Details of the research work duly signed by the applicant, for which the Sun Pharma Research Award is claimed, including references and illustrations (not to exceed 6000 words).

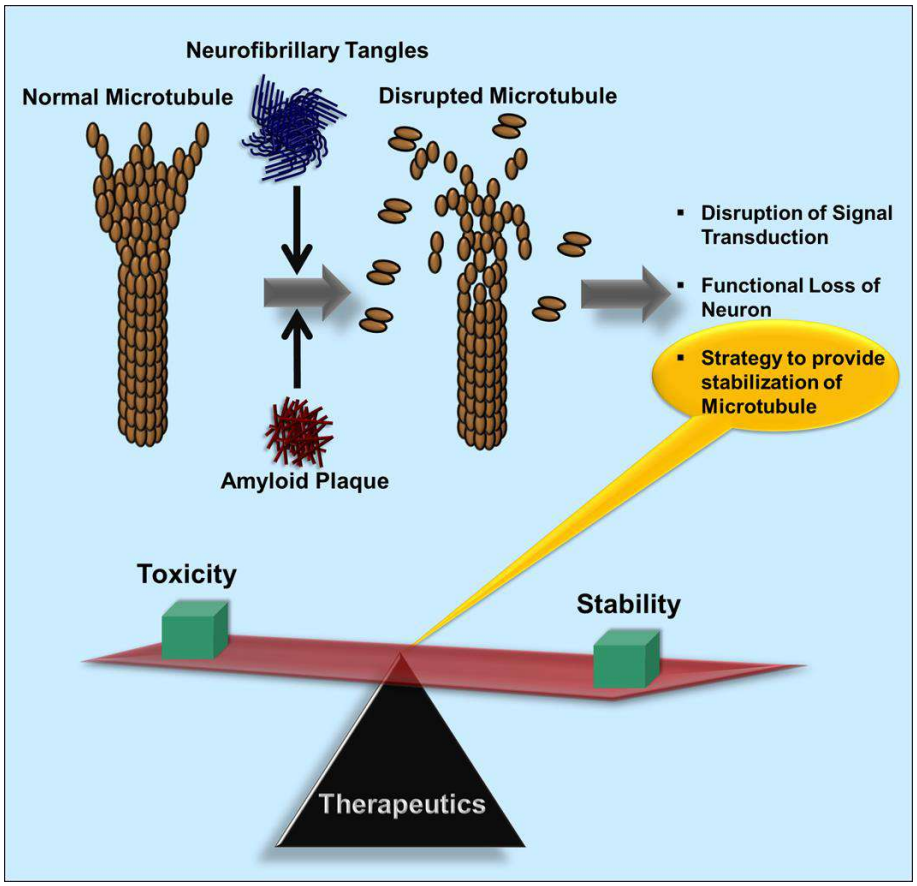
As an independent investigator, I am mainly focusing my research in the areas of Chemical Neuroscience, Chemical Biology and Medicinal Chemistry. I have established an interdisciplinary laboratory, where we performed design of therapeutic molecules using computational tools, followed by synthesis, *in vitro* and *in vivo* validations. For therapeutic target, we have considered microtubules as one of our primary intracellular target along with other targets such as amyloid beta, acetyl cholinesterase, genes involved in neurogenesis, mitochondria, matrix metalloproteinase 9 etc. and for therapeutic delivery we target overexpressed proteins in cancer cells, cell penetrating peptides etc. Towards this journey, in last ten years we have tried to contribute in development of various therapeutic leads, platform for screening neurotherapeutic drugs, nanotechnology based platform for delivery of therapeutic molecules (nanomedicine development), bioengineered hydrogel for drug delivery and repairing of brain injury and reconstitution of artificial cell for understanding disease progression.

Few key contributions are summarized below:

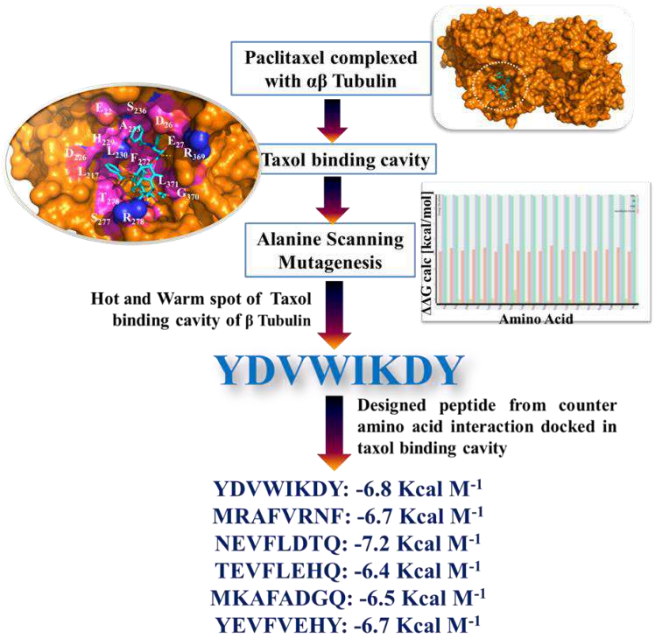
1. **Development of peptide-based neuro-therapeutic lead targeting microtubule:**
   Microtubule stabilization has never really being considered as an avenue for development of neuro-therapeutics in Alzheimer's disease. Microtubules (MTs) are polymers of heterodimeric α and β tubulins that maintain the embroidered morphology of the neurons and undergo continuous phases of growth and shrinkage to attain dynamicity in its structure. Here, we highlight some of these functions and why microtubules can be considered as key players of the neurons:
   - Neurons have complex branched morphology consisting of axons and dendritic arbors, which are further interconnected with other neurons through synapses. In order to maintain this exaggerated network, a robust transport mechanism is required. Microtubules with the help of motor proteins (kinesin and dynein) fulfill that function by serving as railway tracks for the intracellular transport of several neuronal cargos like organelles, neurotransmitter receptors, various cell signaling molecules, synaptic vesicle precursors and mRNAs. Microtubule organization also confers specificity in their transport of cargo into axons and dendrites.
   - In order to develop the much needed functional interconnections, the neurons need to reach out to each other, making neurite outgrowth an extremely important feature. Apart from several other extracellular cues, the most crucial step in the formation of these neurite processes for better interconnectivity is taken care by the pushing and pulling forces of the microtubules and actin filaments present in the extending neurites which ultimately leads to membrane protrusions.
   - Other than neurite outgrowth and intracellular cargo transport in neurons, microtubule stabilization is critical for axonal differentiation, outgrowth and regeneration in case of any injury.

It has been commonly observed that in most of the neurodegenerative diseases, there is a progressive loss of microtubule mass from axons and dendrites. Considering the above mentioned roles of microtubules in neurons, microtubule stabilization is likely to become the new horizon for the development of future neuro-therapeutics. Due to the intense association of microtubule stability with Tauopathies, it has sparked interest in the development of microtubule stabilizing drugs. Already known some of the microtubule stabilizing drugs has shown promising effects, which causes microtubule stabilization like the taxols. In this direction, we adopted an innovative strategy by designing peptide, from the taxol binding pocket of β-tubulin. However, one important point one should keep in mind that, while designing drugs targeted for microtubule stabilization, it must be remembered that while trying upholding the stability of the microtubule so as to prevent any loss of microtubule mass, we must always remember to never comprise on its dynamicity otherwise it will

turn out like a poison like taxol for the cell. Therefore, a careful balance between toxicity and stability should be prerequisite while designing the microtubule targeted neuro-therapeutics.



During progression of Alzheimer's disease (AD), severe destabilization of microtubule occurs, which leads to the permanent disruption of signal transduction process and memory loss. Thus, microtubule stabilization is one of the key requirements for the treatment of AD. Taxol, a microtubule stabilizing anti-cancer drug has been considered as potential anti-AD drug, which



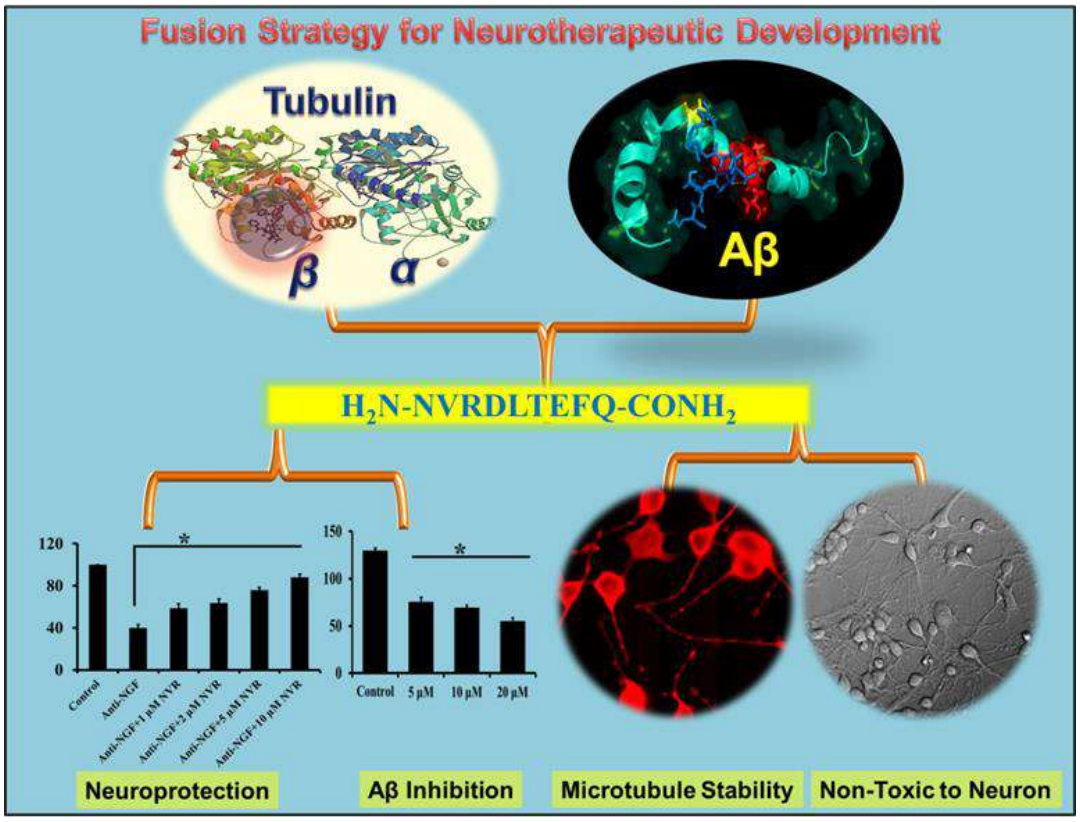
was clinically unsuccessful due to its toxicity. Here, we adopted an innovative strategy for the development of peptide based microtubule stabilizer, considering the taxol binding pocket of β-tubulin and by using alanine scanning mutagenesis technique. This approach lead us to a potential octapeptide, which strongly binds with taxol pocket of β-tubulin, serves as an excellent microtubule stabilizer, Aβ aggregation inhibitor and neuroprotective agent. It showed strong binding with tubulin in the *in vitro* assays with a binding constant of 3.8×10^5 M^{-1}. When applied to PC12 derived neurons, it showed microtubule polymerization, increased expression of acetylated tubulin, neurite outgrowth and neuroprotection against anti-NGF mediated toxicity. What is of consequence here is that in spite of being carved out of the taxol pocket, it doesn't cause toxicity unlike other taxol drugs which may be attributed to its moderate microtubule binding affinity which is 225 times lower at 4 °C and 47500 times lower at 37 °C as compared to taxol. Further, results revealed that this peptide is non-toxic against both PC12 derived neurons as well as primary cortical neurons. We believe that our strategy and discovery of peptide-based microtubule stabilizer will open the door for the development of potential anti-AD therapeutics in future.

Reference:

1a. Prasenjit Mondal, Gaurav Das, Juhee Khan, Krishnangsu Pradhan, and **Surajit Ghosh*.** Crafting of Neuroprotective Octapeptide from Taxol-Binding Pocket of β-Tubulin, ***ACS Chem. Neurosci., 2018, 9, 615-625.***

1b. Gaurav Das and **Surajit Ghosh.*** "Why Microtubule should be Considered as one of the Supplementary Target for Designing Neuro-therapeutics?" ***ACS Chem Neurosci. 2019, 10, 1118-1120.***

2. **Development of peptide-based neuro-therapeutic lead targeting both Amyloid-beta (Aβ) and microtubule:**



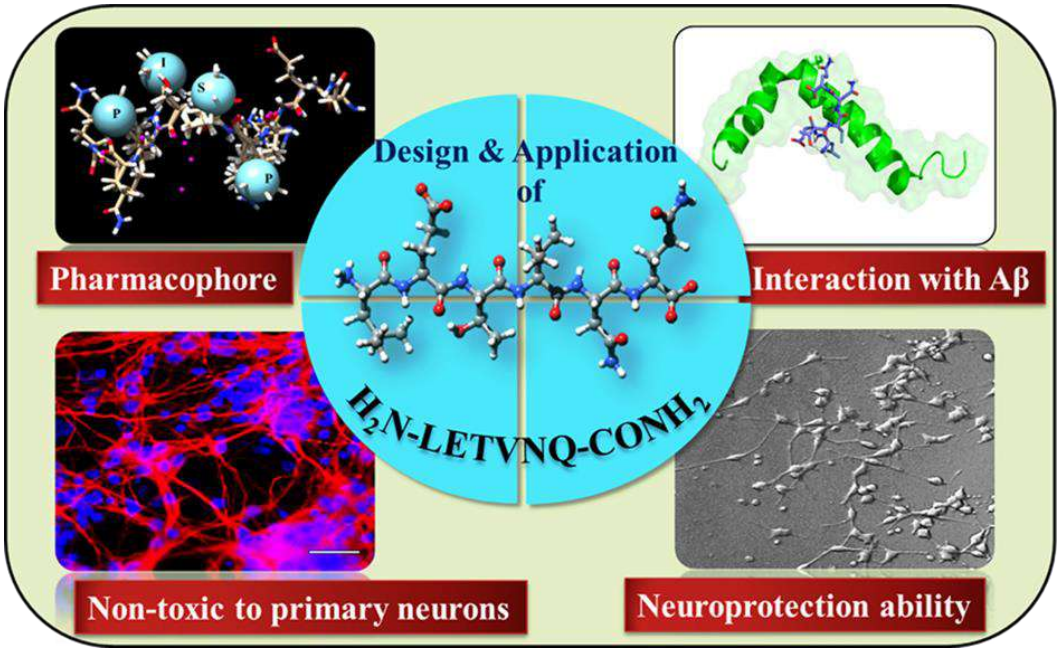
Encouraging results from above discussed designing strategy brings confident that we can introduce additional parameter in our design principle. Amyloid-beta (Aβ) peptide misfolds into fibrillary aggregates (β-sheet) and deposited as amyloid plaques in the cellular environment, which severely damages intraneuronal connections leading to Alzheimer's disease (AD) pathogenesis. Furthermore, neurons are rich in tubulin/microtubule and the intracellular network of microtubules also gets disrupted by the accumulation of Aβ fiber in brain. Hence, development of new potent molecules, which can simultaneously inhibit Aβ fibrillations and stabilize microtubules, is particularly needed for the efficient therapeutic application in AD. To address these issues, here we introduced an innovative fusion strategy to design and develop next generation anti-AD therapeutic leads. This unexplored fusion strategy entails design and development of a potent nonapeptide by taking into account both the hydrophobic core (17-21) of Aβ peptide and the taxol binding region of β-tubulin. *In vitro* results suggest that this newly designed peptide interacts at the taxol binding region of β-tubulin with a moderate binding affinity and promotes microtubule polymerization. It has the ability to bind at the hydrophobic core (17-21) of Aβ, responsible for its aggregation and prevent the amyloid fibril as well as plaques formation. In addition, it interacts at the CAS site (catalytic anionic site) of acetylcholinesterase (AChE) and significantly inhibits AChE induced Aβ fibrillation, stimulates neurite branching, provides stability to intracellular microtubules and extensive protection of neurons against nerve growth factor (NGF) deprived neuron toxicity. Moreover, this newly designed peptide shows good stability in serum obtained from humans and efficiently permeates the blood-brain barrier (BBB) without showing any toxicity towards differentiated PC12 neurons as well as primary rat cortical neurons. This excellent feature of protecting the neurons by stabilizing the microtubules without showing any toxicity towards neurons will make this peptide a potent therapeutic agent of AD in near future.

Reference:

2. Prasenjit Mondal, Gaurav Das, Juhee Khan, Krishnangsu Pradhan, Rathnam Mallesh, Abhijit Saha, Batakrishna Jana and **Surajit Ghosh.*** Potential Neuroprotective Peptide Emerged from Dual Neurotherapeutic Targets: A Fusion Approach for the Development of anti-Alzheimer's Lead. ***ACS Chem Neurosci. 2019, 10, 2609-2620.***

3. Development of peptide-based neuro-therapeutic leads targeting amyloid-beta (Aβ):

Alzheimer's disease (AD) is a constantly recurring neurodegenerative disease that deteriorates over a period of time. In this pathology, connections between neurons got extremely damaged due to the deposition of senile plaques in the membrane region, which results in abnormal signal transduction process. Also, the intracellular microtubule networks got disrupted in hyperphosphorylated tau cascade of AD. Therefore, design and development of potent neuroprotective molecules are extremely important that can instantaneously target multiple



facets of AD pathogenesis to tackle this unmet medical need. Here, we have implemented a pharmacophore based in silico analysis of various neuroprotective peptides known for neurotherapeutic application in AD. Our design originated from amyloid beta peptide. Fascinatingly, we have identified an active core of these peptides and designed a library of hexapeptides. We observed that two hexapeptides "NAVSIQ" and "LETVNQ" have shown significant protection ability against degeneration of neurons. Experimental evidences suggest that these peptides immensely reduced the aggregation rate of amyloid-beta (Aβ) and helped in microtubule polymerization. Intriguingly, this newly designed peptide does not have any cytotoxicity towards differentiated PC12 neurons rather it helps in neurite outgrowth. Further, these hexapeptides helps to maintain the complex microtubule network in cells by promoting the polymerization rate of intracellular microtubule and deliberates excellent protection of neurons even after removal of nerve growth factor (NGF). Finally, we observed that these peptides have substantial stability in physiological condition, and helps to retain the healthy morphology of the primary rat cortical neurons. This excellent piece of work identifies that potent hexapeptides, which have exceptional ability to protect neurons as well as microtubule from degeneration, may come up as a potent therapeutics of AD pathogenesis in future.

References:

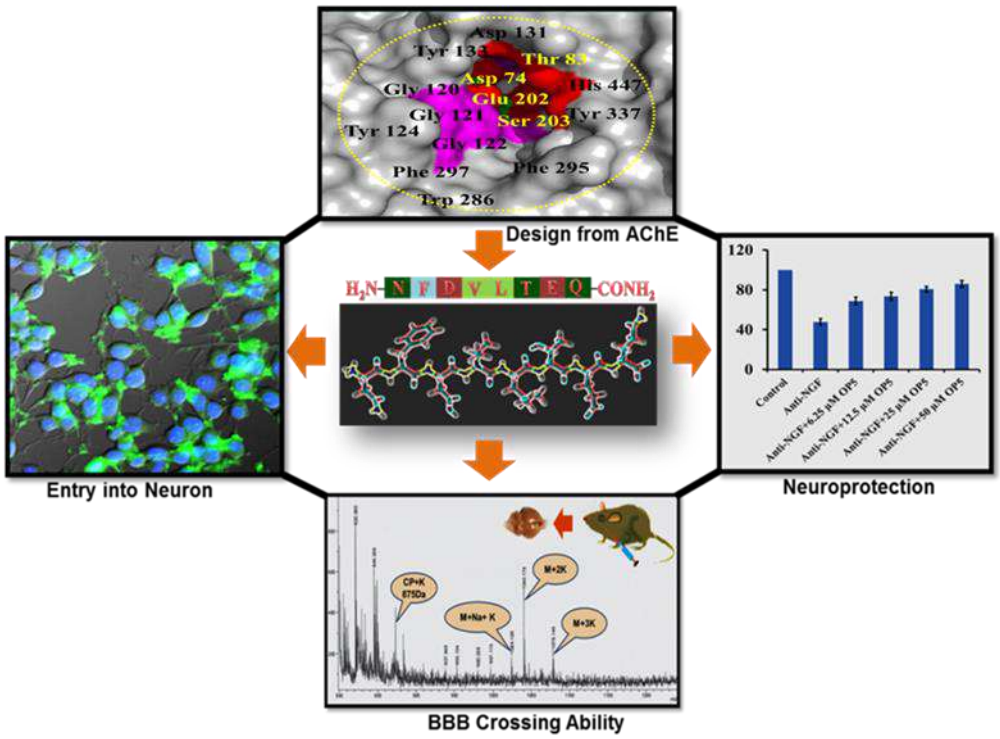
3a. Prasenjit Mondal, Juhee Khan, Varsha Gupta and **Surajit Ghosh.*** In silico Approach for Designing Potent Neuroprotective Hexapeptide. ***ACS Chem Neurosci. 2019, 1063018-3030.***

3b. Atanu Biswas, Prashant Kurkute, Suraiya Saleem, Batakrishna Jana, Saswat Mohapatra, Prasenjit Mondal, Anindyasundar Adak, Subhajit Ghosh, Abhijit Saha, Debmalya Bhunia, Subhash Chandra Biswas, and **Surajit Ghosh*.** Novel Hexapeptide Interacts with Tubulin and Microtubules, Inhibits Aβ Fibrillation, and Shows Significant Neuroprotection. ***ACS Chem. Neurosci., 2015, 6, 1309–1316. (Highlighted in Cover-page)***

3c. Hexapeptide for neuroprotection against a beta toxicity. By **Ghosh, Surajit**; Biswas, Atanu; Jana, Batakrishna; Mohapatra, Saswat; Biswas, Subhas Chandra; Saleem, Suraiya; Mondal, Prasenjit; Adak, Anindyasundar; Ghosh, Subhajit; Saha, Abhijit; et al. ***U.S. Pat. Appl. Publ. (2017), US 20170253631 A1 20170907. [Granted]***

4. **Development of peptide-based neuro-therapeutic lead from active site of catalytic anionic site (CAS) of AChE:**

Design and development of acetylcholinesterase (AChE) inhibitor has tremendous implications in the treatment of Alzheimer's disease (AD). Here, we have adopted a computational approach for designing of peptide based AChE inhibitor from its active site. We identified an octapeptide, which interacts with the catalytic anionic site (CAS) of AChE enzyme and inhibits its activity



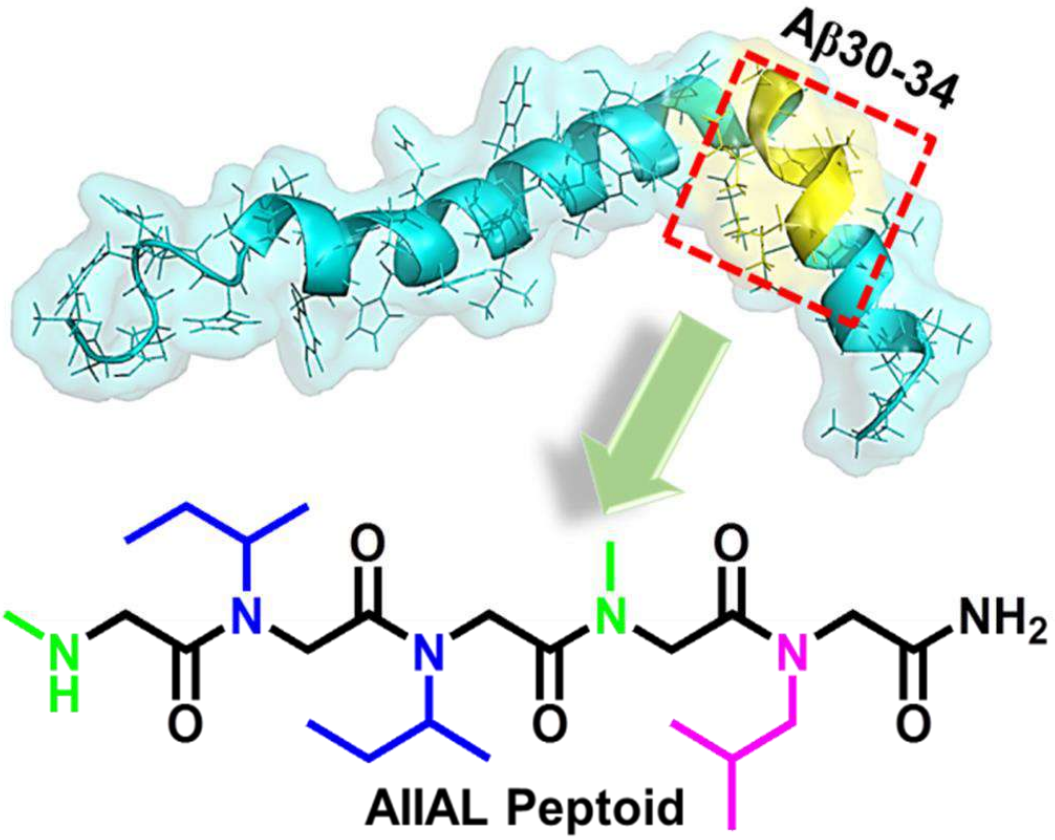
Interestingly, this peptide also inhibits amyloid aggregation through interacting at the 17-21 region of amyloid-beta (Aβ) and stabilizes microtubules by interacting with tubulin as well. Eventually in the PC12 derived neurons, it shows non-cytotoxicity, promotes neurite out-growth, stabilizes intracellular microtubules and confers significant neuroprotection even upon withdrawal of nerve growth factor (NGF). Further, results reveal that this peptide possesses good serum stability, crosses blood-brain barrier (BBB) and maintains the healthy architecture of the primary cortical neurons. This work shows a discovery of excellent peptide-based AChE inhibitor with additional potential as microtubule stabilizer, which will pave the way for the development of potential anti-AD therapeutics.

References:

4. Prasenjit Mondal, Varsha Gupta, Gaurav Das, Krishnangsu Pradhan, Juhee Khan, Prabir Kumar Gharai and **Surajit Ghosh.*** Peptide-based Acetylcholinesterase Inhibitor Crosses Blood-Brain Barrier and Promotes Neuroprotection. ***ACS Chem Neurosci. 2018, 9, 2838-2848.***

5. **Development of peptoid-based neuro-therapeutic lead targeting amyloid-beta (Aβ):**

Till date, key challenge is to develop non-toxic, proteolytically stable amyloid inhibitors which can target multiple pathways involved in AD simultaneously. Various attempts have been made in this direction, however clinical outcome of those attempts have been reported to be poor. Thus, we choose development of peptoid (N-substituted glycine oligomers)-based leads for potential AD therapeutics, which are easy to synthesize, are proteolytically stable and exhibit excellent bioavailability. In this work, we have designed and synthesized a new short peptoid



for amyloid inhibition from 30-34 hydrophobic pocket of amyloid beta (Aβ) peptide. The peptoid selectively binds with 17-21 hydrophobic region of Aβ and inhibits Aβ fibril formation. Various *in vitro* assays suggested that peptoid binds with tubulin/microtubule and promotes its polymerization and stability. This peptoid also inhibits AChE induced Aβ fibril formation and provides significant neuroprotection against neurite growth factor (NGF) deprived neurons derived from rat adrenal pheochromocytoma (PC12) cell line. Moreover, this peptoid shows serum stability and is non-cytotoxic to primary cortical neurons.

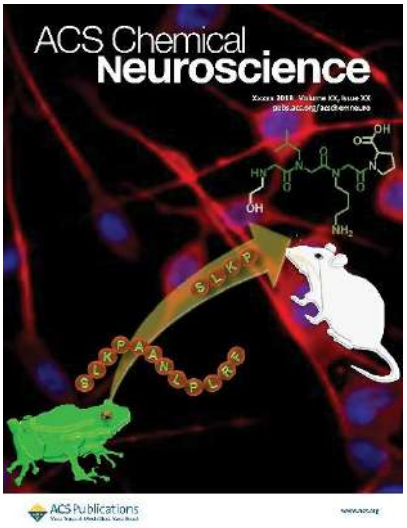
References:

5. Krishnangsu Pradhan, Gaurav Das, Prasenjit Mondal, Juhee Khan, Surajit Barman, **Surajit Ghosh.*** Genesis of Neuroprotective Peptoid from Aβ30-34 Inhibits Aβ Aggregation and AChE Activity. ***ACS Chem Neurosci. 2018, 9, 2929-2940***.

6. **Development of neuroregenerative peptoid from amphibian neuropeptide:**

Development of potential therapeutics for Alzheimer's disease (AD) required multifaceted strategy considering the high level of complexities of human brain and its mode of function. Here, we adopted an advanced strategy targeting two key pathological hallmarks of AD such as senile plaque and neurofibrillary tangles. We derived a lead short tetrapeptide Ser-Leu-Lys-Pro (SLKP) from dodeca-neuropeptide of amphibian (frog) brain. Results suggest that SLKP peptide has superior effect compared to the dodecapeptide in neuroprotection. This result

encourages us to adopt peptidomimetic approach to synthesize SLKP peptoid. Remarkably, we found that SLKP peptoid is more potent than its peptide analogue, which significantly inhibits Aβ fibrillization, moderately binds with tubulin and promotes tubulin polymerization as well as stabilization of microtubule networks. Further, we found that SLKP peptoid is stable in serum, showed significant neuroprotection against Aβ mediated toxicity, promotes significant neurite outgrowth, maintains healthy morphology of rat primary cortical neurons and crosses the Blood-Brain Barrier (BBB). To the best of our knowledge, our SLKP peptoid is the first shortest peptoid showed significant neuroprotection, neuro-regeneration against Aβ toxicity as well as crossed the BBB offering a potential lead for AD therapeutics.



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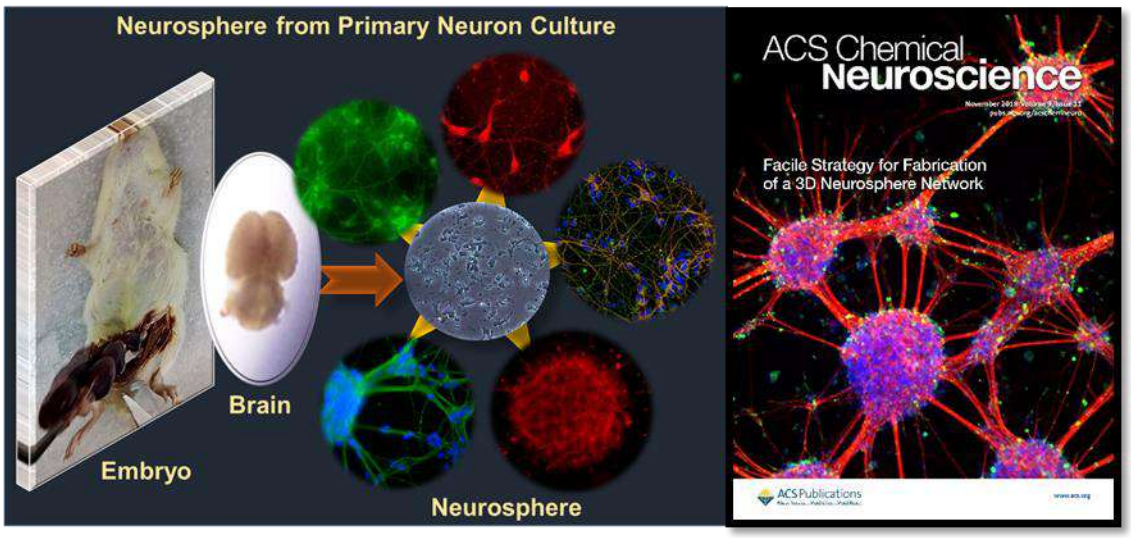
6a. Krishnangsu Pradhan, Gaurav Das, Varsha Gupta, Prasenjit Mondal, Surajit Barman, Juhee Khan, and **Surajit Ghosh.*** Discovery of Neuro-regenerative Peptoid from Amphibian Neuropeptide Inhibits Aβ Toxicity and Crossed Blood-Brain Barrier. ***ACS Chem Neurosci. 2019, 10, 1355-1368.***

6b. Peptoid of formula I, pharmaceutical compositions and method for preparation thereof. By **Ghosh, Surajit;** Pradhan, Krishnangsu; Das, Gaurav; Mondal, Prasenjit; Barman, Surajit; Ghosh, Subhajit. ***Indian Pat. Appl. (2018), IN 201811016874.***

6c. Prepn. of peptoid pharmaceutical compns. for treatment of Alzheimer's disease. **Ghosh, Surajit;** Pradhan, Krishnangsu; Das, Gaurav; Mondal, Prasenjit; Barman, Surajit; Ghosh, Subhajit. ***PCT Int. Appl. (2019), WO 2019211878 A1 20191107.***

7. Neurosphere based drug screening platform:

Reconstitution of complex biological structure or system following simple and facile strategy using minimum physiochemical cues is challenging for in depth understanding of those systems. In particular, brain is a highly sophisticated and complex network of trillions of neurons and glial cells that controls function of our body. Understanding this complex machinery requires innovative and simple bottom-up approach. In this venture, we developed an easy and efficient strategy to culture cortical and hippocampal primary neurons from the E14-E16 embryo of Sprague Dawley rat. This generates spontaneous neurospheres within 6-7 days of primary culture of E14-16 embryo. It further proliferates and forms radial glia like structures, which are known to be the primary neural progenitor cells that differentiate into neurons, astrocytes and oligodendrocytes.



Interestingly, these neurospheres possess a heterogeneous population of glial cells, neurons, neural stem and progenitor cells, bear a closer resemblance to the human brain. Moreover, neurospheres leads to the formation of large projection neurons and radial glia, which mimic the early stage of cortical development in *in vivo* system. Overall, this new facile strategic mixed primary neuron culture method offers a potential platform for understanding the effect of neuro-chemical modulators, which has tremendous future implications in screening of neuro-therapeutics.

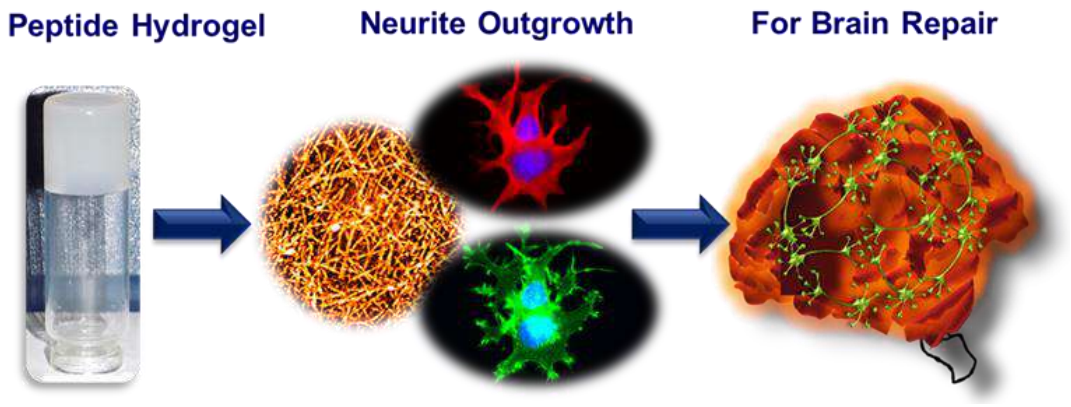
References:

7a. Juhee Khan, Gaurav Das, Varsha Gupta, Saswat Mohapatra, Subhajit Ghosh, and **Surajit Ghosh.*** Neurosphere Development from Hippocampal and Cortical Embryonic Mixed Primary Neuron Culture: A Potential Platform for Screening Neuro-Chemical Modulator. ***ACS Chem Neurosci. 2018, 9, 2870–2878.***

7b. Gaurav Das, Varsha Gupta, Juhee Khan, Deepshikha Mukherjee and **Surajit Ghosh.*** Generation of Neurospheres from Mixed Primary Hippocampal and Cortical Neurons Isolated from E14-E16 Sprague Dawley Rat Embryo. ***Journal of Visualized Experiments: JoVE, 2019, 150.***

8. **Hydrogel for therapeutic delivery and repairing of traumatic brain injury:**
Repairing of traumatic brain injury (TBI) is an immediate challenge due to the limited regenerative properties of the brain. We are working on understanding repair mechanism by exogenous application of a neuroprotective hydrogel to brain injury region in a cryogenic injury mice model. In this direction we have developed few bioengineered hydrogel for repairing neural damage. Some of key developments are as follows:

(a) ***Neuro-compatible Hydrogel:*** A novel neuro-compatible peptide based hydrogel has been designed and developed, which contains microtubule stabilizing and neuroprotective short peptide. This hydrogel shows strong three dimensional cross-linked fibrillary networks, which can capture water molecules. Interestingly, this hydrogel serves as excellent biocompatible

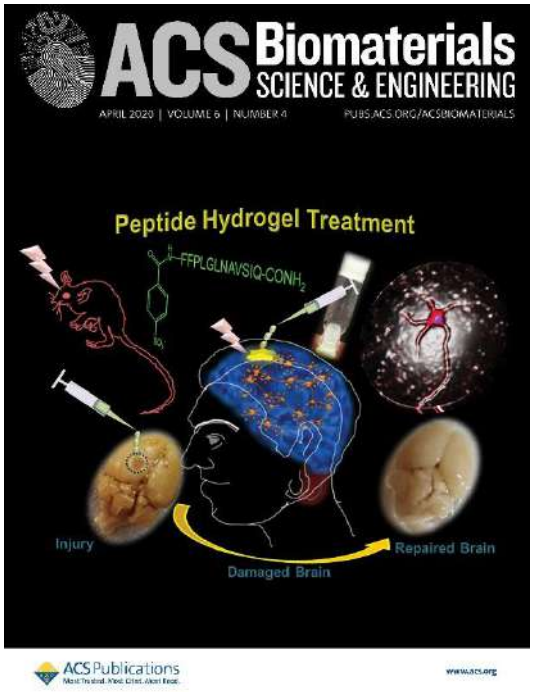


soft-material for 2D and 3D (neuro-sphere) neuron cell culture and provides stability of key cytoskeleton filaments such as microtubule and actin. Remarkably, it was observed that this hydrogel slowly enzymatically degrades and releases neuroprotective peptide, which promotes neurite outgrowth of neuron cell as well as exhibits excellent neuroprotection against anti-NGF induced toxicity in neuron cells. Further, it can encapsulate anti-Alzheimer and anti-cancer hydrophobic drug curcumin, releases slowly and inhibit significantly the growth of 3D spheroid of neuronal cancer cells. Thus, this novel neuroprotective hydrogel can be used for both neuronal cell transplantations for repairing brain damages as well as delivery vehicle for neuroprotective agents, anti-Alzheimer and anti-cancer molecules.

Reference:

8a. Anindyasundar Adak, Gaurav Das, Surajit Barman, Saswat Mohapatra, Debmalya Bhunia, **Surajit Ghosh.*** Biodegradable Neuro-Compatible Peptide Hydrogel Promotes Neurite Outgrowth, Shows Significant Neuroprotection, and Delivers Anti-Alzheimer Drug. ***ACS Appl Mater Interfaces. 2017, 9, 5067- 5076.***

(b) Brain injury can lead to the loss of neuronal functions and connections, along with damage of extracellular matrix (ECM). Thus, it ultimately results in devastating long-term damage and recovery of it is a challenging task. To address this issue, we have designed a sulfo group functionalised injectable biocompatible peptide hydrogel, which not only mimic ECM and supports the damaged neurons but also it releases a neurotropic factor around the injured sites of brain in presence of Matrix Metalloproteinase 9 (MMP 9) enzyme. It has been also observed that the driving force of hydrogel formation is β-sheet secondary structure and π-π stacking interactions between the Phe-Phe moieties. The hydrogel is not only able to promote neurite outgrowth of PC-12 derived neurons and primary neurons cultured in presence of hydrogel, but also it is able to nullify the toxic effects of anti-NGF induced neurons. It also promotes the expression of the key neuronal markers in rat cortical primary neurons, displays substantial potential in neuroregeneration and also promotes fast recovery of the sham injured mice brain. Increase expression of reactive

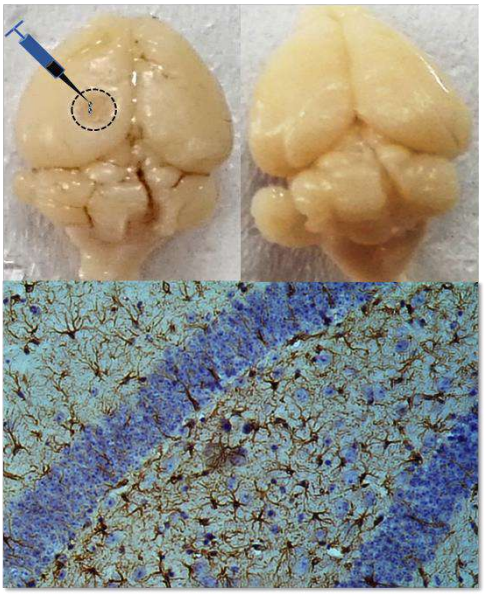


astrocytes in the hippocampal dentate gyrus region of the sham injured brain is clearly suggesting about its tremendous ability in neural repair of the damaged brain. Thus, we can convincingly state that our hydrogel is cable of repairing brain injury by mimicking ECM like environment and providing a neuroprotective effect towards the damaged neurons.

Reference:

8b. Anindyasundar Adak, Gaurav Das, Juhee Khan, Nabanita Mukherjee, Varsha Gupta, Rathnam Mallesh, **Surajit Ghosh*** "Extracellular Matrix Mimicking (ECM) Neuroprotective Injectable Sulfo-functionalized Peptide Hydrogel for Repairing Brain Injury" ***ACS Biomater. Sci. Eng. 2020, 6, 4, 2287-2296. Highlighted in Cover Page.***

(c) Brain damage is associated with spatial imbalance of cholinergic system, which makes severe impact in recovery of damaged neurons of brain. Therefore, maintenance of cholinergic system is extremely important. Here, we fabricated an injectable hydrogel with acetylcholine functionalized graphene oxide and polyacrylic acid. Results revealed that this hydrogel is non-cytotoxic, promotes neurite outgrowth, stabilizes microtubule networks, and enhances the expression of some key neural markers in rat cortical primary neurons. Further, this hydrogel exhibits significant potential in neuro-regeneration and also promotes fast recovery of the sham injured mice brain. Moreover, we found significant enhancement of reactive astrocytes in the hippocampal DG region of the sham injured brain, indicating its excellent potential in neural repair of the damaged brain. Finally, above results clearly indicate that this neuro-regenerative hydrogel is highly capable of maintaining the cholinergic balance through local release of acetylcholine in the injured brain, which is crucial for brain repair.



Reference:

8c. Krishnangsu Pradhan, Gaurav Das, Juhee Khan, Varsha Gupta, Surajit Barman, Anindyasundar Adak, and **Surajit Ghosh.*** Neuro-Regenerative Choline Functionalized Injectable Graphene Oxide Hydrogel Repairs Focal Brain Injury. ***ACS Chem Neurosci. 2019, 10, 1535-1543.***

9. **Chemical modulator for lineage reprograming for the development regenerative medicine for traumatic brain injury:**

Human brain is an inexplicable machine, which contains networks of millions of neurons surrounded by large number of glial cells with neuro-protective functions. This amazing machinery acts as an integrated precise machine that maintains physiological, motor and cognitive functions. Any damage or even slight perturbation to this complex machinery due to stress, injury or neurodegenerative disorders, causes severe malfunction in our psychological, motor and cognitive functions. Indigenous repairing mechanism of brain is limited due to its inadequate regenerative capabilities and complexities, which makes the repairing of the damages even more challenging. Thus, novel approaches in this direction are extremely important. Various approaches have been attempted and some of them are promising. Among them, stem-cell-based approaches has shown maximum promise in repairing and regenerating the damage caused in neurons. These approaches are unique due to their regeneration and repairing capability including replacement of damaged

cells, restoration of neuronal circuitry, reduction of inflammation/gliosis and induction of axonal regeneration. Recently, interesting approaches involving reprogramming of reactive astrocytes into functional neurons using multifaceted approaches have emerged as a new strategy. This approach is attractive given the fact that it reduces the multiple steps of regeneration and transplantation of neurons involved in the stem-cell based approaches. This approach is beneficial due to the resident population of reactive astrocytes already present in the damaged area of brain which will aid in their simultaneous conversion into functional neurons. Chemical-induced conversion of reactive human astrocytes into the functional neurons using a cocktail of nine small molecules that inhibits the glial cells but activates the neuronal signalling pathways to successfully generate a horde of reprogrammed neurons. This chemical reprograming is also governed by epigenetic regulation and activation of transcription factors NeuroD1 and NeuroG2. This astrocyte derived regenerated neurons are functional for few months. However, due to large number of molecules used as cocktail, this may cause adverse effect in longer run as well as understanding the precise effect of individual molecules is challenging. To address this issues, we adopted a joint strategy where we used QSAR and identified the active phamacophore unit and designed a library of molecules. Interestingly, we observed remarkable results where we could bring down into a single effective molecules, which shows neurogenesis. This chemical approach of reprogramming opens up a new field of immeasurable opportunities that can lead to great discoveries in the arena of regenerative medicine.

References:

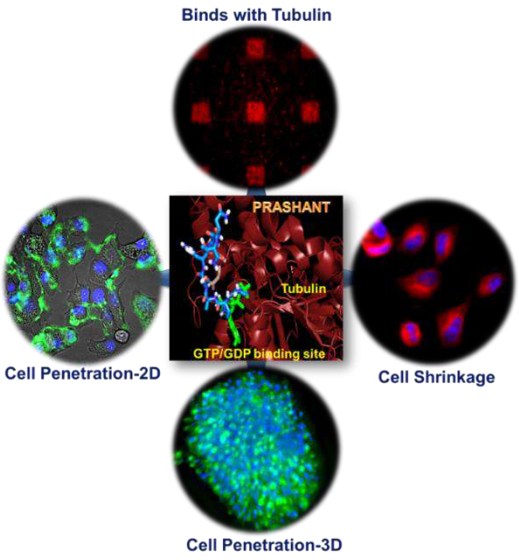
9. Gupta *et al.*, 2020, *Unpublished Work.*

10. Discovery of efficient and short cell penetrating peptide:

Plasma membrane remains a tough barrier for smooth entry of drugs causing poor cellular uptake, requiring high amount of administered drugs to achieve the desired bio-logical effect. Thus, efficient translocations of drugs through the plasma membrane are extremely important for its successful delivery and minimization of side effects on healthy tissues. This issue has been addressed in remarkable studies such as identification of Trans-Activator of Transcription (Tat) protein of the Human Immunodeficiency Virus (HIV) and Drosophila melanogaster Antennapedia homeodomain as important structural sequences for efficient translocation into the cells. Further studies reveal the minimum domain needed for translocation, which popularly termed as the "cell-penetrating peptides" (CPPs). In this direction, we have worked and developed short CCPs that shows remarkable results. Brief overview of those work as follows:

(a) ***Cell Penetrating Octapeptide:*** A novel short cell penetrating peptide (CPP) is designed from exchangeable GTP/GDP binding pocket of tubulin. This octapeptide strongly binds with tubulin at exchangeable GTP/GDP binding site, significantly inhibits tubulin polymerization and reduces kinesin driven microtubule motility. Interestingly, this peptide enters into the cells

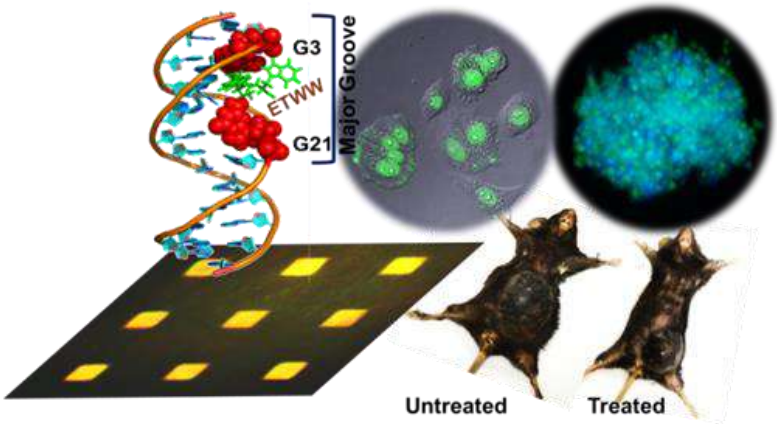
through endocytosis, targets intracellular tubulin and shows moderate cytotoxicity (IC50 37.8 μM) against human breast cancer and other cancer cell lines. In addition, this novel peptide interacts with intracellular tubulin/microtubules, depolymerizes intracellular microtubules, promotes intracellular tubulin aggregation and bundle formation of microtubules, activates mitotic check point, p53 and p21 proteins. Finally, it induces apoptotic death of breast cancer cell, exhibits significant penetration ability in multicellular 3D spheroid of HeLa cells and inhibits its growth significantly. To the best of our knowledge this is the first CPP discovered, which interacts with tubulin strongly and shows antimitotic activity.



References:

10a. Debmalya Bhunia, Saswat Mohapatra, Prashant Kurkute, Subhajit Ghosh, Batakrishna Jana, Prasenjit Mondal, Abhijit Saha, Gaurav Das and **Surajit Ghosh*.** Novel Tubulin-targeted Cell Penetrating Antimitotic Octapeptide**. *Chem. Commun., 2016, 52, 12657-12660.***

(b) Identification of key amino acids is required for development of efficient cell penetrating peptides (CPPs) and has tremendous implications in medicine. Extensive research work enlightened us about the importance of two amino acids, arginine and tryptophan in the cell penetration. Here, we present a top-down approach to show how spatial positions of two tryptophans regulate the cellular entry and nuclear localization. This enables us to develop short nontoxic tetrapeptides with excellent potential of cell penetration and nuclear localization. Among them Glu-Thr-Trp-Trp (ETWW) emerges as most promising one. Result suggests that it enters into the cancer cell following endocytic path-way and binds at major groove of nuclear DNA, where successive tryptophan plays major role. Subsequently, we showed that it is not a P-gp substrate and nontoxic to PC12 derived neurons, suggesting its excellent potential as CPP. Furthermore, its potential as CPP has been validated in multi-cellular 3D cell culture (spheroid) and in *in vivo* mice model. This study provides major fundamental insights about the positional importance of tryptophan and opens new avenues towards the development of next generation CPP and major groove specific anticancer drugs.



References:

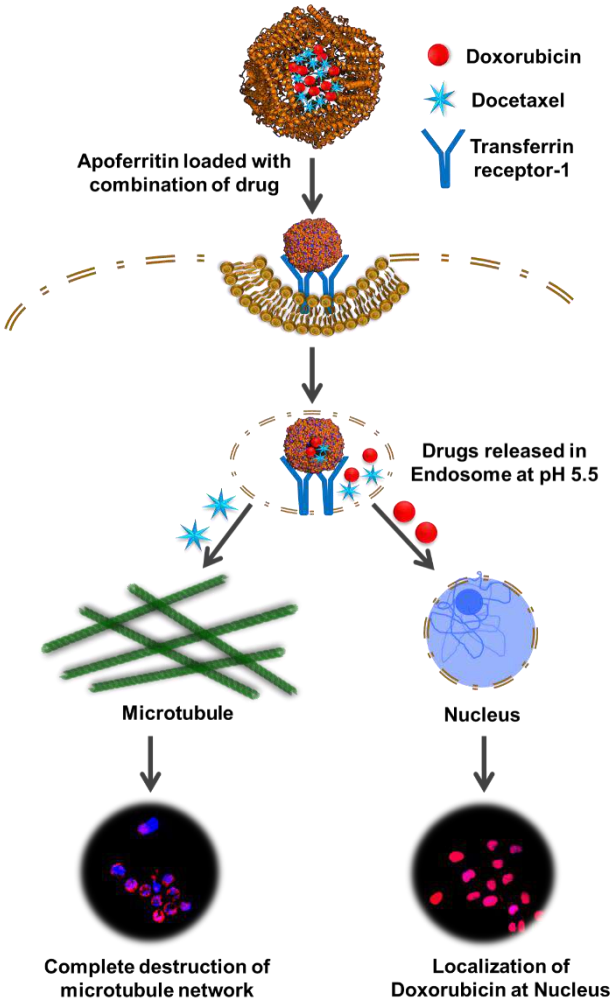
10b. Debmalya Bhunia, Prasenjit Mondal, Gaurav Das, Abhijit Saha, Pallabi Sengupta, Jagannath Jana, Saswat Mohapatra, Subhrangsu Chatterjee, and **Surajit Ghosh.*** Spatial

Position Regulates Power of Tryptophan: Discovery of Major Groove Specific Nuclear Localizing Cell Penetrating Tetrapeptide. ***J Am Chem Soc., 2018, 140, 1697-1714.*** **Selected for JACS Young Investigators Virtual Issue, 2019 by Prof. Peter J. Stang (Editor-in Chief, JACS).**

11. Development of Nanomedicine Leads:

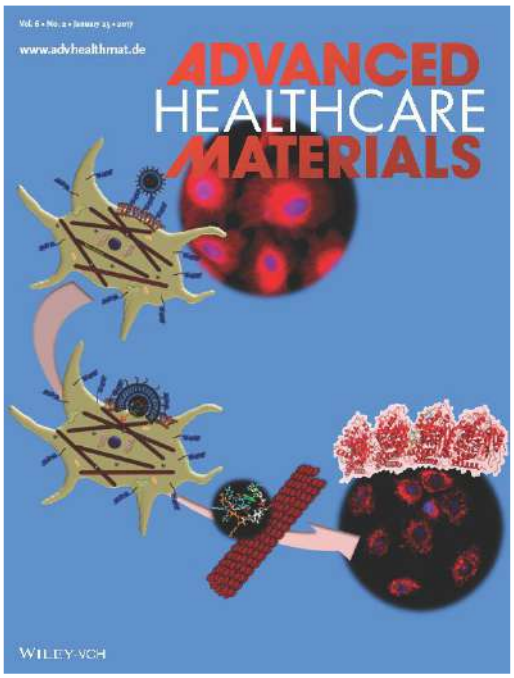
Development of effective therapeutic formulation is always beneficial for the successful clinical outcome. However, while drugs showed potent activity, major problems are still associated with these drugs, such as poor bioavailability and whole body-toxicity causing those poor candidates. In this direction, attempts have been made to increase the bioavailability and delivery of certain drugs through targeted nano-formulations. Various nano-formulation for anti-cancer drug delivery are available but, success rate of these nano-formulations are not satisfactory in terms of clinical use. To address these issues, we are working to develop effective and targeted nano-formulations and few of our recent works are summarized below:

(a) **Appoferritin-based nano-formulation:** An ideal nano drug delivery agent must be potent enough to carry high dose of therapeutics, competent enough in targeting specific cell of interest, having adequate optimized physiochemical properties and biocompatibility. Carrying differentially polar therapeutics simultaneously will make them superior in their class. However, it is of enormous challenge to the researchers to find out such a unique nanocarrier and engineer all the above-mentioned features into it. In this work, we have shown for the first time that Apoferritin (Apf) can carry high dose of doxorubicin (Dox), docetaxel (Doc) and Dox-Doc simultaneously towards cancer cell specific targeting and enhanced killing compared to free drug without any functionalization or property modulation. On the other hand cytotoxicity of these Apf complexes is lesser than free drugs towards normal lung fibroblast cell (WI38). Drug loaded Apf specifically bound and consequently internalized into the cancer cells through receptor mediated endocytosis process and release either single or combination of drug to its specific target. Using molecular docking we have checked the binding efficacy of both the drugs. In addition, we have shown that Apf is non-cytotoxic in nature and binds with intracellular tubulin/microtubule. Further we have studied the efficacy of Apf complexes in 3D multicellular tumor spheroid model. Finally, using fluorescence microscopy we have shown that Apf can deliver combination of drugs inside cancer cells and the drugs exerts their effect thereof.



Reference:

11a. Subhajit Ghosh, Saswat Mohapatra, Anisha Thomas, Debmalya Bhunia, Abhijit Saha, Gaurav Das, Batakrishna Jana and **Surajit Ghosh*.** Apoferritin-nanocage delivers combination of microtubule and nucleus targeting anticancer drugs. ***ACS Appl. Mater. Interfaces, 2016, 8, 30824–30832.***

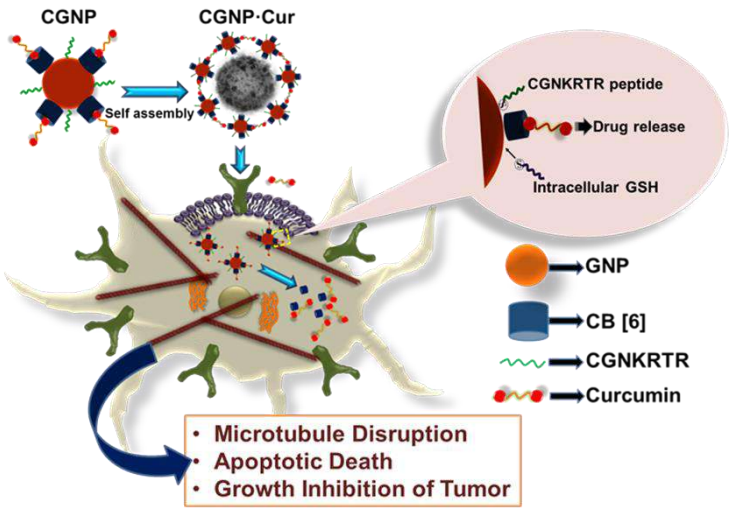
(b) 

Microtubule dynamics play a crucial role in cancer cell division. Various drugs are developed to target microtubule. Although a few of them show potential in treatment of cancer, but success rate is limited due to their poor bioavailability and lack of specificity. Thus, development of highly bioavailable and target specific anticancer drug is extremely necessary. To address these key issues, here, a combination of approaches such as development of a dodecapeptide-docetaxel nano-assembly targeted to tubulin and MUC1 targeting oligonucleotide aptamer conjugated liposome for delivering peptide-docetaxel nano-assembly into the breast cancer cell has been demonstrated. These studies reveal that the peptide forms nano-assembly and entraps docetaxel drug. Further, the liposomal formulation of peptide-docetaxel exerts synergistic anticancer effect, activates key mitotic check point proteins and inhibits bipolar spindle formation, metastatic cancer cell migration and growth of tumor mimicking 3D multicellular spheroid.

Reference:

11b. S Mohapatra, A Saha, P Mondal, B Jana, S Ghosh, A Biswas, **Surajit Ghosh*** Synergistic anticancer effect of peptide-docetaxel nano-assembly targeted to tubulin: Towards development of dual warhead containing nanomedicine. ***Adv Healthcare Mater., 2017, 6, 1600718. Highlighted in Cover Page and Highlighted in Advance Science.***

(c) A multi-arm nanomedicine template has been designed following bottom-up approach, which target neuropolin-1 (Nrp-1) receptor of cancer cells. Through this venture we discovered that cucurbit [6] uril (CB [6]) binds with tubulin close to binding pocket of vinblastine site and perturbs tubulin polymerization. To increase the specificity of gold nanoparticle (GNP) towards Nrp-1 rich cancer cells, we



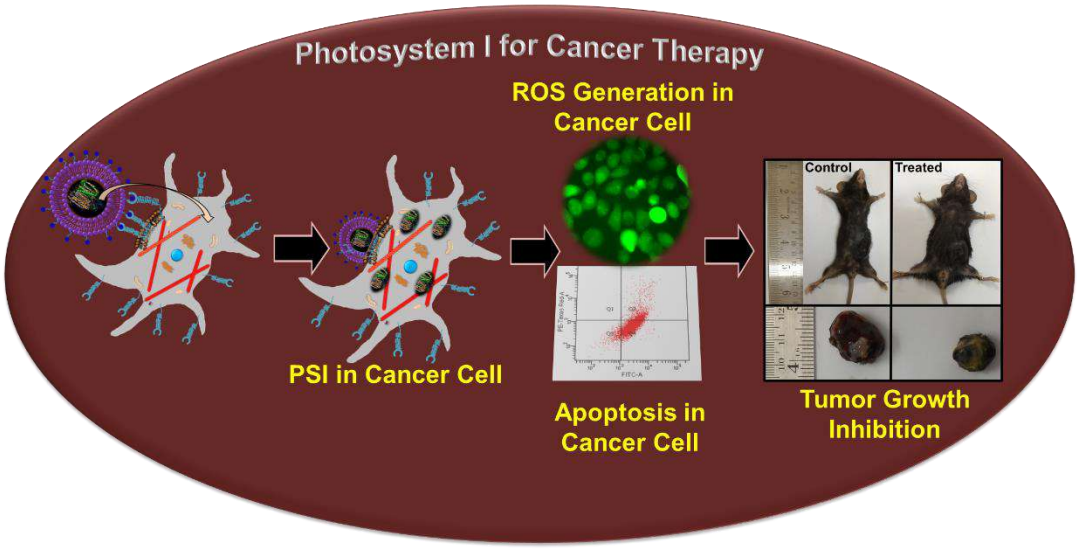
further modified this GNP with Nrp-1 receptor specific short peptide (CGNKRTR). Remarkably, we found an interesting self-assembly process upon addition of curcumin into the CB [6] and peptide functionalized GNP leading to the formation of a spherical nanocapsule (CGNP·Cur). It can deliver and release significantly higher amounts of anticancer drug curcumin in Nrp-1 rich cancer cells. It causes microtubule depolymerization and significant tumor regression in Nrp-1 overexpressed mice melanoma model. These interesting finding shows that nanocapsule has high potential to develop a powerful anticancer nanomedicine and help in its preclinical validation.

Reference:

11c. Surajit Barman, Gaurav Das, Varsha Gupta, Prasenjit Mondal, Krishnangsu Pradhan, Batakrishna Jana, Debmalya Bhunia, Juhee Khan, Deepshikha Mukherjee and **Surajit Ghosh.*** Dual Arm Nanocapsule Targets Neuropilin-1 Receptor and Microtubule: A Potential Nanomedicine Platform. ***Mol. Pharmaceutics 2019, 16, 2522-2531.***

12. Photosystem I (PSI) embedded hydrogel for melanoma:

Many anti-cancer drugs are developed for the treatment of cancer from natural sources. Photo System I (PSI), a protein complex present in the chloroplast is involved in photosynthesis and generates ROS in plant. Here, we have used the ROS generation property of PSI for cancer therapy. We have shown that PSI can enter into the different kinds of cancer cell like human lung carcinoma (A549) and mouse melanoma (B16F10) cell lines and generates ROS inside the cells. It inhibits the proliferation of cancer cell and causes apoptotic death of cancer cells. We have also shown that PSI induces apoptosis through mitochondria dependent internal pathway, induces caspase3, causes DNA fragmentation and arrests cell cycle at SubG0 phase. We also prepared, using C16-LDV lipopeptide [C16 long chain attached on the N-terminal of the tri-peptide containing amino acids leucine (L), aspartic acid (D) and valine (V) as NH2-LDV-COOH] , α4β1 integrin targeted liposomal formulation of PSI which specifically kills the cancer cell without affecting normal cells and it is found to be more potent compared to clinically used drug doxorubicin. Finally, we have found that LDV liposomal formulation of PSI inhibits the growth of tumor in C57BL/6J mice model.



Reference:

12a. Abhijit Saha, Saswat Mohapatra, Gaurav Das, Batakrishna Jana, Subhajit Ghosh, Debmalya Bhunia, and Surajit Ghosh.* Cancer cell specific delivery of Photosystem I through integrin targeted liposome shows significant anticancer activity, **ACS Appl. Mater. Interfaces, 2017, 9, 176-188.**

12b. A LDV peptide liposomal formulation of Photosystem-1 for treatment of cancer. By **Ghosh, Surajit;** Saha, Abhijit; Ghosh, Subhajit; Mohapatra, Saswat; Jana, Batakrishna; Bhunia, Debmalya. **PCT Int. Appl. (2018), WO 2018065993 A1 20180412.**

12c. A LDV peptide liposomal formulation of Photosystem-1 for treatment of cancer. By **Ghosh, Surajit;** Saha, Abhijit; Ghosh, Subhajit; Mohapatra, Saswat; Jana, Batakrishna; Bhunia, Debmalya. **Indian Pat. Appl. (2018), IN 201611034058 A 20180406.**

13. **Reconstitution of artificial cell to understand the disease propagation:**

Reconstitution of artificial cell is challenging but at the same time is rewarding to understand the key cellular events. Construction of artificial cells using reverse emulsion technique required skilled expertise and around the globe only few laboratories can successfully demonstrated this system. In this journey our laboratory is one of the lab from India is capable of produce this artificial cell based system, where currently we are working on various membrane proteins-based problem directly associated with disease. Here, we are showcasing one of the events using this system as described below.

The assembly of native Aβ into fibrillar deposits is governed by structural changes from α-helical to β-sheet, which results in Alzheimer disease. The origin of this disease starts from individual cell, and how the infection spreads from one cell to another is poorly understood. Cell membrane plays a crucial role in aggregate formation and propagation from one cell to another cell. Here, we reconstitute cellular environment with liposome (prepared by reverse emulsion technique) for studying Aβ aggregation and its propagation. This system provides the best platform for studying the Aβ aggregation in cellular like environment, interaction with membrane and transport from one cell to another.

Reference:

13. Abhijit Saha, Goutam Mondal, Atanu Biswas, Indrani Chakraborty, Batakrishna Jana and **Surajit Ghosh.*** In vitro reconstitution of a cellular like environment using liposome for amyloid beta peptide aggregation and its propagation. ***Chem. Commun., 2013, 49, 6119-6121.***

14. Biosensor platform for the detection of Dengue virus:

Dengue virus (DENV) is the most wide-spread arthropod-borne Flavivirus infecting annually 50-100 million humans worldwide. To date, no prophylactic vaccine or selective antiviral chemotherapy exists to prevent/treat DENV infections, which can often lead to life-threatening conditions like DHF or DSS.

Recent studies on characterization of the currently circulating dengue viruses in India suggest that these viruses may be different from those reported earlier or from other countries. For instance, the molecular probes and primer sets designed for the amplification of the non-Indian dengue viruses do not easily amplify these Indian dengue viruses. A recent study from India on the complete genome characterization of Dengue-3 has shown variations in 388 nucleotides (38 amino acids changes) from the reference strain. Therefore, we need to design unique probes to be used in our biosensor to cover these viruses and differentiate Dengue from other co-infecting viruses like Chikungunya. Considering the pandemic nature of Dengue infections throughout the country, development of label free, affordable

and easy-to-use device for point-of-care usage is an urgent need. It will certainly add great value to various stake holders in health care sector such as Industries, Pharmaceutical companies, primary health care centers like Asha workers, remote health centers and hospitals. Currently used ELISA and PCR based assays for Dengue diagnosis are expensive, time-consuming and involves maintenance of cold chain for perishable consumables like enzymes and antibodies.

To address these issues, we have jointly in collaboration with CSIR-CEERI and CSIR-NIIST developing fluorescently labelled and label free sensor platform for facile detection of the DENV virus. Our device can offer flexibility in terms of changing the probes with changing epidemiology of the target viruses more easily compared to other diagnostic approaches.

References:

14. AN EASY-TO-USE DIAGNOSTIC SYSTEM FOR RAPID DENGUE VIRUS DETECTION USING FLUORESCENCE-BASED MOLECULAR PROBE. Biswas Subhajit, **Ghosh Surajit**, Soumi Sukla, Prasenjit Mondal. Indian Pat. Appl. (2020), IN 202011019066.

Date:

Signature of the applicant