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# Crystal Structure Formation of Ten-Eleven Translocation3 Protein: Structure and Functions of TET Family Proteins

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**Original Research Article**

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**Abstract:** Enzymes of Ten-Eleven Translocation family belong to the superfamily of Fe2+- and 2-oxoglutarate (2OG)-dependent dioxygenases. TET proteins are known to have important regulatory roles. Early studies of DNA methylation patterns TET proteins and their role in regulation of DNA methylation/de-methylation patterns. Till now a day no molecule of TET3 protein in case of human is available in PDB. Protein Data Bank contains crystal structure of TET1 and TET2 protein but no one molecule of TET3 available in case of *Homo sapiens*. Formations of 3D structure of TET3 protein in case of human with I-TASER software, it is a first crystal structure of TET3 protein in case of human. Prospect crystal structure of protein validate with X-Ray Crystallography and used too many computational and bioinformatics approaches.
**Keywords:** Ten-Eleven Translocation protein, Oxidation, DNA methylation, 2-oxoglutarate, J-binding protein.

## INTRODUCTION

### Ten-Eleven Translocation Protein

Enzymes of Ten-Eleven Translocation family (TET1, TET2 and TET3) belong to the superfamily of Fe2+- and 2-oxoglutarate (2OG)-dependent dioxygenases all three TET proteins possess a highly conserved carboxy-terminal catalytic region that is composed of a cysteine-rich (Cys-rich) and a double-stranded β-helix (DSBH) domain [1-3]. TET proteins are known to have important regulatory roles. Initial studies of DNA methylation patterns TET proteins and their role in regulation of DNA methylation patterns, TET proteins are large (~180- to 230-kDa) multidomain enzymes.

TET proteins modify the methylation status of DNA by catalyzing consecutive oxidation of the methyl group of 5mC to form 5hmC, which in turn undergoes further oxidation by TET proteins into 5fC and 5caC [4-6]. It's expressed in cells, the catalytic region alone is capable of oxidizing 5mCs in genome. TET proteins require ferrous iron Fe2+ (as an essential cofactor) and 2OG (as an obligatory co-substrate) which bind to the highly conserved 'His-Xaa-Asp-(Xaa) n-His' motif (Xaa refers to any amino acid) and Arg. residues within the DSBH domain, respectively [7].

Once Fe2+ and 2OG are incorporated into their cognate binding motifs in the active site, dioxygen (O2) binds to Fe2+ and oxidizes it to ferric intermediates (Fe3+), stimulating the oxidative decarboxylation of 2OG (to succinate and CO2) and the oxidation of substrate molecules [8]. In addition, TETs alterations are found in B-cell (2%–12%) and T-cell (20%– 83%) lymphomas. Thus, TETs mutations are not limited to a specific disease subtype instead, the mutational landscape suggests that these alterations can be involved in very different disease processes. It can replace multiple transcription factors during cell reprogramming (TET) proteins are α- ketoglutarate dependent dioxygenases [1].

Acquired point mutations and deletion events targeting TET genes are frequently observed, several proteins that interact with TET proteins (such as O-linked β-D-N acetyl glucosamine transferase (OGT)) and with methylated and oxidized cytosines been identified, highlighting their function in the regulation of chromatin structure. The impact of these proteins reaches several aspects of human life—including cell growth regulation, embryonic stem cell maintenance, and cell differentiation—as well as a number of mutations leading to a multitude of diseases, such as those induced by chromosomal translocations. During cytocine modification TETs is able to convert more

than 95% of the 5mC to 5hmC (~60%), 5fC (~30%), and 5caC (5%), but it can only convert about 40% or 25% when 5hmC or 5fC containing DNA was used as a substrate. Metabolic perturbations mutations affect TETs resulting from mutations in genes encoding isocitrate dehydrogenase (IDH), fumarate hydratase (FH) or succinate dehydrogenase (SDH) regulate the TET enzymes and, in turn, DNA de-methylation [9]. Might also be new avenues for research, these enzymes are in the citric acid cycle, are frequently mutated in epigenetic altertation de-methylation proteins TET, and lead to the production of alpha ketoglutarate, which is used as a cofactor for TET and is required for its activity. 2-HG functions as a potent inhibitor of TET proteins that competes with 2-OG *in-vitro* and *in-vivo* [10]. This study report that the new approach crystal structure of human TET3 design. In addition, the catalytic activity of TET proteins is stimulated by ATP [6]. TET proteins was first discovered to convert 5mC to 5hmC in a search of analogous DNA-modifying enzymes based on the analysis of trypanosoma brucei base J-binding protein 1 (JBP1) [1], as a structure–function determinant in TET2, the Thr1372–Tyr1902 scaffold invites comparison to known TET homologs.

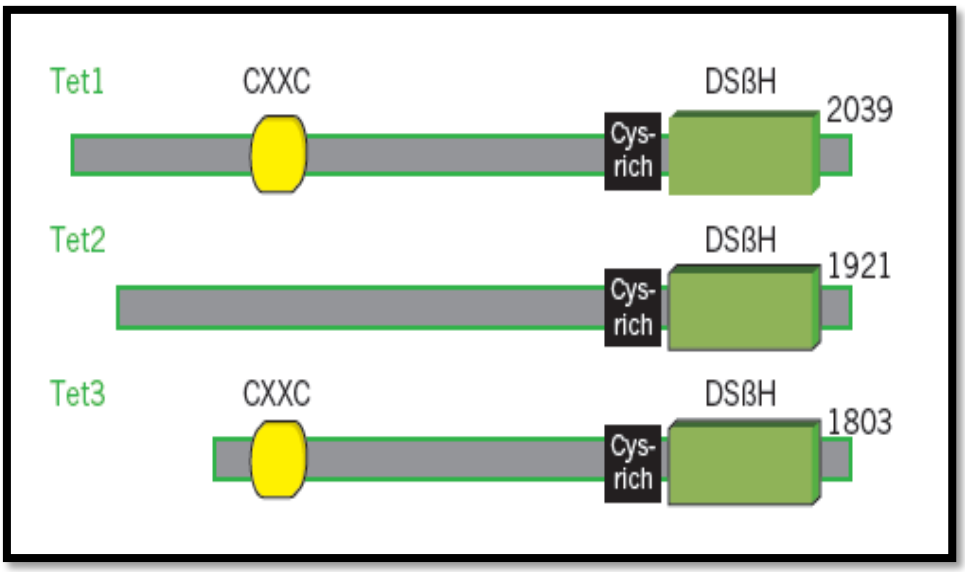



**Fig-1: Structure of TET proteins [1]**

TETs family of protein include a CD that harbors a DSBH domain and a Cys‑rich domain, at the amino terminal of TET1 and TET3 Three conserved domains including CXXC zinc finger, TET2 does not contain a predicted CXXC domain. CD, Catalytic domain; DSBH fold of the 2OG-Fe (II) dioxygenase domain are indicated. Cysteine-rich region (Cys-rich), numbers represent the amino acid numbers [1]. Besides the catalytic domains, the CXXC domains are also involved in TET-mediated gene expression regulation. TET family proteins share highly homologous protein structural features. All TET proteins contain a C terminal double stranded β-helix catalytic domain, TET1 and TET3 also contain a chromatin-associated CXXC domain that is known to bind CpG sequences, whereas TET2 partners with IDAX, an independent CXXC-containing protein **[11],** affected by mutations in TET2 or IDH1 and 2 that decrease TET2 activity, and that this phenomenon has a crucial role in the pathogenesis of diverse myeloid malignancies [12, 13].

**DNA methylation**

DNA Methylation is a major epigenetic mechanism involving direct chemical modification of DNA known as DNA methylation. DNA Methylation is a "transfer of a methyl group onto the C5 position of the cytosine to form 5-methylcytosine", transfer of methyl group with the help of SAM to form 5mC. DNA Methylation take place CpG rich region in the promoter of genes [15, 16], Modified cytosine occurs usually in DNA methylation might be regulate gene expression, also involve in gene regulation and cell differentiation [17]. Cytosine 5 methylation generates 5-methylcytosine, and the reaction is catalyzed by a family of enzymes DNMTs. The conversion of 5mC to 5hmC is facilitated by TETs [1], further oxidize 5hmC into 5fC and 5caC, making these enzymes future targets of pharmacological regulation these steps are catalyzed by the TET enzymes [18]. This oxidation pathway has been proposed as a potential route for the de-methylation of 5mC.

**DNA methylation is reversible**

DNA methylation is a dynamic and reversible process. DNA de-methylation might be induced by TET convert 5-mC to 5-hmC, 5-hydroxymethylcytosine, that can be further deaminated by AID/APOBEC, catalytic polypeptide to produce a DNA mismatch that is repaired by the BER machinery. DNA methylation is reversible because it does not alter the DNA sequence; however, it is heritable from cell to cell. Furthermore,

the reversible nature of methylation offers the potential to revert aspects of the appropriate therapy [19].

**DNA de-methylation**

The conversion of 5mC and its oxidized derivatives back to the unmodified state has been proposed to occur by either "passive" or "active" de-methylatio, "passive" DNA de-methylation refers to the failure to maintain DNA methylation patterns across cell divisions and is believed to result in replication-dependent dilution of 5mC and the other is TET enzyme‐induced "active" de-methyltion through the function of dioxygenase activity, TETs could iteratively oxidize [4, 8]. It was reported that TET proteins are critical regulators for DNA de-methylation by converting 5-mC to 5-hmC in a-KG and in a Fe (II) -dependent manner [1, 20].

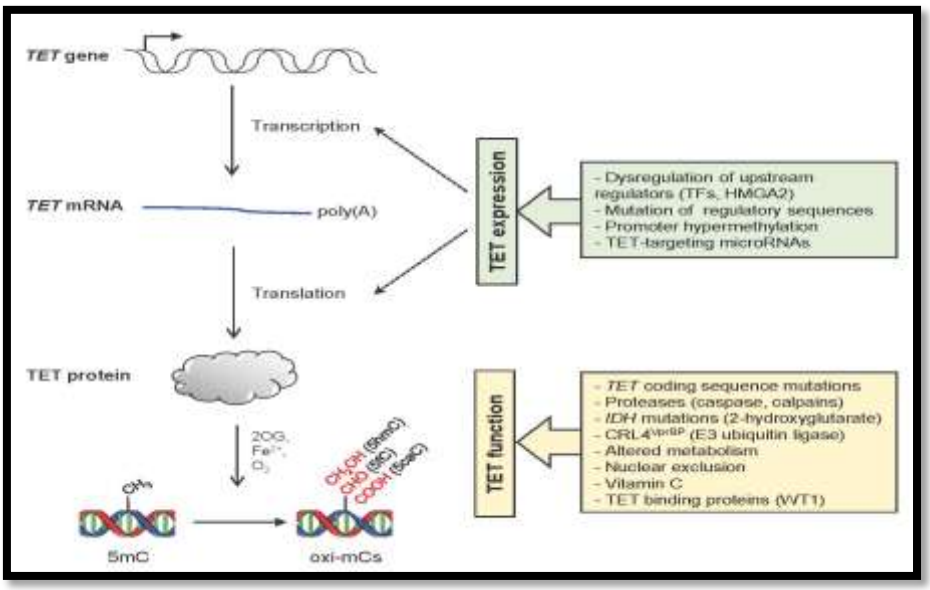



**Fig-2: Non-mutational modulation of TET proteins expression and function, expression and function of TET proteins are regulated on many levels [21]**

TET is a key enzymes play vital roles in DNA de-methylation regulation de-methylation pathway (both TET-dependent and TET-independent) alone [22]. TET enzymes and has epigenetic functions [23]. TETs mediated DNA de-methylation plays an essential role in maintaining DNA methylation, levels at distal regulatory elements [24].

**Regulation of the de-methylation pathway**

Vitamin C enhances TET activity and significantly increases levels of 5hmC/5fC/5caC in suggesting that Vit-C may regulate gene expression, signifying that Vit-C may promote DNA de-methylation. As Vit-C enhances the activity of some Fe (II) 2-oxoglutarate dioxygenases. Vit-C alters the steady-state of DNA methylation and in turn the expression of germline genes in enhancing TET activity. Much work remains to be done to evaluate the ability of Vit-C to modulate TET activity *in vivo* [25].

**METHODOLOGY**

**Model Construction and Preparation**

An on-line version of iterative threading assembly refinement (I-TASSER) is developed for Bioinformatics used to generate protein structure prediction. The I-TASSER server is an integrated platform for automated protein structure and function prediction based on the sequence-to-structure-to-function paradigm. Starting from an amino acid sequence, I-TASSER first generate automated full-length three-dimensional protein structural predictions, atomic models from multiple threading alignments and iterative structural assembly simulations. The function of the TET3 protein is then inferred by structurally matching the 3D models of TET3 proteins [26, 27].

I-TASSER (as 'Zhang-Server') is a hierarchical approach to protein structure and function prediction. It first identifies structural templates from the PDB by multiple threading approach loments. Computational methods for predicting 3D protein structures have been divided into three categories, the structure must be built from scratch by *in-vitro* modeling.

- Based on the availability of template structures in the PDB library.
- In comparative modeling (CM), evolutionarily related homologous templates are identified by sequence.
- High-resolution models generated by copying the framework of the template structures or by satisfying the spatial restraints collected from the template structures no structurally related protein in the PDB library [28, 29].

**Molecular Dynamics Simulation Setup**

- The I-TASSER server is available https://zhanglab.ccmb.med.umich.edu/I-TASSER website.
- Amino acid sequence of TET3 protein chain in the form of FASTA format of respective molecule (TET3) upload. 3. Appropriate design of TET3 protein in the form of PDB. 4. Fill the required form of I-TASSER such as Provide an e-mail address, password and run it. I-TASSER server accepts protein sequences with a length between 10 and 1,500 amino acids so the FASTA sequence must be less than 1500 molecule and more than 500 molecules, so it is run the respectable result [28].

**RESULTS AND DISCUSSION**

**I-TASER software used to 3D modeling of TET 3 protein in case of *Homo sapiens.***

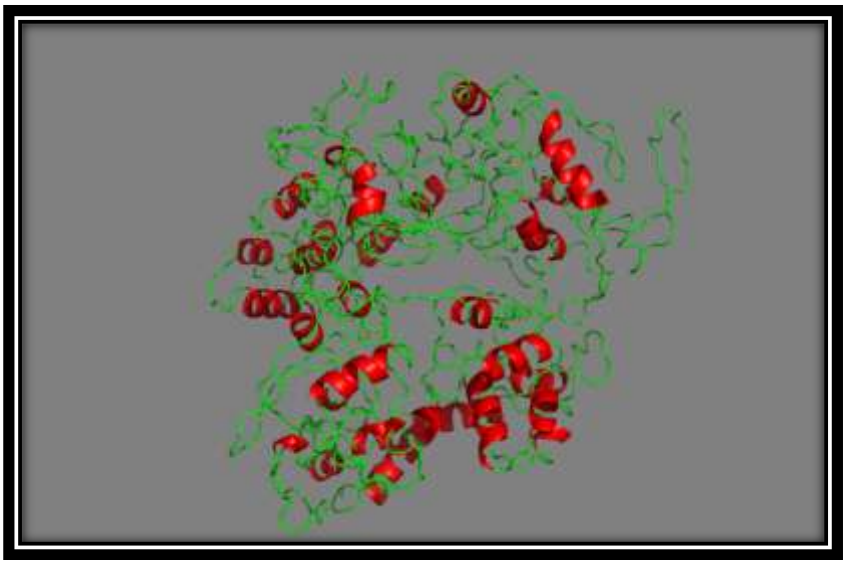
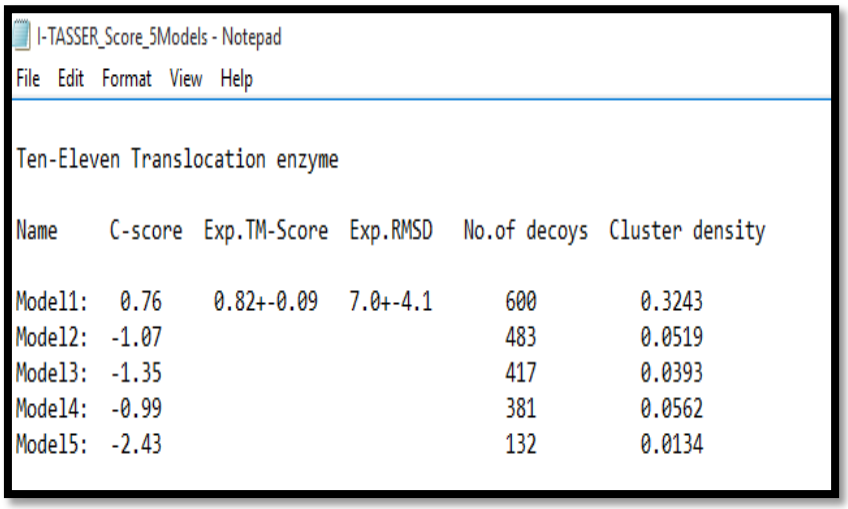


**Fig-3: Crystal structure of human Ten Eleven Translation (TET3) protein**



I-TASSER_Score_5Models - Notepad

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Ten-Eleven Translocation enzyme

| Name | C-score | Exp.TM-Score | Exp.RMSD | No.of decoys | Cluster density |
|---|---|---|---|---|---|
| Model1: | 0.76 | 0.82+-0.09 | 7.0+-4.1 | 600 | 0.3243 |
| Model2: | -1.07 | | | 483 | 0.0519 |
| Model3: | -1.35 | | | 417 | 0.0393 |
| Model4: | -0.99 | | | 381 | 0.0562 |
| Model5: | -2.43 | | | 132 | 0.0134 |

**Fig-4: I-TASER result of Ten Eleven Translation (TET3) model**

Description of 3D protein structure; Ten-Eleven Translocation (TET3) 3D models formation Fig. shows that 5 different model of protein was obtained, first model of protein was seen as best protein model known as standard mode selected on the basis of C-score, TM-score, RMSD value and cluster density is defined as the number of structure decoys at an unit of space in the sicker cluster. First model of TET3 protein contain lesser C-score 0.76, TM-score was 0.82 ± 0.09 and the RMSD value of TET3 7.0 ± 4.1, no of decoys was 600 and the final cluster density of mode1 was 0.3243 is grater then others on the basis of C-score and cluster density model1 is a standard protein molecule contain higher value.

The cluster density is defined as the number of structure decoys at a unit of space in the sicker cluster. A higher cluster density means the structure occurs more often in the simulation trajectory and therefore signifies a better quality model. Model1 containing

0.3243 cluster density it is more higher their other models, so the model1 known as standard model of TET3 protein and the other model of protein present just a reference. The values in the first columns of the above mentioned figure, represents the number of structural decoys that are used in generating each model [29].

## CONCLUSION

Till now a day no molecule of TET3 protein in case of human is available in PDB. Protein Data Bank contain crystal structure of TET1 and TET2 protein in case of human and phages like 5d9y, 5deu etc. and TET3 phages molecule in different species i.e. no. 2WZN, 4HP1 etc. but no one in Homo sapiens molecule of TET3, formations of 3D structure of TET3 protein in case of human with I-TASER software, it is a first crystal structure of TET3 protein in case of human. Prospect crystal structure of TET3 protein validate with X-Ray Crystallography for examining the structure of a regular crystal and submit the Protein Data Bank (PDBe, PDBj, and RCSB) is a crystallographic database for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids (http://www.rcsb.org/). Furthermore crystal structure of TET3 protein used too many computational and bioinformatics approaches.

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