

In order of importance, list of ten best papers of the candidate, highlighting the important discoveries/contributions described in them briefly (not to exceed 3000 words)

Following is a list of selected papers. A short write-up (Abstract/slightly modified abstract) is also provided.

1. Das, B; Roychowdhury, S; Mohanty, P; Rizuan, A; Chakraborty, J.; Mittal, J; & **Chattopadhyay, K*** (2022) A Zn-dependent structural transition of SOD1 modulates its ability to undergo phase separation, **EMBO Journal**, <https://doi.org/10.15252/emboj.2022111185>

The misfolding and mutation of Cu/Zn superoxide dismutase (SOD1) is commonly associated with amyotrophic lateral sclerosis (ALS). SOD1 can accumulate within stress granules (SGs), a type of membraneless organelle, which is believed to form via liquid–liquid phase separation (LLPS). Using wild-type, metal-deficient, and different ALS disease mutants of SOD1 and computer simulations, we report here that the absence of Zn leads to structural disorder within two loop regions of SOD1, triggering SOD1 LLPS and amyloid formation. The addition of exogenous Zn to either metal-free SOD1 or to the severe ALS mutation I113T leads to the stabilization of the loops and impairs SOD1 LLPS and aggregation. Moreover, partial Zn-mediated inhibition of LLPS was observed for another severe ALS mutant, G85R, which shows perturbed Zn-binding. By contrast, the ALS mutant G37R, which shows reduced Cu-binding, does not undergo LLPS. In addition, SOD1 condensates induced by Zn-depletion exhibit greater cellular toxicity than aggregates formed by prolonged incubation under aggregating conditions. Overall, our work establishes a role for Zn-dependent modulation of SOD1 conformation and LLPS properties that may contribute to amyloid formation.

2. Sannigrahi, A; Chowdhury, S; Das, B; Banerjee, A; Halder, A; Saleem, M; Naganathan, A N; Karmakar, S; **Chattopadhyay, K*** (2021) The metal cofactor zinc and interacting membranes modulate SOD1 conformation-aggregation landscape in an in vitro ALS Model **eLife**, 10, E61453 DOI: [10.7554/eLife.61453](https://doi.org/10.7554/eLife.61453)

Highlights in the journal: <https://elifesciences.org/articles/61453#digest>

Highlights in other scientific blogs: <https://www.scisoup.org/article/2021/CSIR-IICB-led-researchers-provide-new-insights-into-ALS-disease.html>

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, we 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. 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.

We 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.

3. Mishra, S.; Sannigrahi A; Ruidas S; Chatterjee S; Roy K; Misra D; Maity BK; Paul R; Ghosh CK; Saha KD; Bhaumik A; **Chattopadhyay K*** (2024) Conformational Switch of a Peptide Provides a Novel Strategy to Design Peptide Loaded Porous Organic Polymer for Pyroptosis Pathway Mediated Cancer Therapy; **Small**
DOI: <https://doi.org/10.1002/sml.202402953>

While peptide-based drug development is extensively explored, this strategy has limitations due to rapid excretion from the body (or shorter half-life in the body) and vulnerability to protease-mediated degradation. To overcome these limitations, a novel strategy for the development of a peptide-based anticancer agent is introduced, utilizing the conformation switch property of a chameleon sequence stretch (PEP1) derived from a mycobacterium secretory protein, MPT63. The selected peptide is then loaded into a new porous organic polymer (PG-DFC-POP) synthesized using phloroglucinol and a cresol derivative via a condensation reaction to deliver the peptide selectively to cancer cells. Utilizing ensemble and single-molecule approaches, this peptide undergoes a transition from a disordered to an alpha-helical conformation, triggered by the acidic environment within cancer cells that is demonstrated. This adopted alpha-helical conformation resulted in the formation of proteolysis-resistant oligomers, which showed efficient membrane pore-forming activity selectively for negatively charged phospholipids accumulated in cancer cell membranes. The experimental results demonstrated that the peptide-loaded PG-DFC-POP-PEP1 exhibited significant cytotoxicity in cancer cells, leading to cell death through the Pyroptosis pathway, which is established by monitoring numerous associated events starting from lysosome membrane damage to GSDMD-induced cell membrane demolition. This novel conformational switch-based drug design strategy is believed to have great potential in endogenous environment-responsive cancer therapy and the development of future drug candidates to mitigate cancers.

4. Chakraborty, R.; Dey, S.; Sil, P., Paul, S. S.; Bhattacharya, D.; Bhunia, A.; Sengupta, J.; **Chattopadhyay, K***. (2021) Conformational distortion in a fibril-forming oligomer arrests alpha-Synuclein fibrillation and minimizes its toxic effects; **Communications Biology** 4, 518 DOI: 10.1038/s42003-021-02026-z

Parkinson's disease (PD)- which is also an important neurodegenerative disease-does not yet have a cure. It is known that an intrinsically disordered protein- α synuclein-forms amyloid fibrils as the end point of its aggregation pathway. A significant number of high resolution structures of α synuclein fibrils is available, although the drug trials targeting them does not seem to work. It is now believed that the fibrils do not possess significant toxicity and these are not necessarily the cause of the disease; they are probably the effect. The fibrillation pathway of α -Synuclein encompasses transient, heterogeneous oligomeric forms whose structural understanding and link to toxicity are not yet understood. We report that the addition of the physiologically-available small molecule heme at a sub-stoichiometric ratio to either monomeric or aggregated α -Syn, targets a His50 residue critical for fibril-formation and stabilizes the structurally-heterogeneous populations of aggregates into a minimally-toxic oligomeric state. Cryo-EM 3D reconstruction revealed a 'mace'-shaped structure of this monodisperse population of oligomers, which is comparable to a solid-state NMR Greek key-like motif (where the core residues are arranged in parallel in-register sheets with a Greek key topology at the C terminus) that forms the fundamental unit/kernel of protofilaments. Further structural analyses suggest that heme binding induces a distortion in the Greek key-like architecture of the mace oligomers, which impairs their further appending into protofilaments and fibrils. Additionally, our study reports a novel mechanism of prevention as well as reclamation of amyloid fibril formation by blocking an inter-protofilament His50 residue using a small molecule. This is the first report of a three dimensional structure of a minimally toxic α synuclein early oligomeric species.

5. Chowdhury S, Sen S, Banerjee A, Uversky VN, Maulik U & **Chattopadhyay K* (2019)** Network mapping of the conformational heterogeneity of SOD1 by deploying statistical cluster analysis of FTIR spectra, **Cellular and Molecular Life Sciences** 76, 4145 DOI: <https://doi.org/10.1007/s00018-019-03108-2>

A crucial contribution to the heterogeneity of the conformational landscape of a protein comes from the way an intermediate relates to another intermediate state in its journey from the unfolded to folded or misfolded form. Unfortunately, it is extremely hard to decode this relatedness in a quantifiable manner. Here, we developed an application of statistical cluster analyses to explore the conformational heterogeneity of a metalloenzyme, human cytosolic copper-zinc superoxide dismutase (SOD1), using the inputs from infrared spectroscopy. This study provides a quantifiable picture of how conformational information at one particular site (for example, the copper-binding pocket) is related to the information at the second site (for example, the zinc-binding pocket), and how this relatedness is transferred to the global conformational information of the protein. The distance outputs were used to quantitatively generate a network capturing the folding sub-stages of SOD1.

6. Mahapatra, A, Sarkar, S., Biswas, SC, & **Chattopadhyay K* (2019)** An aminoglycoside antibiotic inhibits both lipid-induced and solution-phase fibrillation of α -Synuclein in vitro, **Chemical Communications** 55, 11052 DOI: <https://doi.org/10.1039/C9CC04251B>
(**Highlights in Nature India (doi:10.1038/nindia.2019.130)**)

More than 10 million people worldwide are living with Parkinson's disease (PD) – the second most common neurodegenerative disorder. This disease is characterized by the degeneration and death of dopaminergic neurons in the brain leading to impaired release of the neurotransmitter dopamine, which results in eventual loss of motor functions. PD has no cure till date; however some treatments like stem cell therapy or dopamine administration can be used to relieve the symptoms in PD patients. All such treatment options are expensive as well as incapable of actually halting or delaying the progress of the disease.

The complete and exact mechanism behind the pathogenesis of PD is still unclear. However, occurrence of inclusions containing amyloid aggregates of the neuronal protein α -Synuclein in the surviving neurons of the PD patients' brain, and many other evidences from literature, leads us to believe that the misfolding and aggregation of α -Synuclein underlie the disease pathogenesis, and modifying the same is one of the ways to curb disease progression. This has led to extensive research aimed at developing and identifying aggregation-modulating molecules that can eventually serve as prospective therapeutic agents against PD. One major obstacle in the development of such disease-modifying drugs for PD lies in the time-consuming and expensive clinical trials and safety-checks for every new molecule studied, which leads to the mounting cost of the ultimate drug (if any). Another crucial problem lies in the heterogeneous nature of the aggregation landscape of the protein itself. It can aggregate primarily *via* two distinct pathways, following entirely different chemical principles – one in solution and the other in contact with lipid membranes, so that developing or identifying a prospective therapeutic agent that can deter both types of aggregation is very challenging. Usually, an inhibitor effective in one pathway is not effective in the other. Our work has identified a means to circumvent both these problems by making use of the commercially available, inexpensive, aminoglycoside antibiotic kanamycin. Not only is it an already established drug with a known toxicity profile, it has a unique combination of functional groups (primary amine and hydroxyl groups) that can target both types of α -Synuclein aggregation.

We found that kanamycin, due to its unique chemical properties, significantly inhibits the fibrillation (*i.e.* aggregation into amyloid fibrils) of α -Synuclein both in solution and in association with lipid membranes, while also relieving neuronal toxicity caused by pre-formed fibrils of the protein. Lipid-induced fibrillation was inhibited by displacement of α -Synuclein from the membrane surface and/or its conformational alteration; while in solution, kanamycin brought about inhibition of fibril formation by interfering with H-bonding interactions between α -Synuclein monomers, forming less toxic disordered amorphous aggregates instead. Overall, our study points towards the prospective repurposing of kanamycin – traditionally in use for treating a broad range of bacterial infections – as an anti-PD drug, that too an affordable one.

7. Sannigrahi A, Nandi I, Chall S, Jawed JJ, Halder A, Majumdar S, Karmakar S, **Chattopadhyay K*** (2019) Conformational switch driven membrane pore formation by Mycobacterium secretory protein MPT63 induces macrophage cell death, **ACS Chemical Biology** 14, 1601 DOI: <https://doi.org/10.1021/acscchembio.9b00327>

Virulent *Mycobacterium tuberculosis* (MTB) strains cause cell death of macrophages (M ϕ) inside TB granuloma using a mechanism which is not well understood. Many bacterial systems utilize toxins to induce host cell damage, which occurs along with immune evasion. These toxins often use chameleon sequences to generate an environment-sensitive conformational switch, facilitating the process of infection. The presence of toxins is not yet known for MTB. Here, we show that MTB-secreted immunogenic MPT63 protein undergoes a switch from β -sheet to helix in response to mutational and environmental stresses. MPT63 in its helical form creates pores in both synthetic and M ϕ membranes, while the native β -sheet protein remains inert toward membrane interactions. Using fluorescence correlation spectroscopy and atomic force microscopy, we show further that the helical form undergoes self-association to produce toxic oligomers of different morphology. Trypan blue and flow cytometry analyses reveal that the helical state can be utilized by MTB for killing M ϕ cells. Collectively, our study emphasizes for the first time a toxin-like behavior of MPT63 induced by an environment-dependent conformational switch, resulting in membrane pore formation by toxic oligomers and M ϕ cell death.

8. Paul, S.S., Sil, P., Haldar, S., Mitra, S. & **Chattopadhyay, K*** (2015) Subtle change in the charge distribution of surface residues may affect the secondary functions of cytochrome c **J. Biol. Chem.** 290, 14476 DOI: <https://doi.org/10.1074/jbc.M114.607010> (**Highlights in Nature Chemical Biology 2015, doi:10.1038/nchembio.1829**)

Although the primary function of cytochrome *c* (cyt *c*) is electron transfer, the protein carries out an additional secondary function involving its interaction with membrane cardiolipin (CDL), its peroxidase activity, and the initiation of apoptosis. Whereas the primary function of cyt *c* is essentially conserved, its secondary function varies depending on the source of the protein. We report here a detailed experimental and computational study, which aims to understand, at the molecular level, the difference in the secondary functions of cyt *c* obtained from horse heart (mammalian) and *Saccharomyces cerevisiae* (yeast). The conformational landscape of cyt *c* has been found to be heterogeneous, consisting of an equilibrium between the compact and extended conformers as well as the oligomeric species. Because the determination of relative populations of these conformers is difficult to obtain by ensemble measurements, we used fluorescence correlation spectroscopy (FCS), a method that offers single-molecule resolution. The population of different species is found to depend on multiple factors, including the protein source, the presence of CDL and urea, and their concentrations. The complex interplay between the conformational distribution and oligomerization plays a crucial role in the variation of the pre-apoptotic regulation of cyt *c* observed from different sources. Finally, computational studies reveal that the variation in the charge distribution at the surface and the charge reversal sites may be the key determinant of the conformational stability of cyt *c*.

9. Haldar, S & Chattopadhyay K* (2012) Interconnection of Salt-induced Hydrophobic Compaction and Secondary Structure Formation Depends on Solution Conditions **J Biol. Chem.** 287, 11546 DOI: <https://doi.org/10.1074/jbc.M111.315648>

What happens in the early stage of protein folding remains an interesting unsolved problem. Rapid kinetics measurements with cytochrome c using submillisecond continuous flow mixing

devices suggest simultaneous formation of a compact collapsed state and secondary structure. These data seem to indicate that collapse formation is guided by specific short and long range interactions (heteropolymer collapse). A contrasting interpretation also has been proposed, which suggests that the collapse formation is rapid, nonspecific, and a trivial solvent related compaction, which could as well be observed by a homopolymer (homopolymer collapse). We address this controversy using fluorescence correlation spectroscopy (FCS), which enables us to monitor the salt-induced compaction accompanying collapse formation and the associated time constant directly at single molecule resolution. In addition, we follow the formation of secondary structure using far UV CD. The data presented here suggest that both these models (homopolymer and heteropolymer) could be applicable depending on the solution conditions. For example, the formation of secondary structure and compact state is not simultaneous in aqueous buffer. In aqueous buffer, formation of the compact state occurs through a two-state co-operative transition following heteropolymer formalism, whereas secondary structure formation takes place gradually. In contrast, in the presence of urea, a compaction of the protein radius occurs gradually over an extended range of salt concentration following homopolymer formalism. The salt-induced compaction and the formation of secondary structure take place simultaneously in the presence of urea.

10. Halder, S, Mitra, S. & **Chattopadhyay, K*** (2010) The role of the protein stabilizers on the conformations of the unfolded states and its early folding kinetics: An investigation at single molecular resolution **J. Biol. Chem.** 285, 25314 DOI: <https://doi.org/10.1074/jbc.M110.116673>

An insight into the conformation and dynamics of unfolded and early intermediate states of a protein is essential to understand the mechanism of its aggregation and to design potent inhibitor molecules. Fluorescence correlation spectroscopy has been used to study the effects of several model protein stabilizers on the conformation of the unfolded state and early folding dynamics of tetramethyl rhodamine-labeled cytochrome *c* from *Saccharomyces cerevisiae* at single molecular resolution. Special attention has been given to arginine, which is a widely used stabilizer for improving refolding yield of different proteins. The value of the hydrodynamic radius (rH) obtained by analyzing the intensity fluctuations of the diffusing molecules has been found to increase in a two-state manner as the protein is unfolded by urea. The results further show that the presence of arginine and other protein stabilizers favors a relatively structured conformation of the unfolded states (rH of 29 Å) over an extended one (rH of 40 Å), which forms in their absence. Also, the time constant of a kinetic component (τ_R) of about 30 μ s has been observed by analyzing the correlation functions, which represents formation of a collapsed state. This time constant varies with urea concentration representing an inverted Chevron plot that shows a roll-over and behavior in the absence of arginine. To the best of our knowledge, this is one of the first applications of fluorescence correlation spectroscopy to study direct folding kinetics of a protein.