurotrophic Factor (BDNF), the second member of the "neurotrophic" family of neurotrophic factors, binds the tropomyosin-related kinase (trk) receptors, members of the family of receptor tyrosine kinases. BDNF induces trkB receptor dimerization that results is in kinase activation and has multiple functions in the nervous system (Turner *et al*., 2006).

The purpose of this research was in silico engineering of BDNF to improve its neurogenerative bioactivity. Jungbluth, etc., reported that the biological activity of BDNF heterodimers is 10-fold less active than BDNF homodimers. In this study, new structures of BDNF was modeled which showed better interaction with each other to form homodimer. Previous studies have shown that lysin116, Tyrosin112 and 114 Participate in interaction to neutrophins. This is important for the formation of BDNF/ BDNF complex and BDNF/TRKB activity. These amino acids were mutated and thus resulted in a new structure of BDNF. As a result aliphatic amino acids were selected as amino acid exchanges being substituted. The reason is that this region is comprised of more aliphatic amino acids in comparison to other amino acids. Mutations in new structures are depicted in below. The 3Drefine server refines these structures and the Qmean and RAMPAGE server helped to analyze new models of BDNF. Analysis shows that the native BDNF had a QMEAN score of 0.591 and QMEAN score of other structures higher than QMEAN score of native BDNF. Furthermore high resolution X-ray structures on average have a Z score = 0. Results showed Z score data of interactions of atoms, torsion, solvation SSE and ACC agreement are near to 0 and have similarity share in common with the data of BDNF. Therefore the new models were of higher quality than native BDNF. Ramachandran plot demonstrated that the high percentage of amino acids are located in favored

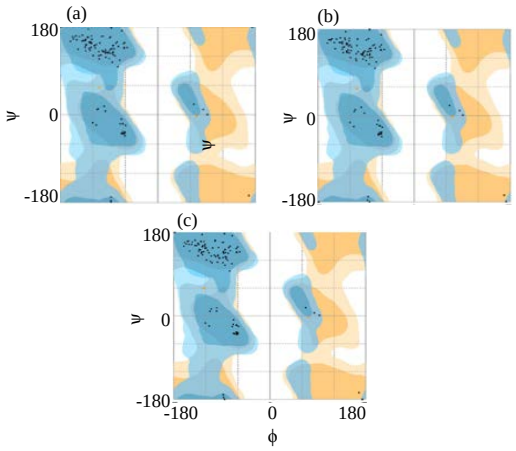

Fig. 1(a-c): Ramachandran plot analysis of new structure of BDNF proteins, (a): Ramachandran Plot of structure 1. (b): Ramachandran Plot of structure 2 and (c): Ramachandran Plot of structure 3

Table 1: Average Z score and Qmean score

Qmean score	Z score	Parameters
-0/59	-1/38	Native BDNF (Structure)
-0/66	-0/72	1
-0/66	-0.71	2
-0/64	-0/90	3

Table 2: Hex results for the docking between new structures of BDNF

E total	Parameters
-738/34	BDNF with BDNF
-761/97	Structure 1 with 1
-883/64	Structure 2 with 2
-827/62	Structure 3 with 3

Table 3: Hex results for the docking between new structures of BDNF with native BDNF

E total	Parameters
-738/34	BDNF with BDNF
-782/61	Structure 1 with BDNF
-797/79	Structure 2 with BDNF
-791/84	Structure 3 with BDNF

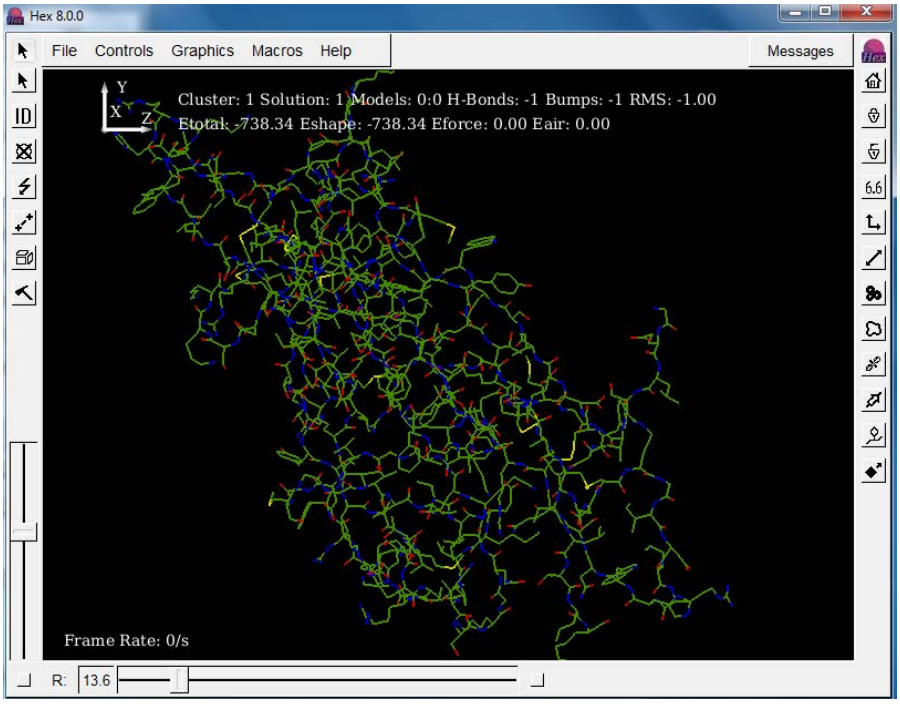

Fig. 2: Three dimensional structure of two BDNF proteins in Hex8.0.0

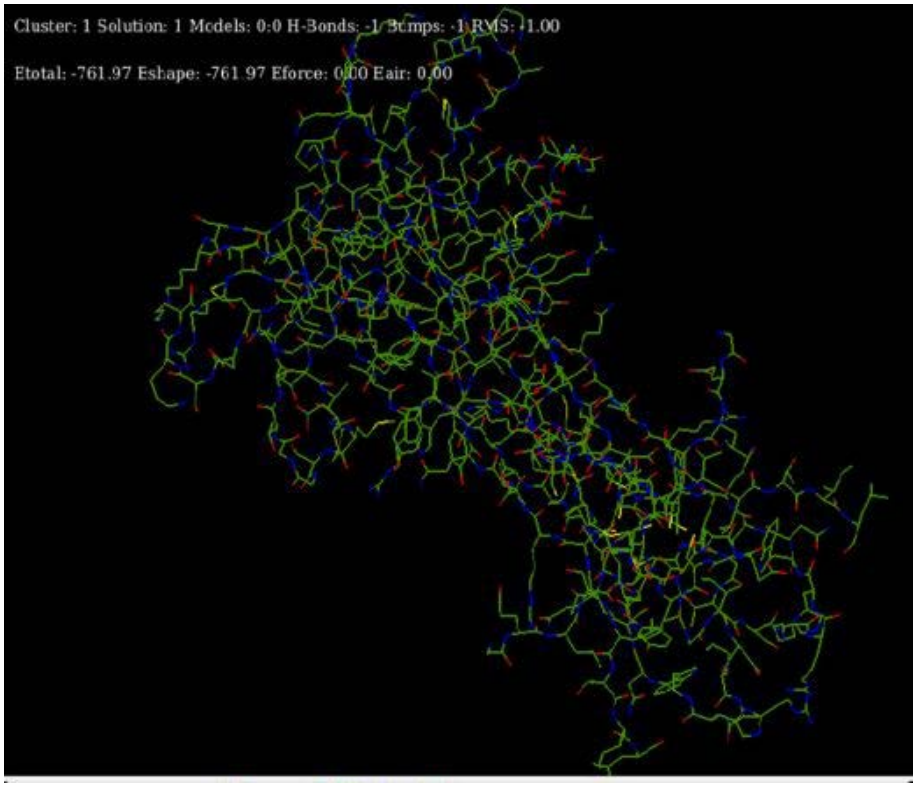

Fig. 3: Continue

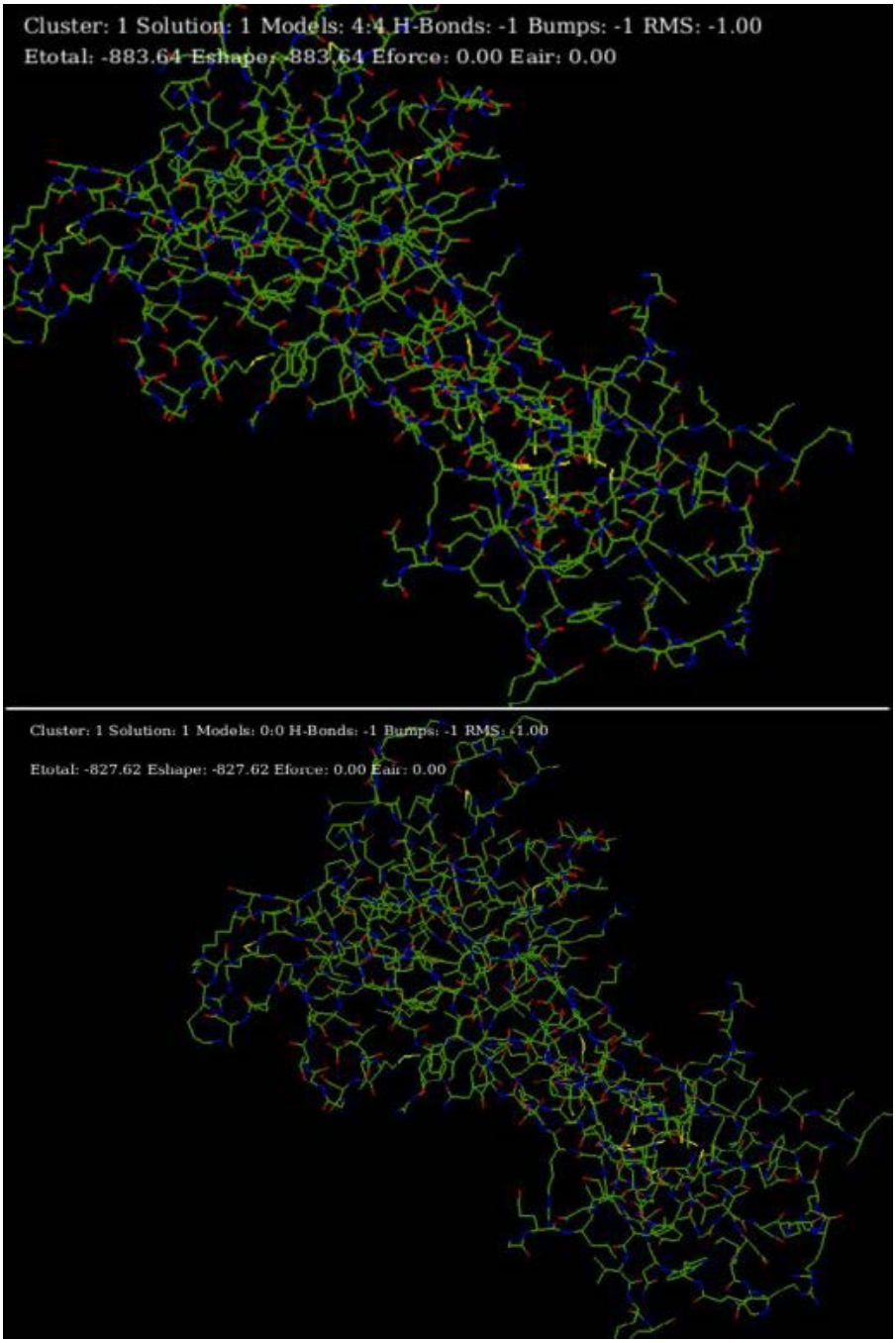

Fig. 3: Three dimensional new structure of two BDNF proteins in Hex8.0.0: structure 1 ensemble. 2: structure 2 ensemble. 3: structure 3 ensemble

region (>90%). Which assures that the built models are of good quality. The results of interaction energy in Table 2 and 3 demonstrated that E total between two native BDNF is higher than data of new structures of BDNF and between these two kinds of BDNF (native and mutated). New structures of BDNF have better interaction with each other to form homodimer. These results suggest that the new models of BDNF Imitate native BDNF and acts as a robust TrkB agonist, providing a powerful therapeutic tool for the treatment of various neurological diseases for example Huntington, Parkinson, Alzheimer and multiple sclerosis.

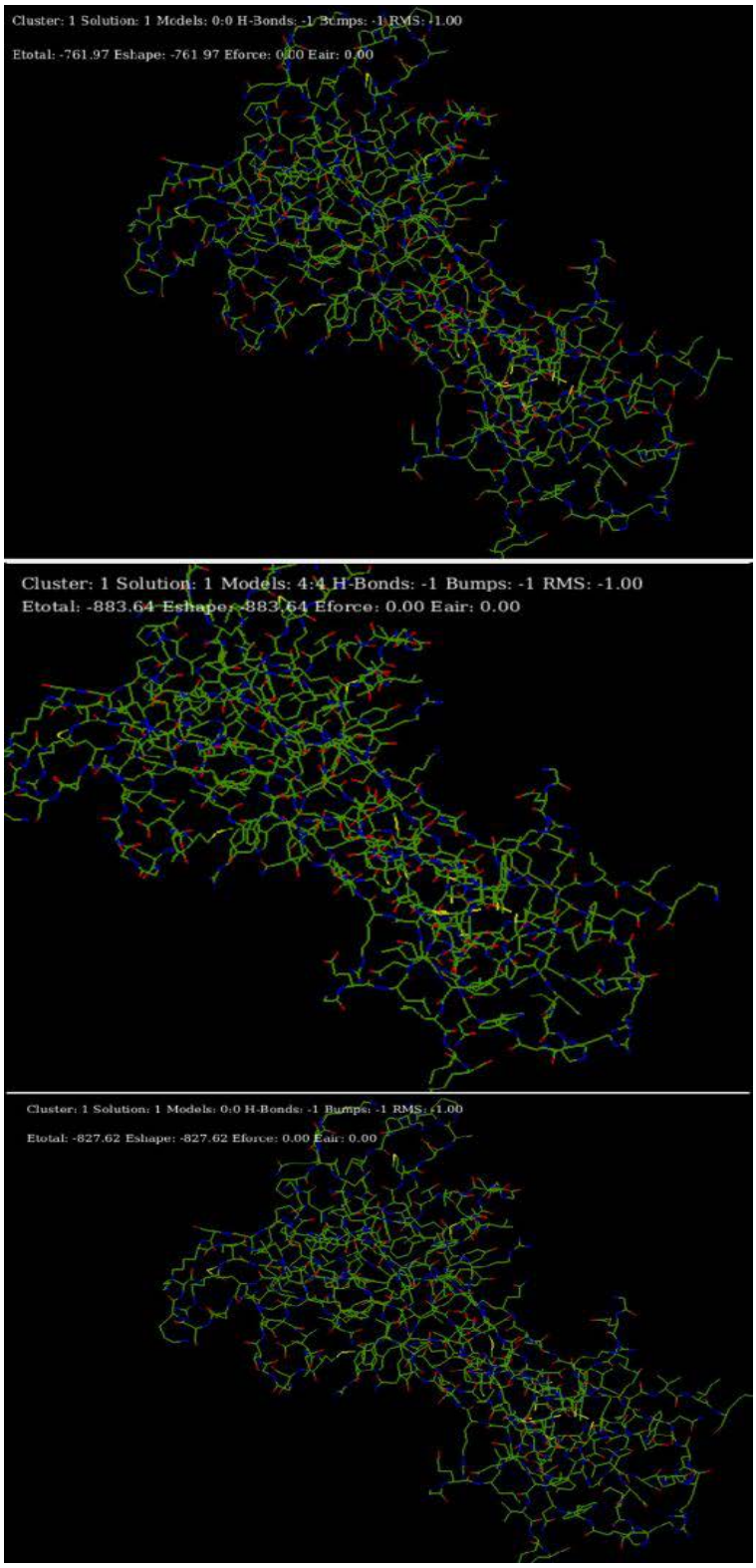

Fig. 4: Three dimensional structure of new structures of BDNF and native BDNF ensembles for docking in Hex8.0.0.1: structure1 with native BDNF. 2: structure 2 with native BDNF. 3: structure 3 with native BDNF

CONCLUSION

In conclusion, this research suggests that these mutant forms of BDNF protein are good choices for the treatment of the nervous system diseases.

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