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In silico Analysis and interaction studies of Functional and Pathological Amyloids

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Abstract: Amyloid proteins have been known to be responsible for many neurodegenerative diseases of mammals including humans. Histopathologically, amyloids are known to be formed due to misfolds, mutations and other induction events concerned with these proteins in the patients. Once formed, these 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 processes. A major amyloid involved in Alzheimer's disease (AD) is the misfolding of human α-synuclein (2N0A). In this report the homologous protein of 2N0A was created and studied for interaction with functional amyloid, TasA from *Bacillus subtilis* (5OF1) using the Z-dock server, and analyzed the interacting aminoacids. This report will help in understanding the self-folding and nucleation process of the functional amyloids in bacteria and further correlate their interaction with human pathological amyloids.

Keywords: Functional amyloids, homology modelling, docking, interaction, Alzheimer's disease (AD)

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]. The main constituents of the Alzheimer's disease (AD) plaques are small peptides 39-43 amino acids long called amyloid β-peptide (Aβ). The early studies on AD focused on the amyloid fibrils but later it was found that Aβ oligomers further formed aggregation of these cytotoxic agents [5]. Monomeric Aβ is disordered intrinsically in aqueous solution. Among all the amino acid sequences, only a few amino acids make up the major repeating models of Aβ monomers [6].

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], [7]. 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], [8].

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

Analysis of the sequences collected

The sequence of α-Synuclein GRCh38.p7 (2N0A) was converted to sensible protein sequences using Expasy software and analysed by Protparam server and their subcellular localization was predicted by using CELLO [9].

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Homology modelling:
The protein sequence of α-Synuclein GRCh38.p7 (2N0A) was 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 was used[10].

Protein models/crystal protein structures used:
Bacillus subtilis TasA, α-Synuclein GRCh38.p7 assembly protein and α-synuclein misfolded homology model were used for further analysis [10].

Docking studies:
Bacillus subtilis TasA was analysed for protein-protein interaction with the normal wild type and misfolded α-Synuclein GRCh38.p7 protein. 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.

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. Result and Discussion

Microbial functional amyloids, since their discovery in late 1980s [11], similar proteins have reported from a number of microorganisms [8]. Majority of these reported functional amyloids are from *Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Bacteroides* but few have been purified and investigated. Thus major functions proposed for FuBA generalized for all bacterial amyloids are still speculations [3], [12]. Recently, the TasA amyloid protein from *Bacillus subtilis* was studied by crystal structure as 5OF1 and 5OF2 [13] 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 PM0081512.

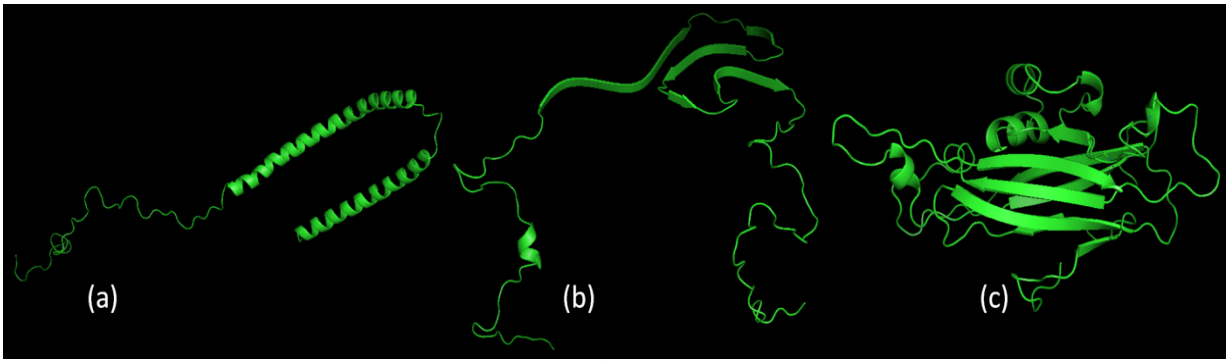

Fig 1: Pathological and functional amyloid protein.
(a)Non-amyloid α-synnuclein protein (2N0A), (b) its homologous amyloid structure model and (c) the TasA amyloid protein from Bacillus subtilis (5OF1).

Tas A239, an amyloid protein crystal structure from *B. subtilis* was edited with PyMOL software for 'SAL' residues and converting it 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, they were further analysed by docking studies.

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. 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.

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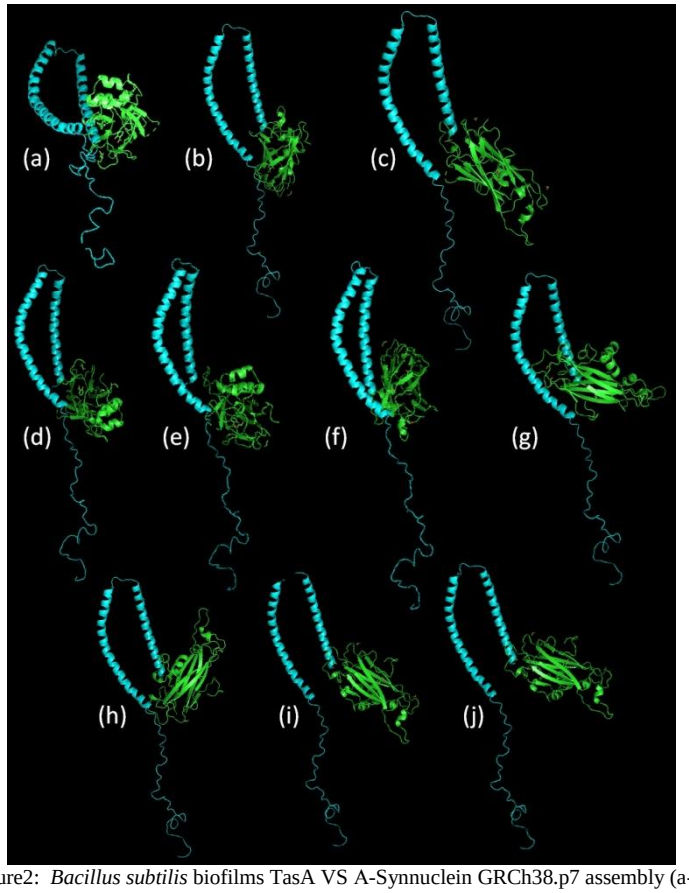

Figure2: *Bacillus subtilis* biofilms TasA VS A-Synnuclein GRCh38.p7 assembly (a-j).

TABLE – 1

DOCKING INTERACTION OF AMYLOID PROTEIN MODELS AND CRYSTAL STRUCTURE

Sr. No	Interacting amyloid proteins			Interacting amyloid proteins amino acids	
	Protein 1	Protein 2	Model No	Protein 1	Protein 2
1	5OF1	α-synuclein	1*	GLU229, THR237,ILE238	PHE4
2	5OF1	α-synuclein	2*	ALA37,PHE39,ASP69,LYS155,GLU 29,THR237	ASP2,ALA9,THR92,LYS97
3	5OF1	α-synuclein	3*	VAL184,GLV229,TRP233,THR237	PHE4
4	5OF1	α-synuclein	4*	PHE39,GLU229,THR237	LYS97,LEU100
5	5OF1	α-synuclein	5*	ASN88,ALA143,GLN225,GLU229,T HR237	MET5,LEU8
6	5OF1	α-synuclein	6*	PHE39,ASP69,THR112,LYS126,ILE1 28,GLN195,GLU197,GLU229,THR2 37	ALA85,ILE88,ALA90,ALA 91,PHE94,VAL95,LYS97
7	5OF1	α-synuclein	7*	PHE39,GLU229,THR237	ALA90
8	5OF1	α-synuclein	8*	PHE161,GLU229,THR237	PHE4
9	5OF1	α-synuclein	9*	GLU229,TRP233,THR237	PHE4,MET5
10	5OF1	α-synuclein	10*	VAL184,GLU229,TRP233,THR237	PHE4,MET5
11	5OF1	α-synuclein	1#	GLU73,VAL116,GLY117,ILE173,AS P174,THR189,GLU229,THR237	TYR39,SER2,LYS43,LYS80
12	5OF1	α-synuclein	2#	LYS35,PHE39,ALA40GLY183,LYS1 86,GLU229,THR237	ALA53,THR54,VAL74,LEU 100,GLV105
13	5OF1	α-synuclein	3#	TYR181,ASP182,GLY183,GLU229,T HR237	TYR39
14	5OF1	α-synuclein	4#	GLY183,GLU229,THR237	VAL3
15	5OF1	α-synuclein	5#	ALA176,PRO179,TYR181,GLU229,	MET5

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16	5OF1	α-synuclein	6#	THR237 TYR181,GLU229,TRP233,THR237	PHE4
17	5OF1	α-synuclein	7#	ASP174,LYS179,TYR181,ASP182,GLY182,ASP192,GLU229,THR237	TYR39,LYS43,THR44
18	5OF1	α-synuclein	8#	ASP36,GLU229,THR237	GLU20,TYR39
19	5OF1	α-synuclein	9#	ASN88,ASP91,GLU103,GLU229,THR237	THR64,THR72,LYS97
20	5OF1	α-synuclein	10#	PHE72,LYS81,ASP182,GLY183,PRO185,GLU229,TRP233,THR237	MET1,PHE4MET5,LYS6LYS10

*normal Wild type protein structure, #misfolded amyloid structure

From table 1 and Figure 1, it is evident that the functional amyloid 5OF1 interacted in each model by GLU229 and THR237 and α-synuclein could by PHE4 before the change in structure. Chen et al., (2016) [14] have reported the possibility of bacterial functional amyloids in increasing the nucleation of α-synuclein as seen in amyloid based prion diseases. Coulson et al., [15] have discussed the similarity/dissimilarity among the eukaryotic/mammalian amyloids while Xia et al., [16], Castillo et al [17] and Ando et al., [18] have discussed the possibility of interaction between the amyloid precursor protein (pre-amyloid α-synuclein) for Alzheimer's disease. Present report gives a thorough amino acid list for the interaction between bacterial functional amyloids and pre-amyloid α-synuclein wild type protein. Thus, figure 2 and table 1 provide the possible sites of interactions among the prokaryotic-eukaryotic protein interactions and hence we can now target the amino acids in pre-amyloid α-synuclein to inhibit the formation of Alzheimer's disease.

As seen in Table 1 and Figure 3, the mis-folded α-synuclein could interact by TYR39 while 5OF1 interacted in each model specifically by using GLU229 and THR237.In our previos study, (in communication), we have already reported the possibility of self and cross interactions among the bacterial functional amyloids. Although amyloid-amyloid interaction could enhance the nucleation process leading to faster formation of fibrils, Seeliger et al., [19] have reported the cross-reaction among different amyloids with Aβ protein of Alzheimer's disease.

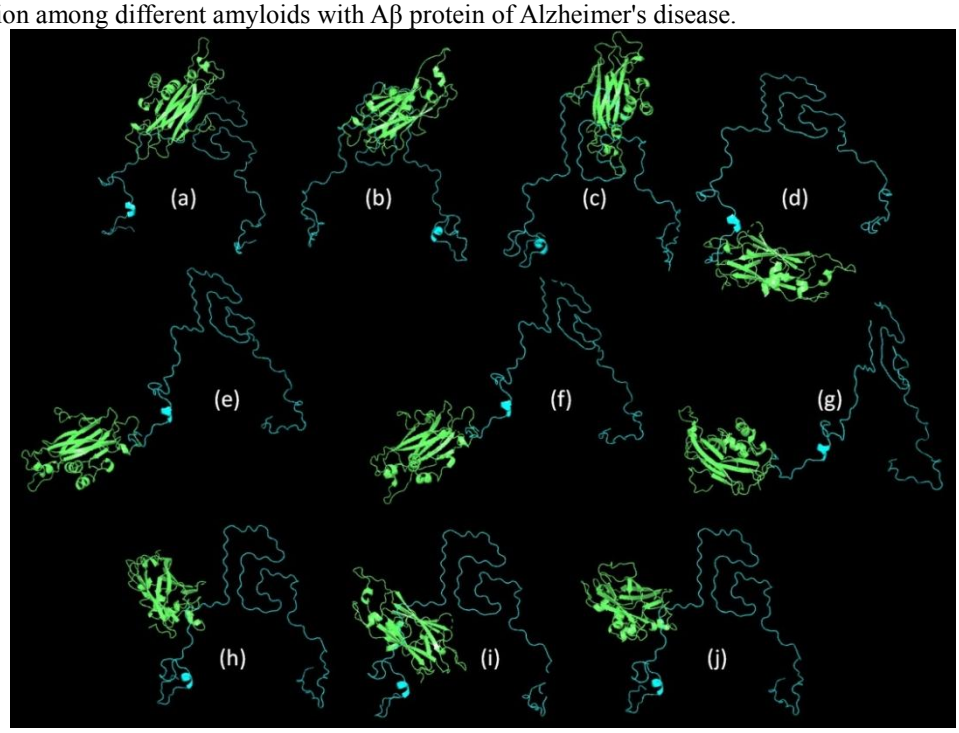

Fig3: *Bacillus subtilis* biofilms TasA VS Alpha-synuclein(a-j)

IV. CONCLUSION

The interaction studies of bacterial functional amyloids with pre and post misfolding of α-synuclein which leads to Alzheimer's disease could help us understand the possibility of onset of this disease and 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 and probable sites of target for future works on prevention of Alzheimer's disease.

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