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In Silico Analysis of Bacterial Functional Amyloids and their Interactions

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Abstract: Amyloid proteins have been known to be responsible for many neurodegenerative diseases of mammals including humans. Histopathologically, amyloid fibres are known to be formed due to mis-folds, mutations and other induction events concerned with these proteins in the patients. Once formed, these amyloid proteins have been well reported to undergo nucleation and form fibrous projections that result in cell-death. In living system (microorganisms and higher organisms alike), similar functional amyloid proteins are known to be produced with similar nucleation process. In this report the homologous proteins were created for functional amyloids from Escherichia coli, Bacillus thuringiensis, Bacillus atropheus, Streptococcus pyogenes and Dinoroseobacter shibae. Using the Z-dock server, analysed their interaction with each other. This report will help in understanding the self-folding and nucleation process of the functional amyloids in bacteria and further correlate the functional amyloids of bacteria with pathological amyloids in mammals.
Key words: Functional amyloids, homology modelling, Docking, interaction

I. INTRODUCTION

Amyloid fibers are reported to be made up of amphipathic proteins that aggregate and since long time have been associated with neurodegenerative diseases such as Alzheimer's, Parkinsons and many other Prion diseases. As seen in a newly described class of 'functional' bacterial amyloids (FuBA), the amyloid formation can be an integral part of normal cellular physiology. Even with varied differences in their primary sequence, many proteins can assemble into amyloid folds. This shows that the amyloid fold has been selected multiple times during the evolution for various functions [1]–[4]. By nucleation (aggregation) - dependent mechanism, elongation of histopathological amyloid proteins occurs into fibrils consisting of structured oligomers and protofibrils. The three detailed amyloid aggregation phases are lag, exponential and stationary. These oligomers and protofibrils are considered as the real cytotoxic species in relation to causing human diseases and cytotoxic bacteria [3], [5]. This cellular toxicity of amyloids is avoided in bacteria by using dedicated and highly controlled pathway for assembling amyloids and extracellular assembly of these proteins. Thus by nullifying the cytotoxic effects of amyloids, they can be used as stable protein structures for many different functions [4], [6]. Only few bacterial species important in relation to human infections have been studied with respect to FuBA with curli like fibrils. This list of bacteria capable of producing FuBA is growing rapidly but only a few of them have been purified and investigated in depth. Thus major functions proposed for FuBA generalized for all bacterial amyloids are still speculations [3]. No major studies are available for bacterial functional amyloids proteins *in silico* and in this study, we are incorporating recently unraveled protein structure of TAS protein from *Bacillus subtilis* for analyzing the nucleation and self-assembly of these bacterial proteins. In the present work, we have analyzed the bacterial functional amyloids (synthesized as models and crystal structures available) for their ability to interact with one another and act as a nucleation initiators for each other.

II. MATERIAL AND METHODS

A. *Database Mining and Collection of Sequences*

The FASTA sequences (DNA and protein) of bacterial amyloids were collected from public databases (NCBI and Uniprot). The collected sequences were filtered against hidden Markov model (hmm) using hmmsearch (http://hmmer.janelia.org/).

B. *Analysis of the Sequences Collected*

The sequences were converted to sensible protein sequences using Expasy software and analyzed by Protparam server and their subcellular localization was predicted by using CELLO [7].

C. *Sequences Used*

FASTA sequences of the amyloid proteins were used for the homology models creation for *Escherichia coli* K12 CsgA protein (P28307), *Bacillus thuringiensis* serovar *kurstaki* YBT-1520 TASA protein(PBMB7635_08), *Bacillus atropheus* 1942 TasA (BATR1942_10625), *Streptococcus pyogenes* MGAS15252 (MGAS15252_1626) and *Dinoroseobacter shibae*

DFL12(Dshi_4130). The PDB file of recently deduced TasA protein of *B. subtilis* as the standard for analyzing the interaction of homologous proteins.

D. Homology Modelling

The protein sequences thus made were converted to PDB files using Swissmodel, Phyre2 and RAPTOR-X servers. The constructed models were verified by using ProSA web. The energy minimization was performed by using Swiss-PdbViewer (DeepView v4.1) and best model was selected. The PDB files thus generated were submitted to Protein Model Database (PMDB) and were used for further analysis [8].

E. Docking Studie

$TasA_{239}$, a bacterial amyloid available as protein crystal with PDB ID 5OF1 and 5OF2. Using Z-dock online server, the docking studies were done for pre-amyloid protein homologues thus prepared among themselves and with the crystal protein of 5OF1 [9].

F. Softwares used for Viewing

Swiss-PdbViewer (DeepView v4.1), Chimera 1.11.2 for image generation, PyMOL(TM) 1.7.4.5 (Schrodinger, LLC) and Rasmol 2.7.5.2.

III. RESULTS AND DISCUSSION

Microbial functional amyloids, since their discovery in late 1980s [10], similar proteins have been reported from a number of microorganisms [6]. Majority of these reported functional amyloids are from Proteobacteria, Actinobacteria, Firmicutes and Bacteroides but only a few of them have been purified and investigated in depth. Thus major functions proposed for FuBA generalized for all bacterial amyloids are still speculations [3], [11]. Recently, the TasA amyloid protein from Bacillus subtilis was studied by crystal structure as 5OF1 and 5OF2 [12] and in present manuscript, its structure has been used for nucleation studies and interaction of other amyloid proteins. The amyloid FASTA protein sequences were found to be valid and converted into PDB files and submitted to Protein model database (PMDB). The models were submitted to PMDB as E. coli K12 CsgA (PM0080890), B. thuringiensis serovar kurstaki YBT-1520 TasA (PM0080885), B. atropheus 1942 TasA (PM0080907), S. pyogenes MGAS15252 (PM0080904) and D. shibae DFL12 (PM0080887). $TasA_{239}$, amyloid protein crystal structure from B. sbtilis was edited with PyMOL software for 'SAL' residues and converting into a monomeric unit. The protein was then analysed with newly synthesized amyloid protein structures. Unlike mammalian mis-folded amyloid proteins, the bacterial FuBa are synthesized with purpose. Hence considering their structure as unique, were analysed further by docking studies.

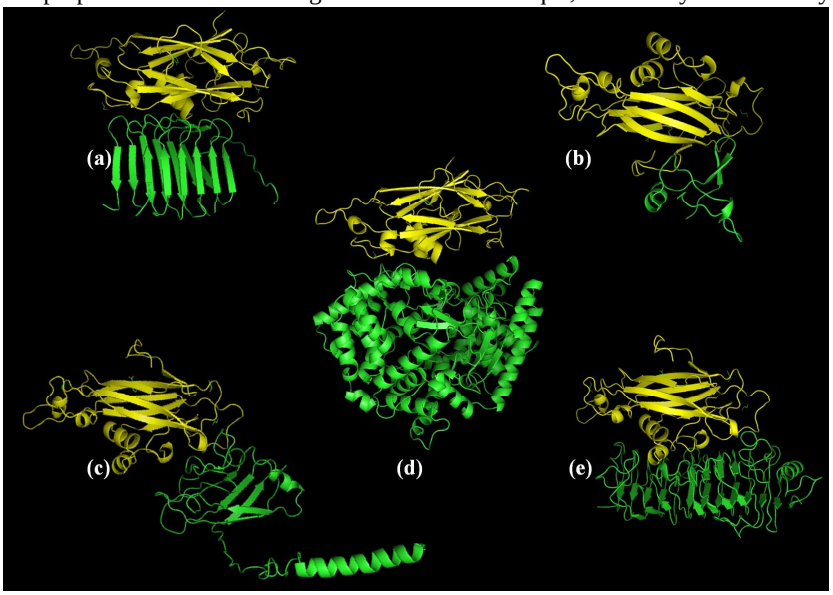

Figure 1: Interaction studies of crystal Bacterial functional amyloid 5OF1 (in yellow) with homology models. The homology models created namely (a) Escherichia coli K12 CsgA protein (P28307), (b) Bacillus thuringiensis serovar kurstaki YBT-1520 TASA protein (PBMB7635_08), (c) Bacillus atropheus 1942 TasA (BATR1942_10625), (d) Streptococcus pyogenes MGAS15252 (MGAS15252_1626) and (e) Dinoroseobacter shibae DFL12(Dshi_4130).

In the field of molecular modelling, docking is a method for the prediction of preferred orientation of two molecules bound to each other to form a stable complex. While protein-protein docking- being computationally oriented (*in silico*), determines the molecular structure of the complex without the need for a wet-lab experiments [9]. Z-DOCK and M-DOCK are rigid-body docking programs predict protein complex structures and symmetric multimers guiding the user in scoring and selection of output models. Z-DOCK is known to achieve high accuracy on protein-protein docking benchmarks [9]. The interaction of B.subtilis TasA protein (5OF1) with other amyloid models from E. coli K12 CsgA (PM0080890), B. thuringiensis serovar kurstaki YBT-1520 TasA (PM0080885), B. atropheus 1942 TasA (PM0080907), S. pyogenes MGAS15252 (PM0080904) and D. shibae DFL12 (PM0080887) by using Z-DOCK server (Figure 1). 5OF1 was found to interact with most of amyloid protein models under study with GLU229 and THR237 amino acids. Which was found to be major contributors in 5OF1 nucleation (data not shown). The interacting aminoacids are listed in Table-1.

TABLE – 1
DOCKING INTERACTION OF AMYLOID PROTEIN MODELS AND CRYSTAL STRUCTURE

Sr. No	Amyloid protein 1	Amyloid protein 2	Amino acids interacting from Amyloid protein 1	Amino acids interacting from Amyloid protein 2
1	5OF1	E.coli CsgA (P28307)	SER52,ASN88,ASP91,TYR139,LYS144,GLU229,THR237	GLN49,GLY53,ASN54,GLY74-ASN77,ARG95, GLY120
2	5OF1	B.thuringiensis TasA (pBMB7635_08)	LYS35,PHE39-ALA40,ASP190,GLU229	LYS19, TYR33-THR34,LYS39
3	5OF1	B.atropheus TasA (BATR1942_10625)	ALA79,VAL189,GLU229,TRP233,THR237	PHE109,GLN110-THR112
4	5OF1	S.pyogenes amyloid (MGAS15252_1625)	ASN88-TYR89, PHE92,PRO102,GLU229,GLN232,THR237	ARG250,MPHE220,GLN232,THR237 TO ARG250,PHE255,THR258,HIS479, HIS483,GLN504, GLU539, Zn1
5	5OF1	D.shibae DFL12 (Dshi4130)	ASN145-ALA147,LYS152,LYS154,PRO159, VAL184,GLU229, TRP233,THR237	ASP100,TYR101, TYR218,HIS220,ASP222,GUN225,TRP227,ASP322,ARG335
6	E.coli CsgA (P28307)	B.thuringiensis TasA (pBMB7635_08)	ILE13,TYR43,ILE47,GLN23,VAL13,ALA8, LYS5	GLU54, ILE55, THR27, LEU26, GLY25, ILE13
7	E.coli CsgA (P28307)	B.atropheus TasA (BATR1942_10625)	PHE97,GLN72,TYR50,TYR26,MET1-LYS2	THR43,LEU44, PHE29, ALA27, GLY23, ALA19, ALA15
8	E.coli CsgA (P28307)	S.pyogenes amyloid (MGAS15252_1625)	LYS5, TYR26,TYR48,TYR50,ASP67,GLN117	TYR212-TYR215, LYS222,ASP242,GLU249-ARG250,HIS479,HIS483, GLU539,ZN1
9	E.coli CsgA (P28307)	D.shibae DFL12 (Dshi4130)	PRO134,PHE137,ASP140,VAL142,ARG151,GLY196,THR215-ASN216, HIS220,TRP227, ARG335	ILE12-SER15, ASW21,HIS32,PRO41,SER55,TRP106,ASN145
10	B.thuringiensis TasA (pBMB7635_08)	B.atropheus TasA (BATR1942_10625)	ILE 13, PHE32, ILE57	PHE92, PHE109-THR115
11	B.thuringiensis TasA	S.pyogenes amyloid	ILE20,GLN30, TYR33	TYR198,TYR215,PHE255,

	(pBMB7635_08)	(MGAS15252_1625)		
12	B.thuringiensis TasA (pBMB7635_08)	D.shibae DFL12 (Dshi4130)	GLN40,LYS19-ASN22	VAL142,HIS220,TYR218,THR320-ASP322
13	B.atropheus TasA (BATR1942_10625)	S.pyogenes amyloid (MGAS15252_1625)	ALA12, LEU16, PHE29, GLU36-LEU44	MET127-LEU131, GLN239-LYS243, LYS423, LYS511-ASP515
14	B.atropheus TasA (BATR1942_10625)	D.shibae DFL12 (Dshi4130)	Leu 18-PHE39	GLN300,TYR275, TYR 218, TYR149-ARG151
15	S.pyogenes amyloid (MGAS15252_1625)	D.shibae DFL12 (Dshi4130)	VAL216,ASP242,ARG250,GLU254,PHE255,HIS479,HIS483,GLU539,ZN1	TYR167, ARG194,TYR275,ASN336
16	E.coli CsgA (P28307)	E.coli CsgA (P28307)	GLN49,TYR50,ASN54, GLN72,ARG95,PHE97,ASN122	ASN31-HIS32, TYR50-ASN54, GLY75-ASN77, PHE97
17	B.thuringiensis TasA (pBMB7635_08)	B.thuringiensis TasA (pBMB7635_08)	ILE13,ARG15,ILE20,SER23,THR27,PHE32-LEU36, LEU46-GLU47,GLU53-ILE55,ILE57,LYS59	ILE13,ARG15,ILE20,SER23,THR27,PHE32-LEU36, LEU46-GLU47,GLU53-ILE55,ILE57,LYS59,
18	B.atropheus TasA (BATR1942_10625)	B.atropheus TasA (BATR1942_10625)	SER107-LEU113, ASN93	SER107-LEU113, ASN93
19	S.pyogenes amyloid (MGAS15252_1625))	S.pyogenes amyloid (MGAS15252_1625)	TYR4,VAL351,GLN464-SER466,HIS479-HIS483,GLU539,ARG563-ASN568	MET127,TYR212-ASP219,GLU249,HIS479-HIS483,LEU498-ASP500,GLN504,GLU539
20	D.shibae DFL12 (Dshi4130)	D.shibae DFL12 (Dshi4130)	ALA61, TYR101, SER116, ARG151-ASN152, ARG176, PRO221, TRP227-THR228,	ARG 55, TYR101, LYR113-LYS115, PRO134, ASP140, ARG151, HIS168, ALA173, ARG194, TRP227, TYR275,

Interaction studies of amyloid models with each other and as dimers showed interesting results. Each of the protein used specific aminoacids for interactions and could form specific structures as given in figure 2 and figure 3. The amino acids involved in the interaction by Z-DOCK server has been given in Table-1.

E. coli K12 CsgA (PM0080890) was found to be interacting with LYS5, PHE97, GLN72, TYR50 and TYR26; B. thuringiensis serovar kurstaki YBT-1520 TasA (PM0080885) was interacting by using PHE32, ILE57 and ILE20; B. atropheus 1942 TasA (PM0080907) interacted using TYR218, PHE29 and LEU44; S. pyogenes MGAS15252 (PM0080904) interacted using ASP242, ARG250, PHE255 and HIS479; while D. shibae DFL12 (PM0080887) interacted using ARG151 and TYR275.

Thus the model proteins and crystal proteins of the bacterial functional amyloids have similar specific aminoacid sequences for interaction and can interact with one another for the nucleation process.

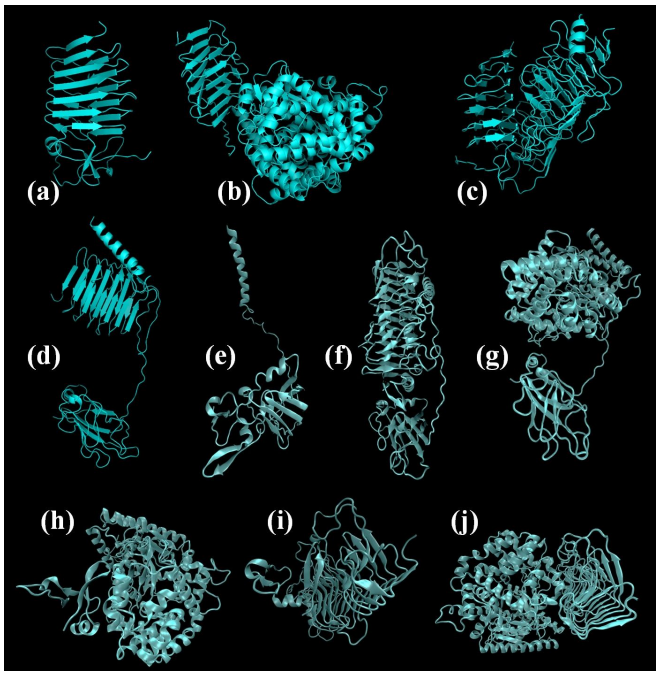

Figure 2: Bacterial functional amyloid docking studies. The interaction image between E.coli CsgA (P28307) with (a) B.thuringiensis TasA (pBMB7635_08), (b) S.pyogenes amyloid (MGAS15252_1625), (c) D.shibae DFL12 (Dshi4130) and (d) B.atropheus TasA (BATR1942_10625) respectively. Also B.atropheus TasA (BATR1942_10625) interaction with (e) B.thuringiensis TasA (pBMB7635_08), (f) D.shibae DFL12 (Dshi4130), (g) S.pyogenes amyloid (MGAS15252_1625); and B.thuringiensis TasA (pBMB7635_08) with (j) S.pyogenes amyloid (MGAS15252_1625) and D.shibae DFL12 (Dshi4130) respectively are also given. Protein docking studies between (j) S.pyogenes amyloid (MGAS15252_1625) and D.shibae DFL12 (Dshi4130) is shown

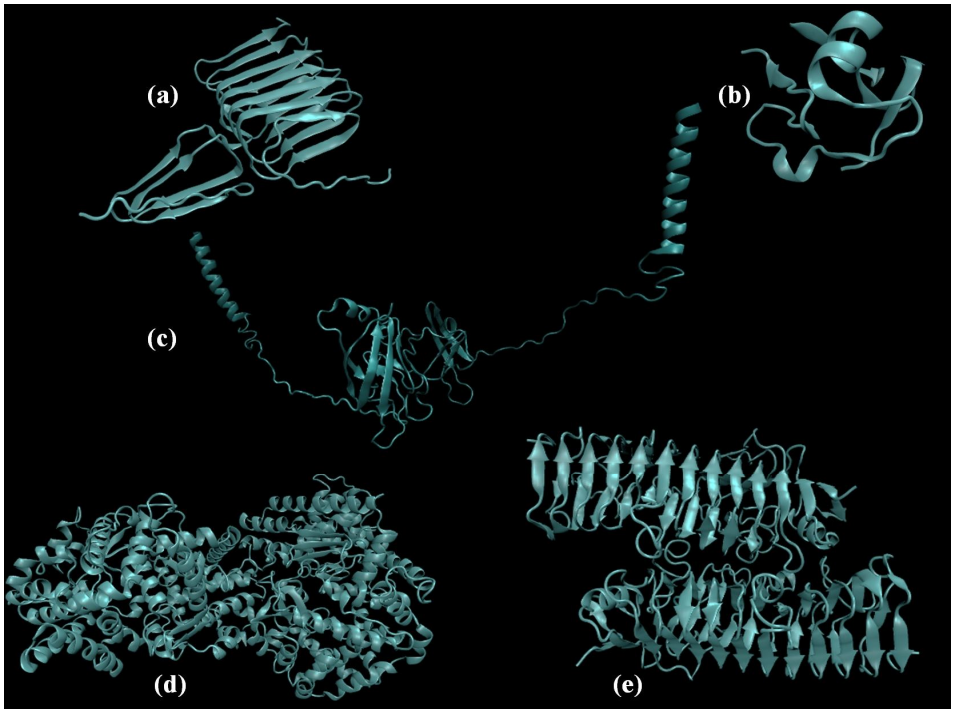

Figure 3: Self- interaction of bacterial functional amyloids. (a) Escherichia coli K12 CsgA protein (P28307) dimer, (b) Bacillus thuringiensis serovar kurstaki YBT-1520 TASA protein (PBMB7635_08) dimer, (c) Bacillus atropheus 1942 TasA (BATR1942_10625) dimer, (d) Streptococcus pyogenes MGAS15252 (MGAS15252_1626) dimer and (e) Dinoroseobacter shibae DFL12(Dshi_4130) dimer.

IV. CONCLUSION

The interaction studies of bacterial functional amyloids can help in understanding the mammalian pathological amyloids and further the knowledge in their nucleation process. Although present *in silico* study in insufficient for confirmed interactions, it gives a basic blueprints for the other works about the possible interactive sites on the amyloid protein chains.

V. ACKNOWLEDGEMENTS

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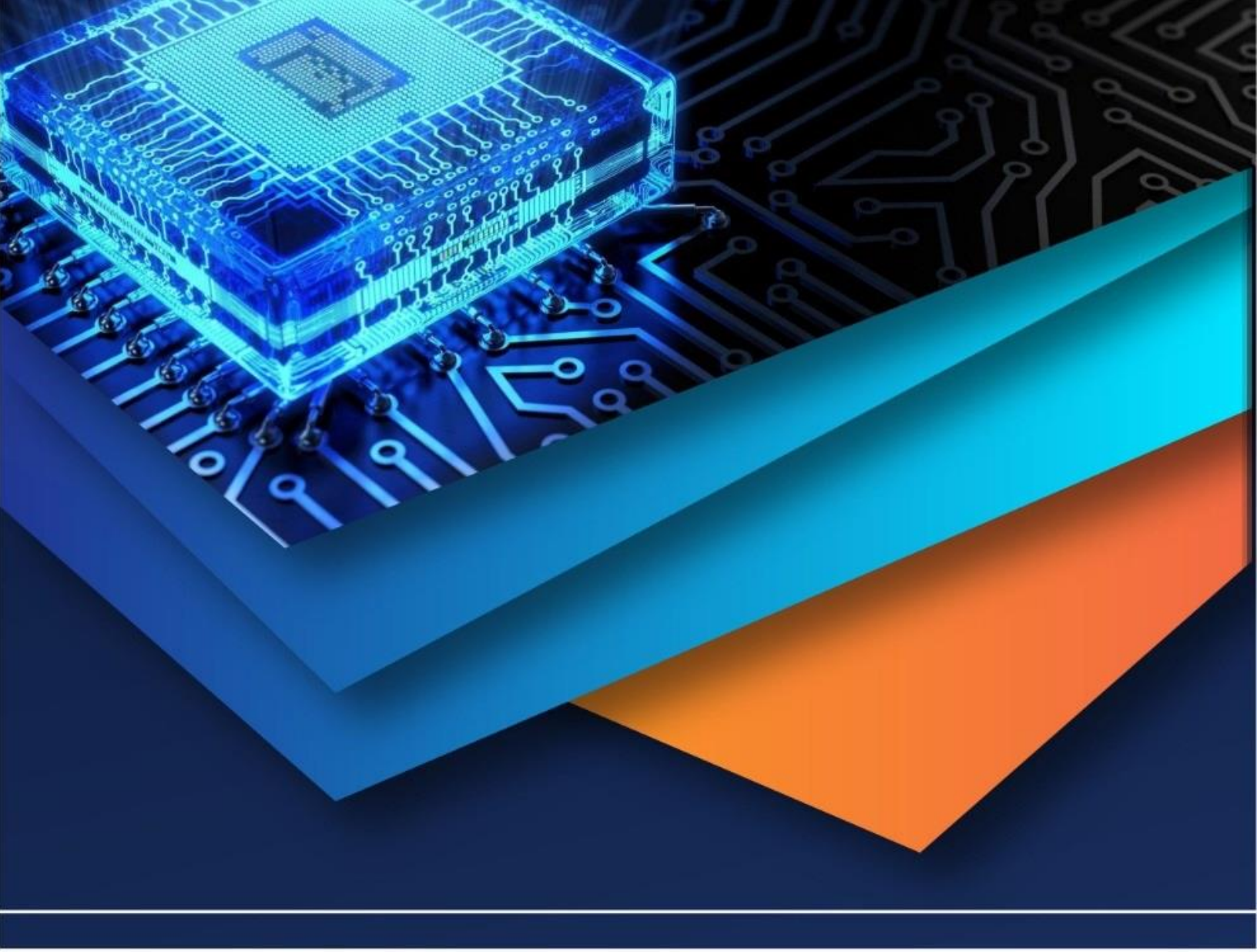

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