RESEARCH ARTICLE

NF1 gene Analysis: New paradigm by computational approach

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Abbreviation:
NF1 : Neurofibromatosis
GRD: GAP related domain

ABSTRACT

Now a day, we are having good stimulation and regulation due to small piece of evolutionarily developed nucleotides working dynamically called gene (eg.NF1). The malfunctioning of NF1is autosomal dominant condition, contributes a set distinct genetic disorder that that cause tumors to grow along various types of nerve. In addition, it can affect the development of non-nervous tissue such as bone and skin. The NF1 gene, encodes for protein called neurofibromine, belongs to family of protein that serve as negative regulators ras oncogene. The GRD region encoded by exons 20-27a, is the function ascribed region. We are aiming to identify and analyze with structure prediction.

Keywords: structure prediction, autosomal dominant, NF1, GRD.

INTRODUCTION

Neurofibromatosis type 1(NF1) is one of the most common genetic disorders in human and is characterized by neurofibromas (Riccardi, 1992) It encompasses a set of distinct genetic disorder within neurons, brains, bones, skins etc that cause tumors to grow various nerves and non-nervous tissue. Neurofibromatosis cause to tumor to grow anywhere on or in the body. The NF1codes for protein neurofibromine, it posses a region that shares a high homology with the family of GTPase–activating proteins, which are negative regulators of RAS function and thereby control cell growth and differentiation (Serra *et al*., 1997). NF1 patients show 'two hit' hypothesis with one allele inactivated and another somatically mutated. While considering importance of impaired regulation (Sebastian, 2011)

We are analyzing NF1 locus in benign neurofibromas in NF1 gene. The further research will helpful in active site prediction and possible outcomes for pharmacokinetics.

MATERIALS AND METHODS

In this analysis, we have retrieved nucleotide as well as protein sequence of NF1 gene from NCBI Gene database (http://www.ncbi.nlm.nih.gov/gene) and Protein database (http://www.ncbi.nlm.nih.gov/protein). After retrival of protein sequence of NF1 gene we analyzed primary structure protein using ProtParam tool, which computes various physico-chemical properties of given protein sequence. It is available online in proteomics category of ExPASy sever http://web.expasy.org/protparam. The secondary structure analysis was carried out by ANTHEPROT integrated protein sequence software. It provides analysis by different method, out of which GOR and DPM method were used in secondary structure analysis. In consequence we predicted motif, domain, coiled region of NF1 protein sequence using Pfam (http://pfam.xfam.org/search/sequence) and Inter Pro Scan (http://www.ebi.ac.uk/Tools/pfa/iprscan5). The PDB File format was used to analyzed active region i.e. Motif and domain for the basis of protein ligand interaction.

RESULTS AND DISCUSSIOINS

Neurofibromin is cytosolic protein with molecular weight of 280kDa. Atomic composition of neurofibromin protein shows 2818 total amino acid. The physico-chemical parameter are specific volume 0.74cm^2 cm/g, Extinction Coefficient 282685/m cm, Estimated half-life >10 hours (E.coli, in vivo), Instability index computed to be 43.35 and GRAVY value is estimated to be -0.129 (Table 1).

Table 1: Physico-chemical parameter of Neurofibromin

Number of amino acids: 2818 Molecular weight: 317032.5 Theoretical pI: 6.90 Total number of positively charged residues (Arg + Lys): 290 Atomic composition: Carbon C 14141 Hydrogen H 22457 Nitrogen N 3813 Oxygen O 4158 Sulfur S 144 Formula: C14141H22457N3813O4158S144 Total number of atoms: 44713	Total number of negatively charged residues (Asp + Glu): 299 Extinction coefficients: Extinction coefficients are in units of M-1 cm-1, at 280 nm measured in water. Ext. coefficient 282685 Abs 0.1% (=1 g/l) 0.892, assuming all pairs of Cys residues form cystines Ext. coefficient 278810 Abs 0.1% (=1 g/l) 0.879, assuming all Cys residues are reduced Estimated half-life: The N-terminal of the sequence considered is M (Met). The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo). Instability index: The instability index (II) is computed to be 43.35 This classifies the protein as unstable. Aliphatic index: 94.36 Grand average of hydropathicity (GRAVY): -0.129

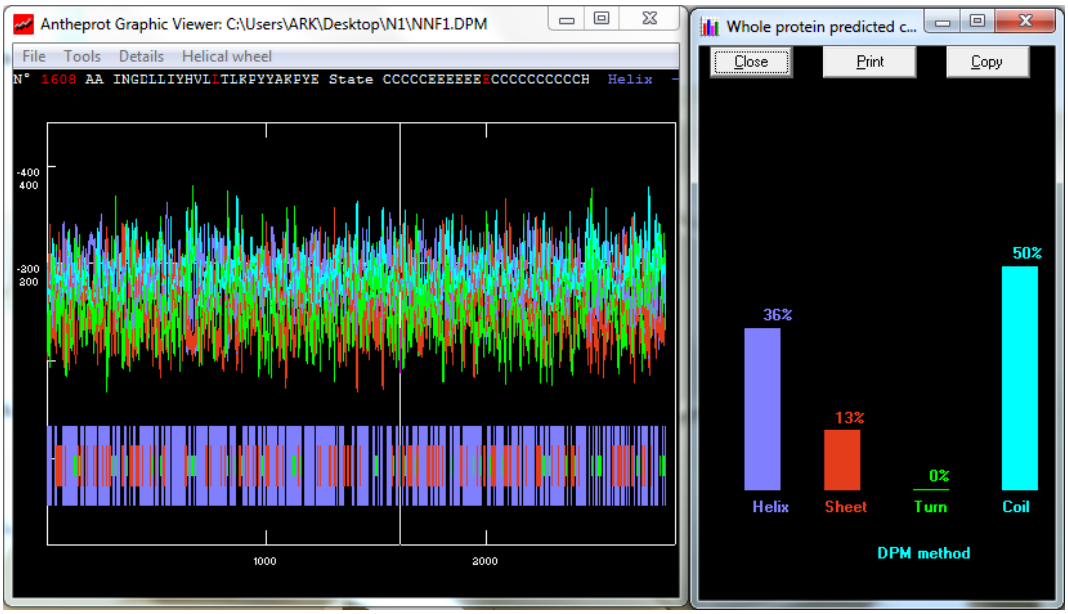

Fig.1: Secondary Structure prediction using Antheprot (a) By DPM method

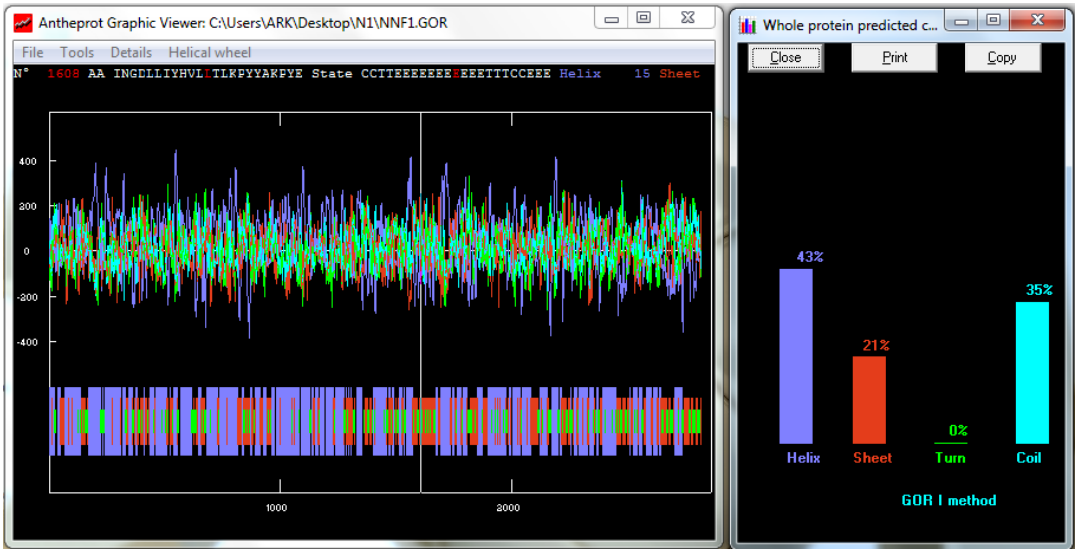

Fig .1: (b) By GOR methods

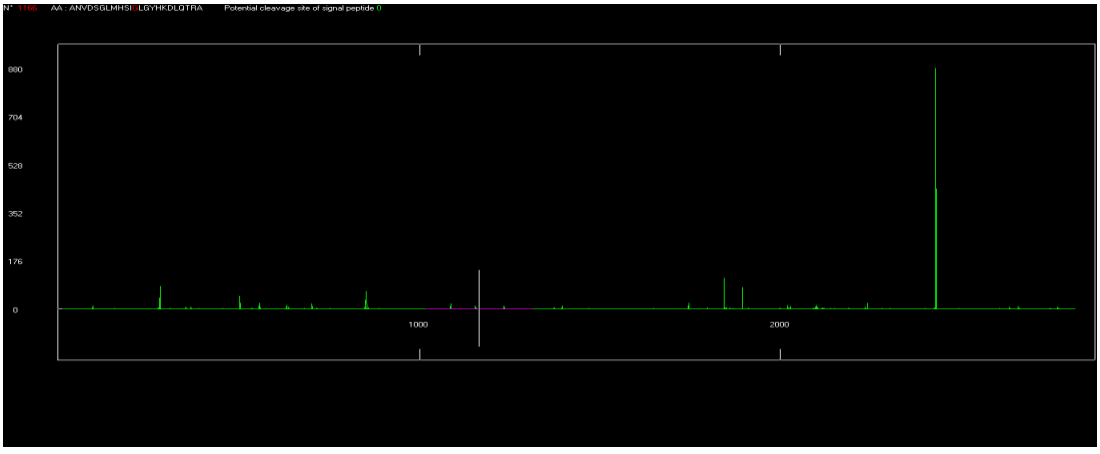

Fig.2: Potential Cleavage Site Using Antheprot (Eukaryotes)

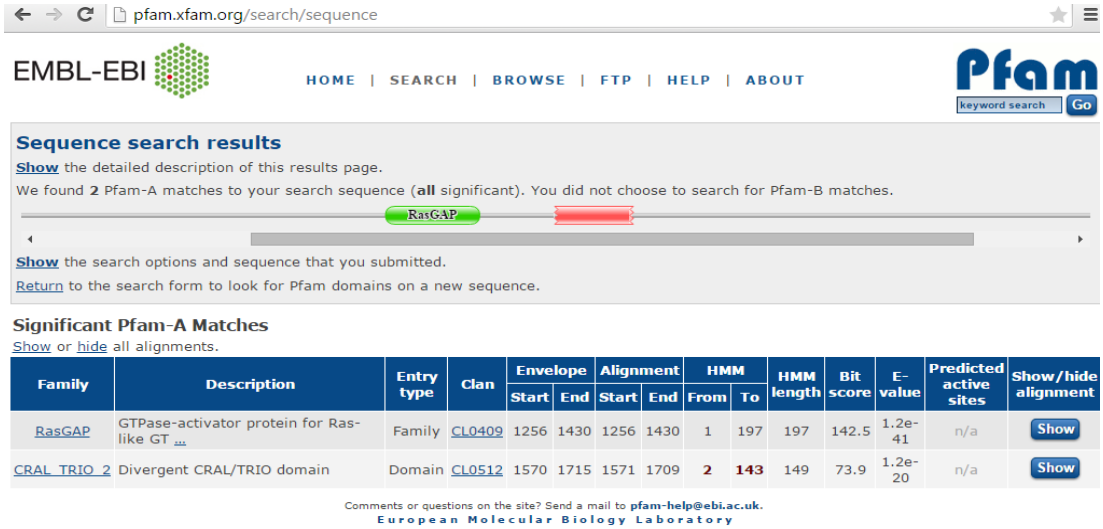

Fig 3: Pfam result showing RasGap related Protein

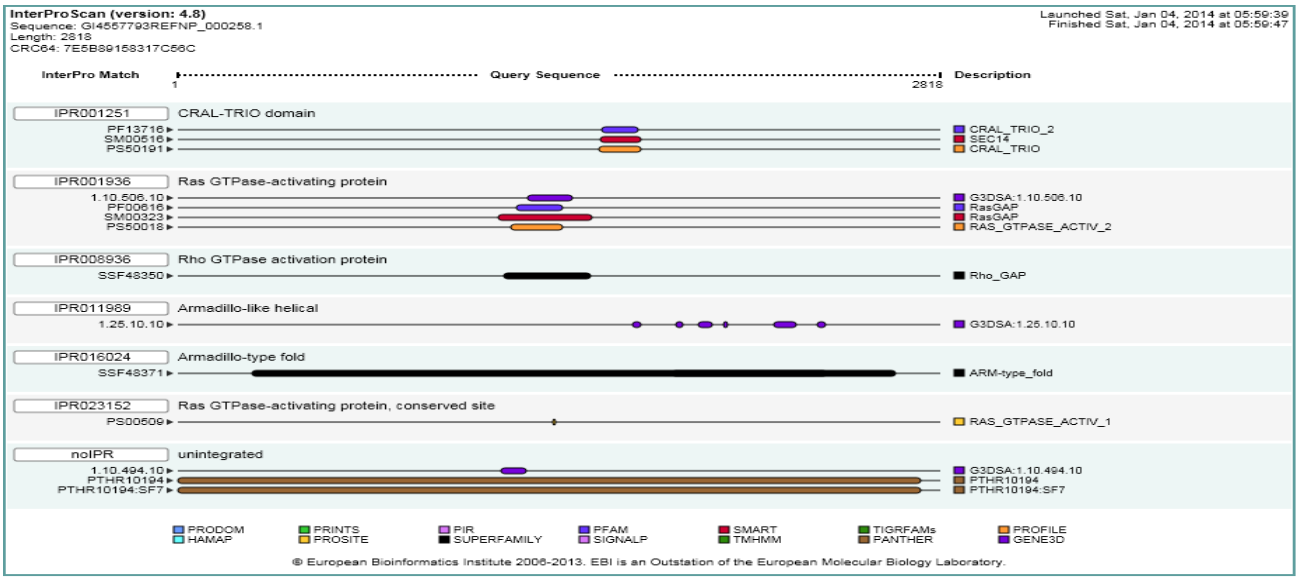

Fig.4: InterPro Scan showing different domain in prote

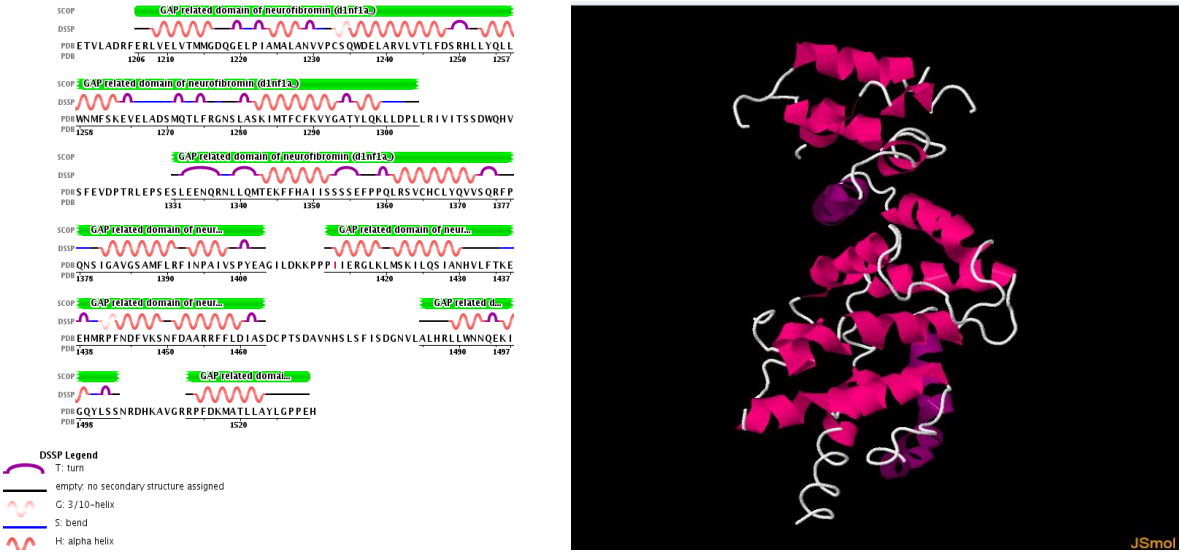

Fig.5: GAP related domain of neurofibromin and 3D view of GAP related domain of neurofibromine using JSmol

The secondary structure comprises alpha helix, β-sheets, turns and coiled region. The analysis shows 36% Helix, 13%Sheet, 0% Turns, 50% coiled region whereas 43% Helix, 21%Sheet, 0% Turns, 35% coiled region according to DPM and GOR method respectively (fig. 1.a & b). The potential cleavage site of signal peptide (Eukaryote) shown in (fig 2). We have found 11 Pfam-A matches to our search sequence (2 significant and 1 insignificant). The graphics below shows (fig.3) the arrangement of matches on our sequence. InterproScan showing different domain in protein (fig.4) GAP related domain of neurofibromin consist of 260 residues (fig.5). 3D Structure of GAP related domain of neurofibromin showing in fig.5.

CONCLUSION

The various protein parameters give significant information about atomic composition, bonding, interactions etc. so it can use to regulate the functioning of neurofibromin protein. This approach is important in new paradigm of computational drug design.

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