Details of the research work duly signed by the applicant, for which the Sun Pharma Science Foundation Research Award is claimed, including references and illustrations

Dr. Chattopadhyay obtained his PhD degree from TIFR-Bombay, where he studied the conformation and function of different heme containing proteins and enzymes by combining optical spectroscopy and bio-electrochemistry. His PhD work has led to one of the initial reports of direct electrochemistry of two non-electron transfer proteins like myoglobin and horseradish peroxidase using electrode coated with neutral surfactants (Chattopadhyay and Mazumdar 2001 Bioelectrochemistry 53, 17-24). This method is widely used now, and the paper has been well cited. In another well cited paper from his PhD study, Dr. Chattopadhyay determined the structural factors, which are responsible for the stability of horseradish peroxidase (Chattopadhyay and Mazumdar 2000 Biochemistry 39, 263-270). As a postdoc at the Washington University School of Medicine, Dr. Chattopadhyay developed applications of Fluorescence Correlation Spectroscopy (FCS) to study protein folding and dynamics in the folded (Chattopadhyay et al 2002 PNAS 99, 14171-14176) and unfolded (Chattopadhyay et al 2005 PNAS 102, 2385-2389) states. This method of combining fluorescence quenching and FCS to study protein dynamics at the microsecond time scale is now used by multiple groups internationally. Before joining CSIR-IICB as a senior scientist (Dec 2006), Dr. Chattopadhyay worked at Pfizer, Inc to characterize and troubleshoot formulation and stability related proteins of several early and late-stage biologics candidates (recombinant proteins and mAbs).

A brief account of the research contributions of his laboratory at CSIR-IICB is discussed below:

a) Molecular Basis of Neurodegeneration: Early Vs. Late Stages of Protein Aggregation:
Neurodegenerative diseases take place when nerve cells in the brain or peripheral nervous system start losing function progressively leading to eventual death. Although currently treatments are used to relieve associated physical or mental symptoms, no disease modifying drugs are available for majority of the neurodegenerative diseases. Aggregation of proteins and their conformational disintegrations is found to be a common theme for neurodegenerative disorders. A major emphasis of Dr. Chattopadhyay's group at CSIR-IICB is to develop ways to monitor the early events of protein aggregation *in vitro* and inside cells to understand its roles in different neurodegenerative diseases with particular emphasis to Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS)

(i) Why is it important to study the early stage of aggregation?

For most neurodegenerative diseases, one or more proteins have been found to become aggregated and deposited in various places of human physiological systems. For example, in PD, as a part of the disease process aggregates of a protein, alpha synuclein (A-Syn) accumulates as Lewy bodies in dopaminergic neurons. A representative example of an aggregation kinetics is shown in **Figure 1**. The product of the aggregation process is often the generation of amyloid fibrils, which can be easily and conveniently probed by absorption (Congo Red staining) or fluorescence assays (using thioflavin T fluorescence measurements). The amyloid fibrils are stable and homogeneous and as a result, many high-resolution structures are available for these species. Both solid state NMR and cryoEM have been used to study amyloid fibrils formed for different proteins including A-Syn. In

addition, last decade has observed many high-profile clinical trials targeting amyloid fibrils, which included the use of small molecules and monoclonal antibody based biotherapeutic molecules. Although many of these molecules efficiently inhibit fibril formation, these have failed to delay disease progression.

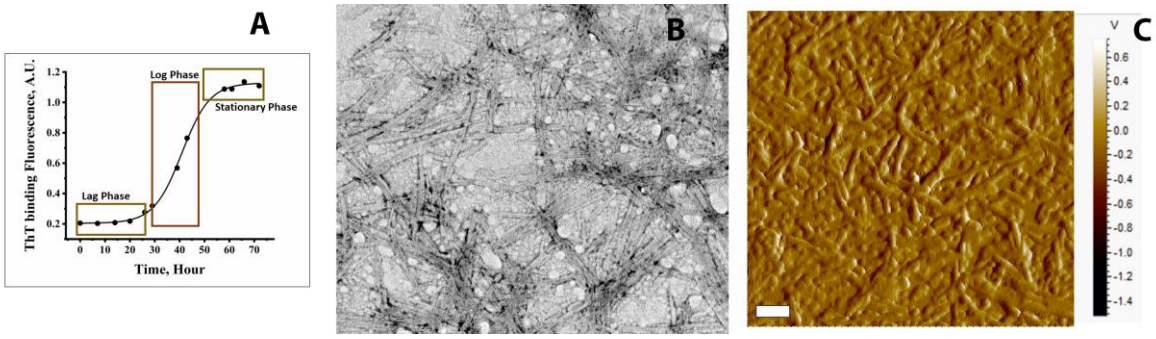

Figure 1: (A) A representative aggregation kinetics of A-Syn monitored by ThT fluorescence. The sigmoidal nature is typical in which the early stage-which does not lead to any fluorescence signal-is called the lag phase. At the end (which is termed here the stationary phase) has been extensively studied in literature. The result of the aggregation is the generation of amyloid fibril. Typical TEM and AFM images of A-Syn are shown in Figure 1(B) and 1(C) respectively. Since fibrils are extensively characterized, most of the drug trials have been targeting these species. Please note this and the majority of the data shown in this document are published and used without permission from the journal. These papers are cited in the text.

These failures and other results have led to the recent belief that amyloid fibrils (which are generated late in the aggregation process) may not necessarily be the most toxic species. Instead, the early phases of aggregation can result in heterogeneous population of rapid and interconverting species, which are responsible for the toxicity. Unfortunately, these species may not respond to the conventional assays (like ThT fluorescence), which combined with their low population, make a lag phase in the kinetics (Figure 1), in which no fluorescence change takes place. Since the early stage is responsible for the toxicity and is undetected by conventional methods, Dr. Chattopadhyay's group has developed the application of different techniques, including that of Fluorescence Correlation Spectroscopy (FCS) to study the early events of protein aggregation using A-Syn and other model proteins.

(ii) <u>Developing the model and methodologies to study the early stage</u>

At the beginning, Dr. Chattopadhyay's group used the help of polymer theory and classical protein folding hypotheses to develop suitable models, which could be experimentally validated. Assuming that the initial contact formation at the unfolded state would lead to the fastest events of protein folding, any wrong contacts can lead to misfolding and subsequent aggregation (Journal of Biological Chemistry 2012). Since the early contact formation at the unfolded state would lead to a compact chain-and both the folding and early oligomer formation result in the reduction of surface area-protein compaction, secondary structure formation and folding and early oligomer formation would share the same heterogeneous landscape. Interestingly, these early events are expected to occur at the late nsec or early microsecond, a time window which has been difficult to

monitor by conventional spectroscopic methods. Dr. Chattopadhyay's group used a combination of fluorescence quenching/FRET and FCS to probe this time kinetics and to determine the relative populations of the compact state, extended state and the early oligomers (Journal of Biological Chemistry 2015). To summarize, his group in these initial experiments could establish that a monomeric protein fluctuates between extended and compact collapsed states with time constants typically in the microsecond scale (Biochemistry, 2009; J. Biol. Chem., 2010). These contacts could either be correct (yielding proper folding), or incorrect, leading to oligomer formation (J. Biol. Chem., 2012; Biochemistry, 2013). They have shown that this landscape depends on multiple factors, including mutational stress (Biochemistry, 2013) and environmental conditions (J. Biol. Chem. 2012).

To validate this model and to solve an unsolved biological problem using this early-stage protein compaction-oligomerization landscape, Dr. Chattopadhyay's group carried out a detailed investigation to understand the secondary function of cytochrome c with respect to the involvement of this protein in the initiation of apoptosis. Although the primary function (electron transfer) of cytochrome c is conserved, its secondary function has been found to vary depending on the source of the protein (e.g., mammals vs. yeast). The pro-apoptotic function of cyt *c* has been well studied in higher (for example, mammals) and lower (for example, *Saccharomyces cerevisiae*) eukaryotes. Although the release of cyt *c* in yeast is common to both proteins, there are striking differences in the downstream mechanisms between the mammalian and yeast systems. Unlike cyt *c* from higher eukaryotes, the release of yeast cyt *c* (y-cyt *c*) does not activate caspases. Instead, the concentration of y-cyt *c*, once it appears in the cytosol diminishes with time (Giannattasio S. et al 2008 Cytochrome *c* is released from coupled mitochondria of yeast en route to acetic acid-induced programmed cell death and can work as an electron donor and a ROS scavenger. *FEBS Lett.* 2008; 582: 1519-1525). The mechanism of the absence of pro-apoptotic activity of the released y-cyt *c* in the yeast, and the observed reduction of the concentration of released y-cyt *c* with time is not yet understood.

Using a combination of heme induced quenching of fluorescence and FCS and employing the above model, Dr. Chattopadhyay's group calculated the populations of the compact, extended, and oligomeric states for both proteins. They found that, although the structure of these proteins are completely superimposable with an extremely high sequence homologies between them, a complex interplay between the conformational distribution and oligomerization of the protein plays the crucial role in the variation of the pre-apoptotic regulation of cytochrome c observed from these two different sources (Figure 2). These data convincingly showed that the population of the extended state plays the predominant role in the modulation of the peroxidase activity (Figure 2). Most interestingly, only a very subtle change in the sequence at the surface is responsible for this large change in the secondary functions of this protein in these two different sources (J. Biol. Chem. 2015; Highlights in Nature Chemical Biology).

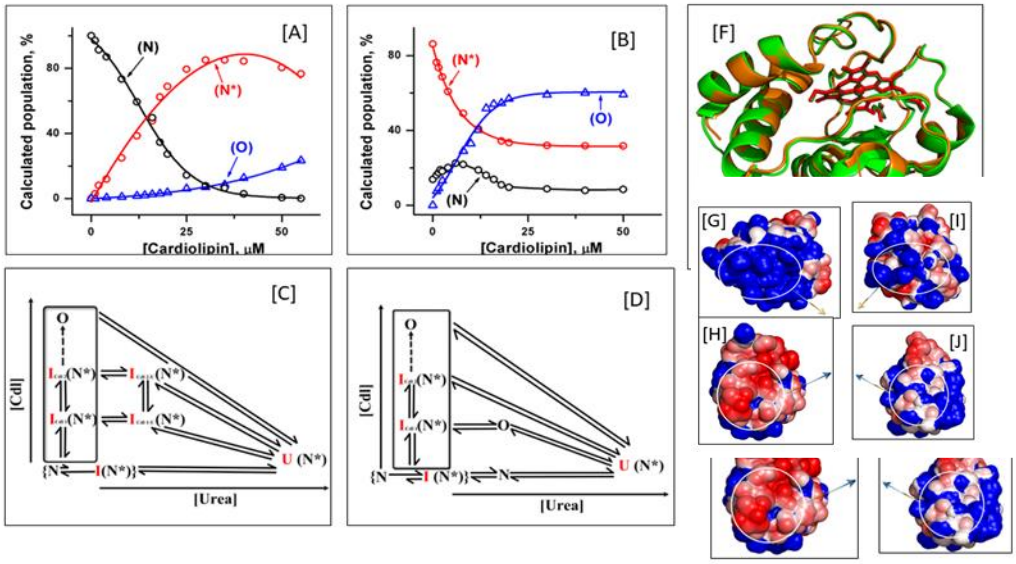

Figure 2: To validate our compaction-oligomerization model, we used FCS to quantify the population of the compact (N), extended (N*) and oligomeric (O) states in horse [A] and yeast [B] cytochrome c as they bind to cardiolopin, and initiates their secondary functions. Although their primary functions are identical, these two cytochrome c proteins differed significantly in their binding-stability landscape ([A] and [C] correspond to horse protein while [B] and [D] show the data of yeast variant. There structure of these two proteins are superimposable [F]. In contrast, the surface charge distribution of yeast protein show large clusters of negative [G] and positive [H] charges, which result in inherent repulsions and hence destability. Subtle changes in the surface amino acids at the horse protein result in distribution of charges ([I] and [J]) minimizing this destabilization effect.

(iii) How the early stage may modulate late events of aggregation in a neurodegenerative disease model.

To determine if the above model would be compatible to the aggregation of a protein in a neurodegenerative disease system, the group studied the early and late stages of aggregation of A-Syn, a protein implicated in PD. FRET-FCS data in combination with large number of secondary structure measurements show that the landscape of A-Syn aggregation is composed of an extended monomeric state, a compact state and aggregates, which is in line with their developed model (Figure 3) (ACS Chem. Neurosci. 2014). Using several disease mutants of A-Syn and solution conditions, they discovered that the early compaction relates to late fibril formation in a specific way, which they characterized further in their next study under in vitro and inside live neuroblastoma cells. Using FCS and small molecules fluorophore labeling of neuroblastoma cells, they have shown that the formation of a compact state during early events inhibited late fibrillization, while favorable generation of early oligomers resulted in more fibrillization in the late amyloid stages (Sci. Rep. 2018) (Figure 3).

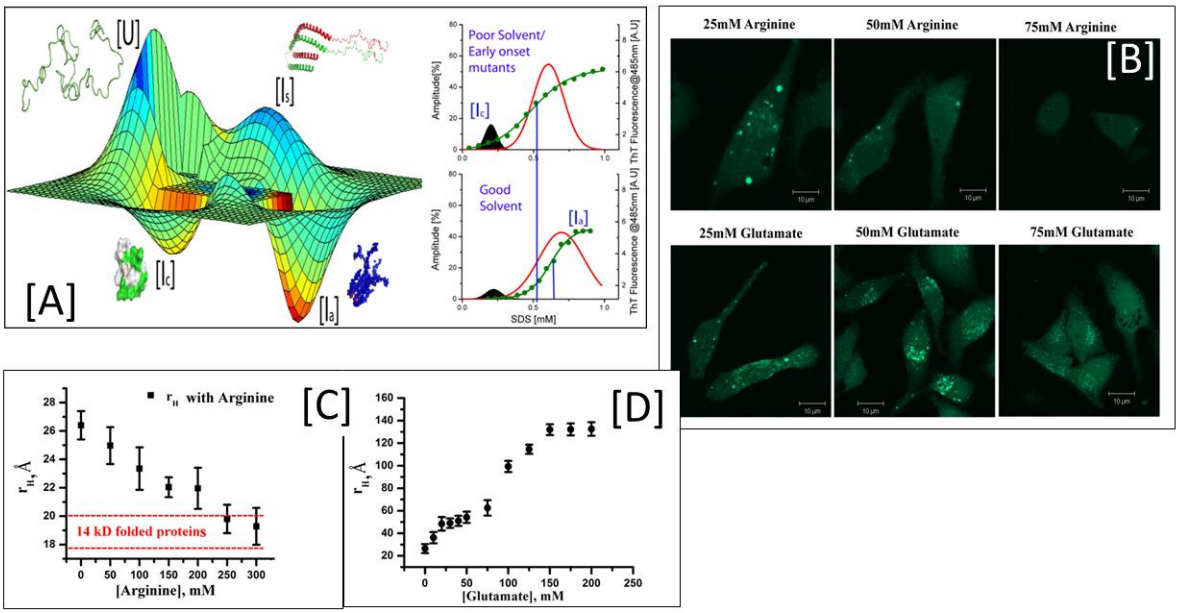

Figure 3: How the early stage modulates the late events: [A] Using FRET-FCS and other spectroscopy, the group has developed the aggregation landscape of A-Syn. In this landscape, [U] corresponds to the native monomeric form, which represents an intrinsically disordered protein. Ic represents a compact state that forms by the chain contact formation at the early stage of aggregation. Is is a helical intermediate which eventually aggregates and form Ia. [B] Late stage of aggregation in live cells: SH-SY5Y cells expressing TC-AS in the presence of different concentrations of Arginine and Glutamate. TC-AS is a small tetra cysteine tagged A-Syn, which was transfected into the cell lines. This1.5 kDa tetra cysteine tag is non-fluorescent but in the presence of FlAsH-EDT_2, it becomes fluorescent. The bright dots in the images correspond to the aggregates, which has been found significantly less in the presence of Arginine. The presence of Glutamate, on the other hand, facilitates late-stage aggregation. [C]-[D]: The early events as monitored by FCS: In the presence of Arginine, the hydrodynamic radius of the monomeric protein decreases suggesting a compaction [C], while in the presence of Glutamate, the protein has been found to facilitate oligomer formation in the early stage. These data were published in Ghosh et al (2018) Scientific Reports 8(1):1-16 and Basak et al (2015) ACS Chemical Neuroscience 6(2): 239-246.

(b) <u>Structural biology of the early oligomers in neurodegeneration:</u>

It may be reemphasized, while several drug discovery attempts have been made to target the late stage of aggregation (the fibrils) in a number of neurodegenerative diseases, these all failed in clinical trials in achieving the clinical end-points. There is no small molecule targeting the early events, and the primary reason being the lack of structural information. A successful determination of the structure of a protein early intermediate requires its population to be monodispersed. In contrast, the early events of protein aggregation are typically heterogeneous with formation and dissociation of many intermediates, which result in very low concentration of a particular species

towards detection and structural characterization. To solve this problem, Dr. Chattopadhyay's group used porphyrin-based heteroannulenes to obtain a homogeneous population of the early oligomers of alpha synuclein, thereby arresting further fibrilization. Using FT-IR and cryo-electron microscopy, they determined the secondary and tertiary 3D structures of these early oligomers, while their toxicity was measured by a combined use of microscopy, biochemistry and cell biology. Their group showed that the toxicity of the early oligomers and their ability towards fibrillization depend on their structural fold (ACS Chem. Neurosci. 2019, Comms.Bio 2021) (Figure 4). They found that the non-toxic versions of the early oligomers are 'mace'-shaped containing a crucial twist at the head-base junction, which prevents their further progression into toxic oligomers, protofilaments and eventually fibrils (Comms.Bio 2021). They further established that a histidine residue in alpha synuclein to which the porphyrin binds, is crucial in inflicting this structural twist. They provided the proof of concept by developing a histidine mutant, in which this histidine would not be coordinated, and this mutant protein has been found to be an aggressive PD variant in which heme coordination would not work (Comms.Bio 2021). This strategy is one of the first internationally, in which the early oligomers (and not the late amyloid fibril of A-Syn) is targeted towards developing an anti PD small molecule.

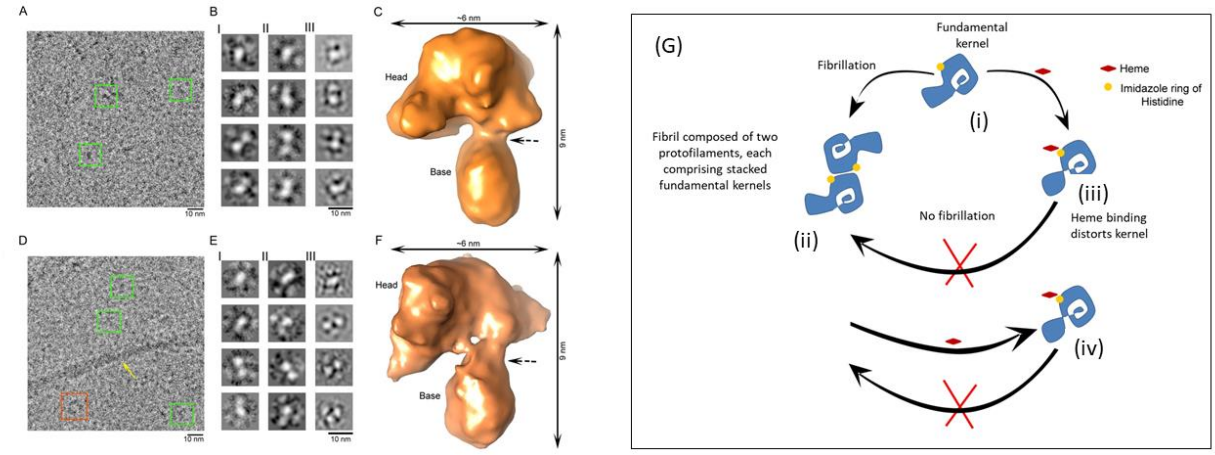

Figure 4: Structural Determination of the Early Oligomers of A-Syn: [A]–[C] The treatment of heme on monomeric A-Syn for 24 h (pre incubation). [A] Raw micrograph showing uniformly distributed small oligomers (green squares, oligomers$_1$). [B] I–III Reference-free 2D class averaging analyses were done by Xmipp, RELION and EMAN2. [C] Cryo-EM density map of A-Syn oligomers$_1$. [D], [E] Heme treatment performed after fibril formation has initiated, i.e., after 48 h of aggregation (post incubation). [D] Raw micrograph showing distribution of small oligomers (oligomers$_2$) similar to oligomers$_1$, as well as semi-annular-shaped oligomers and fibrils are marked by green squares, orange squares and a yellow arrow, respectively. [E] I–III Reference-free 2D class averaging analyses were done by Xmipp, RELION and EMAN2. [F]The density map of A-Syn oligomers$_2$. The figure was taken without modification from the published paper from the group (Chakraborty et al 2021 Communications Biology 4:518; https://doi.org/10.1038/s42003-021-02026)

(c) Developing Novel Methods to detect oligomers:

As mentioned above, these early oligomers are difficult to detect (and traditional assays, like ThT fluorescence would not work) requiring novel and orthogonal method development towards their probing. Keeping this in consideration, Dr. Chattopadhyay's group developed three separate strategies, which are described below:

(i) FCS: While we have mentioned the application of this technique above with respect to our work on developing model, the methodological details are discussed here briefly. This is a sensitive fluorescence technique which can be used at single molecule resolution to study molecular diffusion and conformational fluctuations of labelled biomolecules. Using an optimized setup and suitable protein, the molecular diffusion could be restricted at few hundreds microsec, and hence the conformational fluctuations at the microsecond time scale could be conveniently determined (PNAS 2003, PNAS 2005). Dr. Chattopadhyay's group used FCS to study the diffusional dynamics of A-Syn to show that FCS can pick up small changes in diffusional coefficients, which occur due to the formation of small molecule oligomers at the early stage of aggregation, at which stage ThT fluorescence does not give any fluorescence change. His group then used this method to screen small molecules against the early stages of aggregation in vitro (Langmuir, ACS Chemical Neuroscience).

(ii) Non-linear Z scan technique: Because of the inherent complexity and large dynamic range (between a few microseconds to several days under in vitro measurement conditions), it is difficult for the conventional biophysical and biochemical techniques to sample the entire time window of α-syn aggregation. The early stage of aggregation can be heterogeneous with the presence of different species of varying populations, which necessitates the use of non-classical technologies for their characterization. Here, for the first time, Dr. Chattopadhyay's group collaborated with IIT/ISM Dhanbad to develop the application of the non-linear Z-scan technique as a novel tool to investigate different conformations formed in the early and late stage of temperature and mechanical stress-induced α-syn aggregation, in which different species showed its characteristic nonlinear characteristics. A power-dependent study was also performed to observe the changes in the protein nonlinearity. The perceived nonlinearity was accredited to the thermal-lensing effect. A switch in the sign of the refractive nonlinearity was observed for the first time as a signature of the late oligomeric conformation, a prime suspect that triggers cell death associated with neurodegeneration. This is a simple, inexpensive, and sensitive method and the team is trying to test the applicability of this method using patient samples (ACS Chemical Neuroscience 2020).

(iii) Using a small molecule synthetic fluorophore targeting the early oligomeric intermediates: Here, Dr. Chattopadhyay's group designed a new hybrid ligand (SCG) which can target the early stage selectively using a cysteine monitoring chemistry. The molecule was synthesized and characterized in collaboration with Dr. Biswadip Banerjee of CSIR-IICB. SCG was found effective in detecting early intermediates of A-Syn *in vitro*, and inside live neuroblastoma cells (Biochemistry 2019).

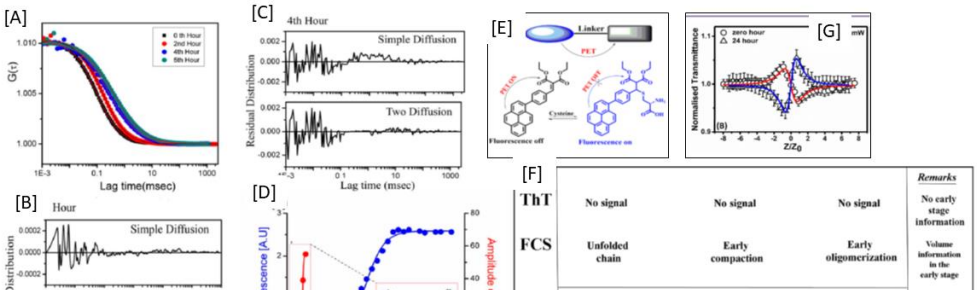

Figure 5: [A]-[D] The use of FCS to monitor the early oligomerization of A-Syn. [A] As the protein was subjected to aggregation condition, the correlation functions were found shifted towards right [B]-[C] Although the diffusion analyses show the presence of monomeric protein in aqueous buffer at time zero, the data at hour 4 could only be fit to a sum of two diffusing components: the monomer and an oligomer; [D] While the FCS data (red) show an increase the population in the multimer, the data monitored by ThT fluorescence (blue) suggested no change in fluorescence at the early hours indicating that the early multimers do not have cross beta structures. [E] Structure and the mechanism of action of SCG [F] A comparison between FCS, SCG and ThT fluorescence detection methods in their ability to probe different events of A-Syn aggregation [G] Z-scan measurements show a reversal in the sign of non-linearity as the population of late stage oligomers increase. The figure has been prepared using data published in Ghosh et al (2020) ACS Chem Neurosci.; Chatterjee et al (2019) Biochemistry and Joshi et al (2015) Langmuir.

(d) Developing the molecular understanding of ALS using a cofactor derived membrane interaction model:

Amyotrophic lateral sclerosis, or ALS, is an incurable neurodegenerative disease in which a person slowly loses specialized nerve cells that control voluntary movement. It is not fully understood what causes this disease. However, it is suspected that aggregates of a protein called Superoxide Dismutase (SOD1) in nerve cells may play a crucial role. More than 140 mutations in the gene for SOD1 have been linked to ALS, with varying degrees of severity. But it is still unclear how these mutations cause SOD1 aggregation or how different mutations influence the survival rate of the disease (the time span between the disease onset and the death of the patient). The protein SOD1 contains a copper ion and a zinc ion, and it is possible that mutations that affect how these two ions bind to SOD1 influences the severity of the disease. To investigate this, the nominee's group genetically engineered mutants of the SOD1 which each contain only one metal ion. Experiments on these mutated proteins showed that the copper ion is responsible for the protein's role in neutralizing harmful reactive molecules, while the zinc ion stabilizes the protein against aggregation (EMBO Journal 2023). They found that when the zinc ion was removed, the SOD1 protein attached to a structure inside the cell called the mitochondria and formed toxic aggregates. They then used these observations to build a computational model that incorporated different mutations that have been previously associated with ALS. The model suggests that mutations close

to the site where zinc binds to the SOD1 protein increase disease severity and shorten survival time after diagnosis. This model was then experimentally validated using two disease variants of ALS that have mutations close to the sites where zinc or copper binds. This discovery could help design new ALS treatments that target the zinc binding site on SOD1 or disrupt the protein's interactions with the mitochondria (eLife 2021).

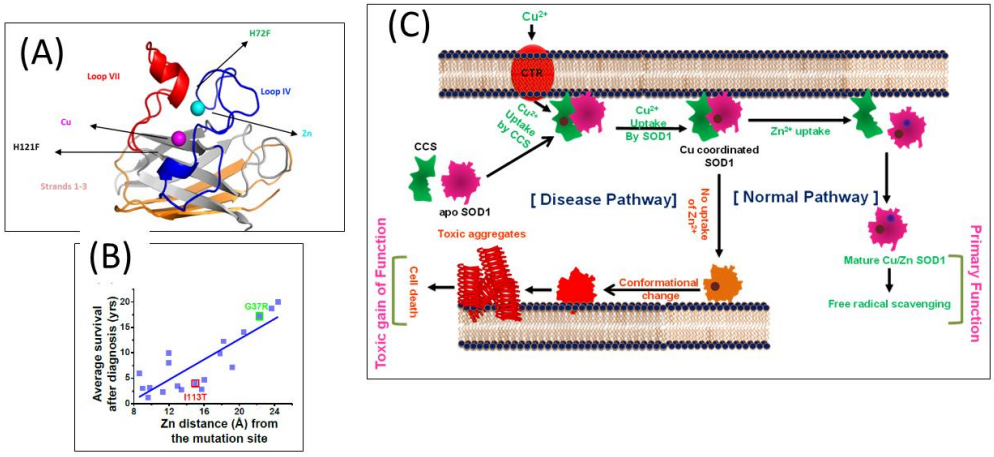

Figure 6: (A) The structure of SOD1 monomer showing different mutants used in this study (not discussed here). (B) The ALS disease severity in terms of average survival time after diagnosis has been plotted against the distance parameters of the mutation points from Zn centre for the disease mutants. It shows a good correlation. Two mutants (G37R and I113T) are selected from this plot for detailed experimental validations. (C) The Cofactor Mediated Membrane Association Model of SOD1 aggregation. The Figures were taken from Sannigrahi et al (2021) eLife 10:e61453

(e) Host-pathogen interaction and its implication in infectious diseases

Protein conformational changes during protein-lipid interactions play ubiquitous roles in governing a number of cellular events. Using two proteins of unknown functions, Dr. Chattopadhyay's group investigated the role of protein-lipid interactions to define the mechanistic pathway regarding disease progression. Their findings suggested that KMP-11-membrane interaction is modulated by the cholesterol content of lipid bilayer which is directly linked with the mechanism of parasite survival strategy (J. Phys. Chem. B 2017). On the other hand, environment-dependent binding events between immunogenic KMP-11 and membrane ergosterol resolved the missing link between ergosterol biogenesis and immune suppression (ACS Omega 2019). In another context, they established that MPT63 loses its immunogenic responses through surface binding, and this happens through the environment-sensitive conformational switching from native beta sheet to helical conformation (Langmuir 2018). Nevertheless, this environment-dependent switch event of MPT63 is also responsible for host cell death through membrane pore formation. The investigation has revealed that the helix conformer of MPT63 creates toxic oligomers in order to perforate host membranes (ACS Chemical Biology, 2019) (Figure 7).

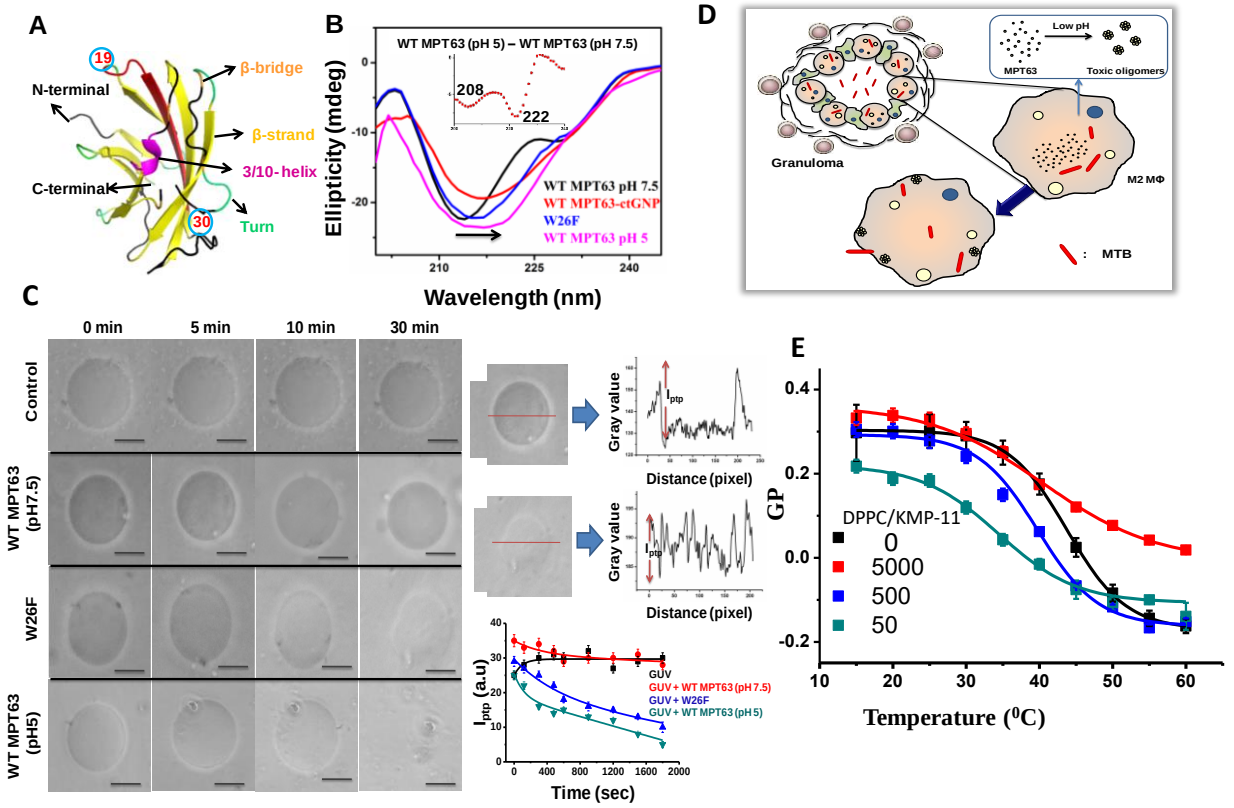

Figure 7: Conformational changes and pore formation of MPT63 in different conditions. (A) Crystal structure of WT MPT63 (PDB ID: 1LMI) showing the secondary structure components. (B) Far-UV CD spectra of WT MPT63 at pH 7.5, WT MPT63-ctGNP, W26F, and WT MPT63 at pH 5 proteins. For WT protein at pH 7.5, the CD spectrum shows a minimum at 216 nm, which is a characteristic of β-sheet protein. In other conditions, the spectra showed an enhancement of ellipticity at 222 nm. A difference spectrum, as shown in the inset, shows clearly the appearance of double minima at 222 and 208 nm as a result of helix formation. The difference spectrum also shows a decrease at 216 nm, indicating the loss of β-sheet.(C) The GUVs show different behavior due to the pore-formation process in vesicles with different protein variants outside (WT MPT63 at pH 7.5, W26F, WT MPT63 at pH 5). The bars represent 20 μm.(D) Model Showing the Proposed Pathway of Release of MTB from Granuloma (F) Measurement of phase transition events in DPPC membranes in presence of different KMP-11 concentrations.

- Protein-excipient interactions and Biopharmaceutical Formulation

The translational research component of Dr. Chattopadhyay's laboratory initially revolved around his studies on protein-excipient interactions, which is an important consideration of Biopharmaceutical formulation development. These excipients are typically added onto protein pharmaceutical drug substances in order to stabilize them in solution or as lyophilized powder. His laboratory has shown that Arginine can be a good excipient by inhibiting protein aggregation by inhibiting a protein misfolding pathway, which can conveniently be probed by FCS (Biochemistry 2009, JBC 2010). His group showed that arginine possesses a combination of 'unfolded state destabilization' and 'denaturant-like' properties (Biochemistry 2009; Scientific Reports 2013), while other excipients (like sucrose) satisfy only the first criteria. By virtue of these two properties, arginine has been found to destabilize the aggregation prone intermediate states in the protein folding pathway. These studies have been well cited by researchers from Academia and Industries.

- Developing methods to probe the early events of alpha synuclein aggregation: A step towards finding biomarkers of Parkinson's Disease (PD)

Parkinson's Disease, like most other neurogenerative diseases, does not currently have a confirmed diagnosis. There are extensive efforts to develop methods to detect the toxic oligomeric molecules, which are believed to form at the early stage of alpha synuclein aggregation. Unfortunately, these molecules are difficult to detect because of their transient nature and the inherent heterogeneity of the aggregation. Dr. Chattopadhyay's group are developing three different methods towards the detection of the early events of alpha synuclein aggregation. In the first method, his group collaborated with Dr. Biswadip Banerjee (from the Organic and Medicinal Chemistry Division of CSIR-IICB) to design small molecules fluorescent probes, which changes its fluorescence character when its binds to the early oligomers (Biochemistry 2019). In the second method, they used FCS, which can probe oligomeric molecules by virtue of high sensitivity of its single molecule detection (Langmuir 2014). For the third method, Dr. Chattopadhyay's group collaborated with the scientists of the department of Physics, IIT (ISM)-Dhanbad and developed an application of non-linear Z scan techniques (ACS Chemical Neuroscience 2020). His group is now in the process of testing these three methods using Parkinson's Disease patient samples.

- Developing inhibitor molecules against Parkinson's Disease (PD) and Atherosclerosis:

While it was traditionally believed that an effective intervention against PD would come from designing inhibitors against alpha synuclein fibrillation, this view has changed. This is now believed that the toxic species are generated early presumably at the lag phase, although it has been found difficult to develop inhibitors against these early oligomers. The nominee's group then attached small molecule stabilizers onto magnetite nanoparticles and found that coating of nanoparticles with small molecules results in the inhibition of early events of alpha synuclein aggregation. Although there are many available small molecules targeting the late events of fibril formation, this is the first report of the successful application of any small molecule inhibiting the early events of amyloidosis of alpha synuclein (Langmuir 2014). In addition, they have explored the possibility of repurposing Kanamycin, a commonly used antibiotic molecule, towards the inhibition of membrane induced fibrillation of alpha synuclein (Chemical Communication 2019).

In collaboration with Dr. Arun Bandyopadhyay of CSIR-IICB, nominee's group have recently developed pathogen inspired synthetic HDL molecule, which has shown significant reversing of atherosclerosis in animal studies. This is a novel biologics molecule, which we are in the process of patenting. In addition, they are now trying out an extensive formulation screening to improve its stability as a liquid at room temperature.

Signed

Krishnananda Chattopadhyay
(The Nominee)