#	Name	Title
1	Anant K. Paravastu	Distinguishing oligomeric assembly pathways from fibrillar assembly for the Alzheimer's amyloid-β peptide
2	Andrea Contreras-Marciales	FKBP51 inhibition: A potential therapy to improve resilience in the tauopathic brain?
3	Ansgar B. Siemer	Intrinsic disordered domains in amyloid fibrils: their structural ensemble and chaperone interactions
4	Ayyalusamy Ramamoorthy	Effects of Gangliosides on Amyloid Aggregation of Insulin
5	Baum Jean	Mechanistic insight into α-synuclein aggregation and inhibition processes in neurodegenerative disease
6	Bernd Reif	Aggregation kinetics and amyloid fibril structure probed by solution and MAS solid-state NMR spectroscopy
7	Christian Griesinger	Protein aggregation and interference with it for treatment studied by NMR and beyond
8	Corinne Lasmezas	Targeting neuronal bioenergetics is a novel therapeutic approach for protein misfolding neurodegenerative diseases
9	Daniel Raleigh	Exploiting Evolutionary and Mechanistic Analysis to Develop Next Generation Amylin Analogs For Therapeutic Applications
10	David S. Libich	Liquid-liquid phase separation of the oncogenic fusion protein EWS:FLI1 is modulated by its DNA-binding domain.
11	Dmitry Kurouski	Lipids Determine the Toxicity of Human Islet Polypeptide Aggregates in Vivo
12	Dmitry Kurouski	The toxicities of A30P and A53T alpha-synuclein fibrils can be uniquely altered by the length and saturation of fatty acids in phosphatidylserine
13	Dmitry Kurouski	Potential Neuroprotective Transition Metal Dichalcogenide Nanoflowers Therapeutic for Parkinson's Disease
14	Ehud Gazit	Peptide-Metabolite Interactions and Metabolostasis in Amyloid Diseases
15	Erwan. Bezar	Differential pathological dynamics triggered by distinct Parkinson patient-derived α-synuclein extracts in non-human primates.
16	Eunsook Lee	REST as a new therapeutic target for neurodegenerative disorders: REST affords protection against manganese-induced neurotoxicity.
17	Gopal Thinakaran	Alzheimer's disease risk factor BIN1 in tauopathy
18	Gunnar F. Schröder	Cryo-EM studies of amyloid fibrils from mouse models
19	Holger Wille	Translating structural biology into rationally-designed vaccines for neurodegenerative diseases
20	Jennifer C. Lee	Amyloid formation of alternatively spliced variants of α-synuclein
21	Jennifer C. Lee	Water bend-libration as a Raman probe of intracellular amyloid fibrils
22	Jin Hae Kim	Elucidation of the lipid-mediated aggregation mechanism of transthyretin
23	Jin Hyung Lee	Solving brain circuit function and dysfunction with computational modeling and optogenetic fMRI
24	John E. Straub	Exploring the role of cholesterol, phase separation, and lipid rafts in the amyloid cascade and Alzheimer's disease
25	Karen Tsai	Role of Water in Directing Pathological Protein Aggregation

26	Kendra Frederick	Structural Determination of Neurodegenerative Disease-Associated Proteins Inside Cells
27	Kenji Sugase	Site-Specific Kinetic Analysis of Protein Amyloid Fibrillation Under Flow Using High-Sensitivity Rheo-NMR
28	Kenjiro Ono	Protofibrils of Amyloid-β are Important Targets of a Disease-Modifying Approach for Alzheimer's Disease.
29	Laura J. Blair	Tau's Tamers: The Role of Molecular Chaperones in Slowing Tau Accumulation
30	Lucie Khemtemourian	Insight into the cross-seeding molecular mechanism between the islet amyloid polypeptide and other amyloid forming proteins.
31	Magdalena Ivanova	Protein Aggregation: Key Challenges and Research Approaches
32	Martin Muschol	Self-assembly of amyloid plaques from monomers versus isolated fibrils
33	Martin Muschol	Using Amyloid β 42 Growth Kinetics to Identify Oligomer-Selective Dyes
34	Rakesh Kumar	Elucidation of the role of fatty acids in aggregation of amyloidogenic proteins
35	Rakez Kayed	Clearance of intracellular tau protein pathology through novel immunotherapy approaches
36	Rakez Kayed	Intrinsic Disorder in p53 and its Mutants Causes Aggregation and Inactivation of DNA Repair
37	Rezaei Human	Prion dynamics and the consequences of coexisting multiple prion conformations
38	Sapun Parekh	Quantifying amyloid polymorphism with microscopy and spectroscopy
39	Sophie Lecomte	Tau selectively aggregates on membranes and induces membrane damage
40	Sungsool Wi	Short- and Long-Range 2D ^{13}C-^{13}C, 13C-15N, ^{15}N-^{15}N, 1H-1H NMR Correlations in Peptide Groups Using $^{13}C/^{15}N$-Labeled and Naturally Abundant Samples
41	Takahiro Muraoka	Protein-incorporating self-assembling peptides for injured brain regeneration
42	Takahiro Watanabe-Nakayama	Single molecule observation of tau aggregation
43	Thomas Caulfield	Leveraging AI-Driven Molecular Glue Design for Targeted Protein Degradation of Amyloid-β in Alzheimer's Disease Therapy.
44	Thomas L. Rothstein	My quarter-century of fameFAIM: from apoptosis to proteostasis
45	Vijayaraghavan Rangachari	Designer Peptides to Study the Redox-Controlled phase separation of Biomolecular condensates
46	Vijayaraghavan Rangachari	Heterotypic amyloid formation between alpha Synuclein and TDP-43
47	Yan Li	Immuno-metabolic regulation of brain region-specific organoids with isogenic microglia-like cells
48	Yan Li	Human brain organoid-derived extracellular vesicle therapeutics for combating neuronal cell type associated senescence
49	Conor B. Abraham	Elucidating the mechanism of recognition and binding of heparin to amyloid fibrils of serum amyloid A
50	Dana Wolfe	Investigating FAIM's Role in Insulin Aggregation: Mechanisms of Inhibition in Protein Aggregation Disorders
51	Jhinuk Saha	Non-micellar lipids influence insulin aggregation pathways

52	Samuel D. McCalpin	Modulation of the aggregation and toxicity of islet amyloid polypeptide by ganglioside lipids

1. Distinguishing oligomeric assembly pathways from fibrillar assembly for the Alzheimer's amyloid-β peptide

Yuan Gao, Alicia S. Robang, Ramesh Prasad, Tarunya Rao Sudarshan, Peter S. Randolph, Jens O. Watzlawik, Cong Guo, Shirin Kamalaldinezabadi, Scott M. Stagg, Huan-Xiang Zhou, Terrone L. Rosenberry, & Anant K. Paravastu
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I will present a structural model of a 32-mer oligomer of the 42-residue variant of the Alzheimer's amyloid-β peptide. This model is based on structural constraints obtained from a combination of solid-state nuclear magnetic resonance (NMR) and cryo-electron microscopy (EM) measurements. This oligomer is "off-pathway" to fibril formation. We will use our structural model and results of pathways-probing experiments to discuss why the oligomer and fibrillar assembly differ.

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2. FKBP51 inhibition: A potential therapy to improve resilience in the tauopathic brain?

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Tau protein aggregates in the brain are the primary feature in a group of progressive neurodegenerative disorders known as Tauopathies, including Alzheimer's disease (AD). In human AD brain tissue, the 51 kDa FK506-binding protein (FKBP51), a co-chaperone of the Hsp90 complex involved in stress response regulation, is upregulated. We demonstrated that FKBP51 overexpression in tau transgenic mice promoted the accumulation of toxic tau oligomers. Furthermore, FKBP51 KO mice showed lower phosphorylated and total tau compared to wild type (WT) mice. Based on this, we hypothesize that FKBP51 inhibition will provide resilience in the brains of tau transgenic mice. To test this, we evaluated the effects of a pharmacological inhibitor of FKBP51, SAFit2, on tau levels and cognitive activity in PS19 tau transgenic mice. Male and female 7.5-month-old PS19 and WT mice were i.p. injected twice a day for 28 days with 10 mg/kg of SAFit2 or vehicle (n = 12 mice/treatment/genotype/sex). Two weeks into the treatment, blood serum was collected before and after acute tube restraint stress, to measure the potential protective effects of SAFit2 on corticosterone levels after stress, assessed by ELISA. The final week of treatment, Open Field Test, Novel Object Recognition, Tail Suspension Test, and Radial Arm Water Maze with Reversal were performed for cognition and affective-like behaviors. Finally, brain tissue and blood serum were collected 1 hour after the last treatment. Behavioral and tissue analyses are currently ongoing. According to our preliminary data, we expect to find that chronic SAFit2 treatment induces stress-resilience and lowers phospho- and total tau levels. We also expect to see an improvement in cognitive skills of SAFit2-treated compared to vehicle-treated PS19 mice, while no changes are expected in the WT controls. This study will help clarify the role of FKBP51 inhibition as a possible therapeutic intervention for the treatment of tauopathies.

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3. Intrinsic disordered domains in amyloid fibrils: their structural ensemble and chaperone interactions

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In the past decades, structural biology of amyloid fibrils has mostly focused on their relatively ordered cross-β core. However, most amyloid fibrils contain large intrinsically disordered regions (IDRs) adjacent to their core. Depending on their size, these IDRs can become the dominant feature of the fibril surface mediating the interactions of these fibrils with other molecules such as chaperons and antibodies.

Results from us and others have highlighted the importance of the IDRs for protein-amyloid interaction resulting in a need to determine their conformational distribution and understand how this distribution is affected by fibril formation and fibril core conformation. Therefore, we are developing a combined NMR, EPR, and MD simulation approach in which conformational ensembles from MD simulations will be benchmarked using key experimental data. Finally, I will present data showing the binding of the co-chaperone DNAJB1 to IDRs of amyloid fibrils and how fibril-type specific this interaction is.
REFERENCES:

4. Effects of Gangliosides on Amyloid Aggregation of Insulin

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Amyloid aggregation of insulin is a key concern in Type-2 Diabetes (T2D), contributing to complications such as tissue necrosis, infections, and reduced drug efficacy. While gangliosides, glycosphingolipids with sialic acid residues, are known to influence amyloid formation in neurodegenerative diseases, their role in insulin aggregation remains underexplored. This study investigates the effects of GM3 and GD3 gangliosides on the amyloid aggregation of insulin. We employed Thioflavin T (ThT) fluorescence assays, Fourier Transform Infrared Spectroscopy (FT-IR), Circular Dichroism (CD), and Transmission Electron Microscopy (TEM) to characterize the aggregation kinetics, structural transitions, and morphological features of insulin aggregates in the presence of these gangliosides. Results revealed that GM3 and GD3 accelerated insulin aggregation, producing short, beaded aggregates distinct from the fibrils formed in lipid-free conditions. CD and FTIR analyses indicated that gangliosides preserved or promoted alpha-helical structures, contrasting with the beta-sheet-dominated aggregates observed otherwise. This work highlights the role of gangliosides in modulating amyloid polymorphism and provides insights into their potential impact on T2D treatment strategies. Future studies will focus on the molecular mechanisms underlying these interactions and the toxicity of lipid-induced aggregates.
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5. Mechanistic insight into α-synuclein aggregation and inhibition processes in neurodegenerative disease

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The seeding of alpha synuclein (α-Syn) fibrils is key to disease progression of various synucleinopathies including Parkinson's disease (PD). Understanding the mechanism of inhibition for fibril seeding is critical to the development of therapeutics against disease progression. Using an integrative approach, including solution NMR experiments, liquid high-speed video-rate scanning (VRS) AFM and TEM, we demonstrate that the disordered C-terminal flanking regions of the fibril play a critical role in monomer recruitment in the α-Syn fibril seeding process. Furthermore, we highlight the role of chaperones, in particular HtrA1, as inhibitors of αS aggregation and as a possible therapeutic approach against disease progression.
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6. Aggregation kinetics and amyloid fibril structure probed by solution and MAS solid-state NMR spectroscopy

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Systemic antibody light chains (AL) amyloidosis is characterized by deposition of amyloid fibrils derived from a particular antibody light chain. It has been shown that soluble oligomeric protein has a direct cytotoxic effect on cardiomyocytes prior to protein aggregation and organ malfunction. Removal of circulating pathogenic light chains by chemotherapy yields a drastic reduction of the concentration of biomarkers reporting on cardiac dysfunction.
Solution and MAS solid-state NMR experiments are used to characterize the aggregation of a particular patient protein. The focus is put on a human protein sequence for which adipose and heart tissue material is available from a patient. It is shown that ex vivo material allows to reproduce the amyloid fibril structure in vitro by employing a seeding procedure. MAS solid-state NMR experiments yield information on the conformation of the amyloidogenic core and allow to probe interactions with small molecules that potentially interfere with the aggregation process. Using solution-state NMR spectroscopy, we follow the individual steps involved in protein misfolding at atomic resolution. We show that the natively folded protein first partially unfolds, before it converts into a high molecular weight molten globule like structure. Oligomer formation implies high local concentrations of aggregation prone regions which catalyze the subsequent conversion into amyloid fibrils. We show that the topology of the aggregated state is determined by balanced electrostatic interactions in the core of the fibril, resulting in an anti-parallel arrangement of the β-sheets around the conserved disulfide bond.
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7. Protein aggregation and interference with it for treatment studied by NMR and beyond

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We have studied the process of aggregation of a-synuclein and Ab on membranes in vitro and identified key time points in the aggregation process, that enable targeted isolation of a so called intermediate I and the fibrillar endpoint (1). Intermediate I has the characteristics of a toxic oligomer and the structure and stoichiometry will be presented (2). In addition, we determined the structure of lipidic Ab fibrils in the absence (3) and presence of anle138b (4) and will compare with the structures determined for a-synuclein in the past. (5). Comparison of the binding site of anle138b with compounds that bind even tighter to α-synuclein fibrils and might therefore be useful for diagnostics will be discussed.

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8. Targeting neuronal bioenergetics is a novel therapeutic approach for protein misfolding neurodegenerative diseases

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Our studies on the pathogenic mechanisms of amyloid diseases uncovered that NAD deprivation is a major effector of toxicity induced by misfolded proteins. Based on these findings, we implemented a high-throughput screening campaign to discover small molecules correcting the NAD deficit induced by proteotoxic injury. We identified a compound restoring physiological NAD levels and complete cellular viability at nanomolar concentration. Structure activity relationship studies were then performed focusing on removing off-target effects while maintaining neuroprotective potency and improving drug metabolism and pharmacokinetic properties. We then tested the in vivo probe in the SOD1*G93A murine model of amyotrophic lateral sclerosis (ALS). Oral administration of our NAD restoring compound significantly increased muscle strength and motor function of ALS mice. Analysis of NAD levels in blood and brain provided proof of target engagement and restoration of brain NAD levels. In summary, our data suggest that NAD restoration is a new disease-modifying therapeutic approach for protein misfolding neurodegenerative diseases.
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9. Exploiting Evolutionary and Mechanistic Analysis to Develop Next Generation Amylin Analogs For Therapeutic Applications

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Amylin is a neuropancreatic polypeptide hormone produced and secreted by the pancreatic beta-cells. The peptide normally acts as an endocrine partner to insulin, but forms islet amyloid in type 2 diabetes. The process of amylin aggregation leads to the generation of toxic oligomeric species which contribute to b-cell dysfunction and death and to the progression of type 2 diabetes. The hormone is largely absent in type 1 diabetes, owing to the dysfunction and destruction the of b-cells. Amylin replacement therapy is an attractive therapeutic strategy for treating diabetes and there is considerable interest in the use of amylin-based therapeutics to target obesity. An analog of human amylin, pramlintide (also known as Symlin), is approved for the treatment of diabetes, but suffers from poor solubility and cannot be co-formulated with insulin. We demonstrate a rational strategy for the design of bioactive, non-amyloidogenic, non-toxic analogs of human amylin with improved solubility, based on the incorporation of a minimal number of proline and charged residues. A set of bioactive analogs that are non-amyloidogenic, activate a human amylin and human calcitonin receptor, and are not toxic to b-cells. The designed peptides are considerably more soluble than human amylin and pramlintide.

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10. Liquid-liquid phase separation of the oncogenic fusion protein EWS:FLI1 is modulated by its DNA-binding domain.

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Ewing sarcoma (EwS) is an aggressive cancer of bone and soft tissue that predominantly affects children and young adults. A chromosomal translocation joins the low-complexity domain of the RNA-binding protein EWS (EWSLCD) with the DNA-binding domain of FLI1 (FLI1DBD) creating EWS::FLI1, a potent fusion oncoprotein essential for EwS development and responsible for over 85% of EwS tumors. The self-associative properties of the EWSLCD, mediated through tyrosine residues, are essential for the aberrant behavior of EWS::FLI1 [1]. In particular, the expression of EWS::FLI1 in EwS directly interferes with the biological functions of EWS leading to excess alternate splicing events and defects in DNA-damage repair pathways [2,3]. Though the EWSLCD is capable of phase separation, a direct interaction between FLI1DBD and EWSLCD was found to enhance phase separation and drive rapid condensate coarsening. This effect was conserved for three related ETS DBDs while DNA binding blocked the interaction with EWSLCD and inhibited EWS::FLI1 phase separation. NMR spectroscopy and mutagenesis studies confirmed that ETS DBDs transiently interact with EWSLCD via the ETS DBDs "wings". Together these results provide a physical and structural explanation for the dominant-negative

effect EWS::FLI1 exerts on EWS and describes a novel mechanism of how an oncogenic protein alters the activity and physical properties of a biomolecular condensate.
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11. Lipids Determine the Toxicity of Human Islet Polypeptide Aggregates in Vivo

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The onset and progression of type 2 diabetes is linked to the accumulation and aggregation of human islet amyloid polypeptide (hIAPP) in the pancreas. Amyloid oligomers and fibrils formed as a result of such aggregation exert high cytotoxicity. Although some pieces of evidence suggest that lipids could alter the rate of hIAPP aggregation, the effect of lipids on the aggregation properties of this peptide remains unclear. In this study, we investigate the effect of sphingo-, anionic and zwitterionic phospholipids with different lengths of fatty acids on the aggregation of hIAPP. We found that anionic lipids drastically accelerate peptide aggregation, whereas this effect was substantially weaker for sphingo- and zwitterionic phospholipids. Biophysical analysis revealed that the presence of lipids resulted in substantial differences in morphology and secondary structure of hIAPP fibrils compared to the protein aggregates grown in the lipid-free environment. We also found that zwitterionic phospholipids drastically increased cytotoxicity of hIAPP aggregates, whereas this effect was less evident for sphingo- and anionic phospholipids. Our results showed that drastic differences in lipid-determined cytotoxicity of hIAPP aggregates were linked to molecular mechanisms of autophagy, exocytosis, and unfolded protein response. These findings suggest that molecular candidates that could disrupt protein-lipid interactions would allow for deceleration of the onset and progression of type 2 diabetes.
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12. The toxicities of A30P and A53T alpha-synuclein fibrils can be uniquely altered by the length and saturation of fatty acids in phosphatidylserine

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Progressive degeneration of dopaminergic neurons in the midbrain, hypothalamus, and thalamus is a hallmark of Parkinson's disease (PD). Neuronal death is linked to the abrupt aggregation of α-synuclein (α-syn), a small protein that regulates vesicle trafficking in synaptic clefts. Studies of families with a history of PD revealed several mutations in α-syn including A30P and A53T that are linked to the early onset of this pathology. Numerous pieces of evidence indicate that lipids can alter the rate of protein aggregation, as well as modify the secondary structure and toxicity of amyloid oligomers and fibrils. However, the role of lipids in the stability of α-syn mutants remains unclear. In this study, we investigate the effect of phosphatidylserine (PS), an anionic lipid that plays an important role in the recognition of apoptotic cells by macrophages, in the stability of WT, A30P, and A53T α-syn. We found PS with different lengths and saturation of fatty acids accelerated the rate of WT and A30P aggregation. At the same time, the opposite effect was observed for most PS on A53T. We also found that PS with different lengths and saturation of fatty acids change the secondary structure and

toxicities of WT, A30P, and A53T fibrils. These results indicate that lipids can play an important role in the onset and spread of familial PD.
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13. Potential Neuroprotective Transition Metal Dichalcogenide Nanoflowers Therapeutic for Parkinson's Disease

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Parkinson's disease (PD) is the largest increasing neurological disorder and is characterized by the degeneration of dopaminergic neurons in the midbrain. While the exact etiology of neurodegeneration isn't completely understood, the build-up of alpha synuclein (α-syn) protein aggregates into cytotoxic oligomers and fibrils has been heavily implicated. Presence of aggregates induces cellular stress, mitochondrial dysfunction, disruptions in signaling pathways, and eventually cell death. Current treatments for PD are limited in that they are only used to mitigate symptoms, but none are capable of preventing or slowing the progression of toxic aggregates to other cells. Thus, the need for a neuroprotective therapeutic is immediate. Transition metal dichalcogenide (TMD) nanoflowers (NFs) are 2D nanomaterials that increase production of mitochondria through biogenesis, decrease measured cellular stress levels, and improve mitochondrial health via gene regulation. The exact molecular mechanisms in which molybdenum disulfide (MoS2) and molybdenum diselenide (MoSe2) enact their neuroprotective properties are currently under intense research. TMD NFs have shown no cellular toxicity, an ability to decrease reactive oxygen species, and increase mitochondrial health in both healthy cells and modeled PD cells impregnated with α-syn, making them a promising neuroprotective treatment for PD.
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14. Peptide-Metabolite Interactions and Metabolostasis in Amyloid Diseases

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The ability of metabolite to form amyloid-like structures was observed with handful of metabolites that are associated with inborn error of metabolism disorders (IEMs). The formed structures share biological, chemical and physical properties with protein and peptide amyloids including ultrastructural morphology, the binding to indicative dyes, mechanism of assembly, auto fluorescence, membrane interactions, cytotoxicity, inhibition by polyphenols, piezoelectricity and more [1]. The pathological relevance of the assemblies is reflected by their occurrence as deposits post mortem and the production of specific antibodies towards these assemblies in patients. Furthermore, specific polyclonal antibodies could be raised by immunization of rabbit with metabolites assemblies and monoclonal antibodies that neutralize the toxicity of the assemblies are produced by hybridoma techniques. The occurrence of metabolite amyloids in patients with genetic disorders who have metabolite level of orders of magnitude higher than individual of normal metabolism is gaining further support [2]. More recently we have been interested in interactions of these assemblies with protein amyloids as metabolomics studies suggested abnormal concentrations of metabolites that is associated with age-related classical amyloid-diseases [3]. We could clearly see the co-localization of homocysteine and β-amyloid

in animal models of Alzheimer's disease [3]. The interplay between metabolite and proteins/polypeptides in the case of age-related disease prompted us to the development of the concept of metabolostasis (the homeostasis of metabolites) in its role in heath and disease [4]. Finally, our study of metabolite assembly was translated into the development of drug candidate for the treatment of metabolite amyloid disease [5]. We demonstrated that the inhibition of metabolite assembly in model animals of phenylketonuria significantly improved the cognitive and motoric performance of these animals [5].

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15. Differential pathological dynamics triggered by distinct Parkinson patient-derived α-synuclein extracts in non-human primates.

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The presence of α-synuclein (α-syn) aggregates, such as Lewy bodies in Parkinson's disease (PD) patients, contributes to dopaminergic cell death. Injection of PD patient-derived α-syn in non-human primates has illustrated the exquisite vulnerability of primate dopaminergic neurons. Here, we aimed to elucidate the temporal and spatial pathological changes induced by two distinct α-syn pathogenic structures, different in size (large or small). To unravel the underlying molecular pathways, we conducted a proteomic analysis of the putamen and the entorhinal cortex, two brain regions carrying significant α-syn pathology. We demonstrate that distinct assemblies of α-syn aggregates drive unique pathogenic changes that ultimately result in a comparable extent of nigrostriatal degeneration at the level of nigral dopaminergic neuron cell bodies and striatal dopaminergic terminals. More broadly, our findings identify pathogenic trajectories associated with large or small α-syn aggregates, suggesting the existence of several possible concomitant pathogenic routes in PD, hence questioning the quest for unique polymorphs for developing therapeutic strategies.

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16. REST as a new therapeutic target for neurodegenerative disorders: REST affords protection against manganese-induced neurotoxicity.

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Recent emerging evidence reveals that dysregulation of transcription factor RE1-silencing transcription factor/neuron-restrictive silencer factor (REST/NRSF) is linked to Alzheimer’s disease (AD) and Parkinson’s disease (PD). We investigated the role of REST in dopaminergic neurotoxicity induced by environmental neurotoxin manganese (Mn), causing neurological disorders with PD-like symptoms, as well as cognitive impairment which may contribute to the development of AD. Therefore, we focused on dopaminergic toxicity by investigating if REST modulates Mn toxicity using in vitro neuronal cultures

and in vivo mouse models. Our findings reveal that Mn decreased REST expression along with its toxicities, such as oxidative stress and apoptosis, while REST overexpression attenuated Mn-induced toxicities in Cath. -a-differentiated (CAD) neuronal cells. In addition, REST increased the transcription of tyrosine hydroxylase (TH), a rate-limiting enzyme for dopamine synthesis in CAD neurons, while REST overexpression attenuated the Mn-decreased TH expression. To investigate the role of REST in vivo, we focused on dopaminergic REST as Mn preferentially accumulates in the basal ganglia of the brain and causes dopaminergic toxicity. We generated dopaminergic neuron-specific REST conditional knockout (REST-cKO) mice which were exposed to Mn (330 µg, intranasal, daily for 3 weeks). REST loxP mice were used as wild-type (WT) controls. The results showed that Mn decreased REST expression in the dopaminergic neuron-containing midbrain and caused behavioral deficits, such as impaired locomotor activity and motor coordination as well as novel object recognition in WT, which were further decreased in REST-cKO mice. Mn decreased dopamine levels in the nigrostriatal tissues of WT mice with exacerbation in REST cKO. At the cellular levels, Mn induced mitochondrial insults, apoptosis, and oxidative stress in WT, which were further pronounced in REST-cKO mice. On the other hand, REST restoration in the substantia nigra in the midbrain of REST-cKO mice with neuronal REST AAV vector infusion attenuated Mn-induced neurotoxicity. Given that REST appears to be critical for neuroprotection, we investigated if pharmacological agents increase REST expression. Tamoxifen, a selective estrogen receptor modulator, increased REST via Wnt signaling in CAD cells. These novel findings suggest that dopaminergic REST in the nigrostriatal pathway plays a critical role in protecting against Mn toxicity, as well as other neurological disorders associated with REST dysfunctions such as PD and AD.

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17. Alzheimer's disease risk factor BIN1 in tauopathy

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BIN1 is a major genetic risk factor for late-onset Alzheimer's disease (LOAD) and is expressed in neurons, oligodendrocytes, and microglia. BIN1 encodes an adaptor protein that regulates membrane dynamics in the context of endocytosis, recycling, and neurotransmitter vesicle release. In vitro evidence suggests that BIN1 can directly bind to tau in the cytosol. Studies in human subjects indicate that BIN1 LOAD variants increase the risk for AD by influencing tau pathology. BIN1 has been found to limit the uptake and spread of extracellular tau seeds in cultured neurons. To explore the roles of BIN1 in tau pathogenesis and tauopathy-mediated neurodegeneration in vivo, we generated conditional knockout mice in the P301S human tau transgenic background (line PS19). Intriguingly, the loss of Bin1 expression in the forebrain excitatory neurons and oligodendrocytes of PS19 mice mitigated the accumulation of pathogenic tau in select regions, including the hippocampus, entorhinal and piriform cortex, and amygdala. This reduction in pathology attenuated

hippocampal synapse loss, neuronal death, neuroinflammation, and brain atrophy. Conversely, tau pathology was worsened in the somatosensory cortex, thalamus, spinal cord, and sciatic nerve. Tau seeding experiments conducted in young PS19 mice confirmed that the loss of neuronal BIN1 expression attenuated tau spread through connected brain regions in the cortex and hippocampus. On a molecular level, the loss of forebrain BIN1 elicited complex neuronal and non-neuronal transcriptomic changes, including altered expression of neuroinflammatory genes, concomitant with an impaired transition of microglia to the disease-associated microglial phenotype. These findings indicate that BIN1 expression in the forebrain neurons promotes tau pathogenesis and neuroinflammation in the hippocampus.

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18. Cryo-EM studies of amyloid fibrils from mouse models

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A variety of mouse models are used in Alzheimer's disease (AD) research. However, little is known about the structural differences in aggregated Aβ between mouse models and humans or in vitro structures. These differences might help to understand why fibril-targeting drug candidates show efficacy when tested in mouse models but often fail to show the desired effect in clinical trials. We determined the structures of nine ex vivo Aβ fibrils from six different mouse models by cryogenic-electron microscopy (cryo-EM) [1]. We found novel Aβ fibril structures in the APP/PS1, ARTE10 and tg-SwDI models, whereas the human type II filament fold was observed in the ARTE10, tg-APPSwe and APP23 models. Interestingly, the tg-APPArcSwe mice showed an Aβ fibril structure that resembles the human type I filament found in patients with sporadic Alzheimer's disease. A detailed assessment of the Aβ fibril structure is key to the selection of appropriate mouse models for the preclinical development of novel plaque-targeting therapeutics and positron emission tomography imaging tracers in AD.

In addition to protein deposits, such as plaques in AD and Lewy bodies in α-synucleinopathies like Parkinson's disease (PD), a high concentration of lipids is also found, suggesting a potential role for lipids in disease pathology. Cryo-EM studies of Aβ40 and α-synuclein fibrils formed in the presence of liposomes have revealed novel fibril polymorphs [2,3]. These structures provide detailed insights into fibril-lipid interactions and show that the fibrils can take up significant amounts of lipids during formation, which results in lipid-decorated fibrils. This supports the notion that lipid extraction from cell membranes may be a mechanism contributing to fibril toxicity. Understanding these fibril-lipid interactions offers structural insights into disease-relevant processes in AD and PD, and sheds light on how lipid extraction during fibril formation may drive neurodegenerative pathology.

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19. Translating structural biology into rationally-designed vaccines for neurodegenerative diseases

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Many neurodegenerative diseases are caused by the misfolding of particular proteins, which, in the diseased state, adopt a disease-specific β-sheet rich conformation, often resulting in protein fibrillization, neuronal dysfunction, and ultimately death of the afflicted individual. In recent years, cryo electron microscopy and other techniques provided high-resolution structures for many of the disease-specific protein conformations. These structures allowed us to develop structure-based vaccines for the respective neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease, and the prion diseases). For this novel vaccine design approach (Wille et al., 2024), we selected only surface-exposed residues from the disease-specific misfolded protein states and inserted them in a structure-specific, discontinuous manner into an innocuous scaffold protein.

The resulting vaccine candidates were expressed in E. coli, affinity purified, refolded in vitro, and subjected to extensive quality control measures to ascertain proper folding of the antigens. The disease-specificity of the engineered antigens was confirmed by immunizing wild-type mice and testing their post-immune antisera against brain tissue samples from patients who died of the respective neurodegenerative disease. Efficacy trials are being conducted in transgenic mouse models that recapitulate many aspects of disease and have shown prophylactic protection and significant health- and life-span extensions (Flores-Fernández et al., 2024; Pesch et al., 2024). Current efficacy trials also pursue the potential for therapeutic vaccinations, which could provide substantial benefits to patients in early stages of disease.

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20. Amyloid formation of alternatively spliced variants of α-synuclein

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Intracellular accumulation of α-synuclein (SNCA) amyloid fibrils is a key feature of synucleinopathies, namely, Parkinson's Disease, Lewy body dementia, and multiple system atrophy. Through exon skipping, the gene SNCA is alternatively spliced, generating three additional variants of SNCA which are missing either exon 3 (SNCAΔ3) or 5 (SNCAΔ5), or both exons 3 and 5 (SNCAΔ3Δ5). While some studies suggest that alternative splicing is upregulated in synucleinopathies, the proteins involvement in disease etiology remain ill-defined.

To determine the effect of alternatively splicing on amyloid formation, aggregation kinetics of SNCAΔ3, SNCAΔ5, and SNCAΔ3Δ5 were examined by thioflavin-T fluorescence, and structural characterization by circular dichroism, Raman, and infrared spectroscopy as well as transmission electron microscopy were performed. All spliced variants exhibit enhanced aggregation propensity compared to SNCA (SNCAΔ3Δ5 ~= SNCAΔ5 > SNCAΔ3 > SNCA), and SNCAΔ3Δ5 and SNCAΔ5 fibrils adopted distinct morphologies. Upon co-mixing, full length SNCA aggregation is stimulated by SNCAΔ5 and SNCAΔ3Δ5 but not SNCAΔ3. Interestingly, only SNCAΔ5 fibrils were competent seeds for SNCA monomer, which is explained by their similar protease resistant core residues as determined by liquid-chromatography mass spectrometry using proteinase-K and lysosomal cathepsins. We suggest that the enhanced aggregation propensity of SNCAΔ5 and its capability in promoting SNCA amyloid formation can contribute to pathogenesis and disease progression.
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21. Water bend-libration as a Raman probe of intracellular amyloid fibrils

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Protein misfolding, aggregation, and amyloid accumulation are implicated in many neurodegenerative disorders. One example is α-synuclein, the primary amyloidogenic protein implicated in Parkinson's disease, multiple systems atrophy, and dementia with Lewy bodies. As solvation plays a crucial role in protein structure and stability, understanding interactions between water and amyloid fibrils are foundational. However, investigating water involved in biological processes is technically challenging due to the scarcity of direct probes and compatibility with cellular studies. One label-free approach is Raman spectral imaging, which reports on intrinsic molecular vibrations with the ability to obtain spatial information on water and other biomacromolecules (e.g., nucleotides, lipids, and proteins) simultaneously in a single experiment. Recently, we have demonstrated that the bend-libration of water, an environment-sensitive vibration located in a spectrally quiet region in cells, can be used as a Raman imaging probe of intracellular hydration, uncovering distinctive water characteristics in various subcellular compartments.1 In this talk, I will discuss our latest efforts in mapping the effect of α-synuclein fibril uptake on the intracellular hydration network. Remarkably, these β-sheet-rich protein assemblies are associated with more ordered water structure in the cytosol, clearly indicating the impact of amyloid inclusions on intracellular environment. Broadly, we have shown that the bend-libration of water is a robust and unique cellular Raman probe, detailing aggregation-related hydration that may give insights into cytotoxicity and disease progression.
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22. Elucidation of the lipid-mediated aggregation mechanism of transthyretin

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Transthyretin (TTR), an indispensable transporter protein of thyroxine and retinol molecules in humans, constitutes a homo-tetrameric complex in its native conformation. However, this protein is also well known for its aggregation-prone propensity, which causes amyloidopathic diseases, such as senile systemic amyloidosis and familial amyloid polyneuropathy/ cardiomyopathy. It is generally accepted that reduced stability of the tetrameric state of TTR and subsequent dissociation into the monomeric state is the primary limiting step of TTR aggregation. Monomeric TTR appears to have unstable tertiary conformation, thus being followed by misfolding and aggregation into amyloid fibrils. Several factors can compromise TTR's tetrameric stability, such as genetic mutation, low pH, increased temperature, and proteolytic cleavage. Here, we propose that lipids may also work as an important contributor for TTR aggregation. To this end, we employed

solution NMR spectroscopy and various biochemical approaches to investigate the interaction between TTR and lipid molecules. We found that a lipid micelle selectively interacts with the aggregation-competent state of TTR and stabilize a highly dynamic monomeric state. Moreover, we observed that aggregation-prone species of TTR significantly reduced the structural integrity of a liposome membrane, suggesting that the cytotoxicity of TTR aggregates may come from this interaction between monomeric TTR and lipid molecules. Taken together, we revealed the detailed interaction mechanism of how TTR interacts with lipids in its aggregation pathway. In addition, our work provided direct evidence of how TTR exhibits cytotoxicity during its aggregation pathway, which may help to develop novel therapeutic strategies to treat or prevent TTR amyloidosis.
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23. Solving brain circuit function and dysfunction with computational modeling and optogenetic fMRI

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Neurological and psychiatric disorders are dramatically increasing in prevalence due to aging population and social isolation. However, to date, there is no cure for any of the brain disorders. The goal of brain disorder treatments is to restore the brain's function. Therefore, a key challenge is to quantify the brain function underlying behavior. Once the brain function algorithms underlying behaviors of interest can be quantitatively defined, minimizing the normal and diseased brain function difference can be defined as the objective function for the brain disorder treatment. The variables then can be optimized to minimize the objective function. In order to quantify the brain function algorithms underlying behavior, cell type specific whole brain function measurements are necessary. We utilize optogenetics combined with fMRI (ofMRI) to enable such measurements. Through computational modeling of ofMRI data, the functional interactions among different regions of the brain was then quantified. In combination with electrophysiological measurements, we further model brain function at a cellular level. In order to further understand the circuit, pathology relationship, we also utilize brain clearing methods to longitudinally quantify and model pathology. Through these efforts, we aim to enable systematic design of therapeutic interventions necessary for the treatment of brain disorders.
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24. Exploring the role of cholesterol, phase separation, and lipid rafts in the amyloid cascade and Alzheimer's disease

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Cholesterol has been conjectured to play a critical role in the amyloid cascade, the aggregation of amyloid-β (Aβ) protein, and the onset of Alzheimer's Disease. The role proposed for cholesterol has typically included the formation of specific protein-cholesterol complexes. In contrast to these prior conjectures, we propose that cholesterol impacts the genesis of Aβ and the amyloid cascade indirectly by inducing liquid-liquid phase separation and the formation of liquid ordered domains. The heterogeneity created through phase separation serves to partition proteins into domains and compartments, ultimately impacting key endocytotic pathways. We explore the full process of Aβ genesis in the context of liquid ordered phases induced by cholesterol, including protein partitioning to lipid domains, mechanisms of endocytosis facilitated by lipid domains and secretases, and pH-controlled activation of secretase enzymes in specific endocytotic environments. Outstanding questions regarding the essential role of cholesterol in the amyloid cascade are identified for future studies.
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25. Role of Water in Directing Pathological Protein Aggregation

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A grand challenge in developing therapeutics for tauopathies is the inability to rationally generate tauopathy shape-selective fibrils as targets to develop therapeutic strategies. I will present a unique approach to rationally design chemically precise tau prions to build tauopathy shape-specific fibrils, relying on two novel hypotheses. The first hypothesis posits that the complex tauopathy fold in tauopathy fibrils does not emerge all at once and spontaneously, but via a critical tau segment that adopts a dominant shape and initiates in-register stacking of β-sheets. We refer to the fibrils made of such a minimal tau peptide a mini-tauopathy prion fibril. The second hypothesis posits that key to targeting tauopathy-specific fibrils lies in the topology of the activity map of the fibril end cross-section, strongly influenced by solvation thermodynamics. A dominant localized hotspot is needed to align and pin tau proteins to stack in-register to β-sheet fibrils. We posit and verify that the signature of a hotspot is localized structuring of water wrapping over a hydrophobic site. This structured water exhibits greater tetrahedrality, slower dynamics, and lower entropy compared to bulk water, and is attractive for assembly by entropy gain driven by expulsion of this water from the protein surface. Our study identifies such a binding hotspot and uncovers the binding mode and folding sequence in seeded, templated, aggregation. Ultimately, our work targets a critical bottleneck in developing treatment strategies for tauopathies: the knowledge gap to rationally generate tau fibrils adopting disease-specific fibril structures. We focus on identifying a short tau peptide segment that forms a minimal fibril and uncovering the mechanism of the prion-like action of such a fibril to recruit and misfold naive tau. The success of this study will accelerate the discovery of strategies to diagnose or halt the progression of tauopathies.

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26. Structural Determination of Neurodegenerative Disease-Associated Proteins Inside Cells

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The misfolded proteins associated with neurodegenerative disease can adopt a variety of different conformations, some of which are toxic. Because these proteins have identical amino acid sequences, the cellular environment clearly influences the final state, yet most structural studies do not include the cellular context and, perhaps because we are not studying the correct conformation, not a single therapeutic strategy for these diseases addresses the underlying protein misfolding pathology. Using DNP-enhanced solid state NMR, we study protein structure in native environments - inside living cells - to reveal how both healthy and disease-relevant cellular environments influence protein structure. Because NMR reports quantitatively, with

atomic level precision, on all sampled conformation, it can not only report on structural polymorphisms but also provide experimental restraints on regions of intrinsic disorder, complementing insights from cryo-electron microscopy and tomography. Using this approach, we found that amyloid fibrils of the protein a-synuclein were polymorphic. When these fibrils were used to seed amyloid propagation inside mammalian cells, the minority polymorph in the purified setting became the majority polymorph inside cells. Moreover, a region that was intrinsically disordered in vitro was reordered when fibrils were propagated in cellular settings. With this approach we can understand the mechanism of protein-based inheritance of amyloid aggregates and correlate phenotype with conformational ensembles.
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27. Site-Specific Kinetic Analysis of Protein Amyloid Fibrillation Under Flow Using High-Sensitivity Rheo-NMR

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The environment within our bodies is a dynamic solution with physical fluctuations such as blood flow and intracellular flow. From a fluid mechanics perspective, confined flow generates shear forces that can distort molecules. making it likely that proteins in vivo experience mechanical perturbations due to flow. Recent studies show that flow, similar to that in neurons, induces amyloid fibril formation. However, the behavior of proteins under flow, especially at the atomic level, is still poorly understood. Amyloid fibrillation, which has atomic origins, has yet to be fully captured in situ under flow due to technical limitations.
To address this issue, we developed a high-sensitivity Rheo-NMR instrument capable of measuring NMR signals while generating flow in samples [1,2]. In previous research, we successfully applied Rheo-NMR to monitor the site-specific amyloid fibrillation process of SOD1 in real time [3]. This study revealed residue-specific differences in fibrillation rates and identified residues that form the core of oligomers. However, we were unable to determine the kinetics of individual steps in the fibrillation process.
Here, we present real-time monitoring of the amyloid formation process of α-synuclein, a protein associated with Parkinson's disease, using Rheo-NMR. We determined kinetic parameters such as the primary nucleation rates of specific amino acid residues. Remarkably, the primary nucleation rate of residues in the N- and C-terminal regions, which are not part of the final amyloid fibril structure, was faster than that of other regions. In addition, by using polyethylene glycol to create α-synuclein liquid droplets, we monitored amyloid fibrillation from these droplets in real time and further elucidated the kinetic parameters by Rheo-NMR analysis.
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28. Protofibrils of Amyloid-β are Important Targets of a Disease-Modifying Approach for Alzheimer's Disease.

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The Clarity AD phase 3 trial showed that lecanemab which targets protofibril of amyloid β-protein (Aβ) reduced amyloid markers in early Alzheimer's disease (AD) and resulted in less decline on measures of cognition and function than placebo. Aβ molecules tend to aggregate and subsequently form low MW (LMW) oligomers, high MW (HMW) oligomers including protofibrils (PF), and ultimately fibrils. These Aβ species can generally form amyloid plaques implicated in the neurodegeneration of AD, but therapies designed to reduce

plaque load have not enough demonstrated clinical efficacy. We previously reported that HMW oligomers of Aβ 1-42 disturbed membrane integrity by inducing reactive oxygen species generation and lipid peroxidation, resulting in decreased membrane fluidity, intracellular calcium
ysregulation, and synaptic toxicity. Next, we have reported that lecanemab binds to PFs and surrounds them resulting in the reduction of cytotoxicity using mainly high-speed atomic force microscopy. Finally, we succeed in characterization of PF of Aβ captured by lecanemab in human cerebrospinal fluid (CSF) from living participants with AD, which enable an enhanced understanding of the dynamic changes of ecanemab-associated Aβ-PF (Lec-PF) in vivo. The CSF Lec-PF levels significantly increased in the groups of Aβ-positive with mild cognitive impairment and Aβ-positive with AD dementia compared to cognitively unimpaired Aβ-negative group. Thus, the therapeutic reduction of PFs may prevent the progression of AD by ameliorating neuronal damage and cognitive dysfunction.
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29. Tau's Tamers: The Role of Molecular Chaperones in Slowing Tau Accumulation

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Pathological aggregation of the microtubule-associated protein tau is a hallmark of Alzheimer's disease (AD) and other tauopathies. This aggregation is a significant contributor to cognitive dysfunction and neuronal loss in AD. Molecular chaperones, including heat shock proteins (Hsps), regulate aberrant tau directly and indirectly. We have systematically identified chaperones that impact tau. Our prior work focused on Hsp90 co-chaperones, the majority of which promote tau aggregation. Recently, we found that the small heat shock protein Hsp22 slows tau aggregation in recombinant assays and preserves cognitive and neuronal health in tau transgenic mice through pathways important to neuronal health. We have now completed a screen for regulators of tau seeding, which is implicated in the replication and spread of aberrant tau. Using ~50 proteins representing five major molecular chaperones families, Hsp90α, FKBP19, DnaJA2, DnaJB1, and DnaJB6b significantly affected tau seeding. Notably, DnaJB1 and DnaJB6b were also found to decrease tau levels in tau-overexpressing cell models. Inverse relationships were observed between DnaJB1, DnaJB6b levels, and tau accumulation. DnaJB6b, in particular, demonstrated potent antagonistic effects against tau aggregation in aggressive cellular models and was found to form complexes with tau. We are now investigating the effects of DnaJB6b in vivo using PS19 tau transgenic mice. So far, we have found some behavioral benefits of DnaJB6b overexpression in the tau transgenic brain and are currently working to process the tissue to understand the pathological changes. Overall, this study will shed light on the potential of DnaJB6b as a candidate therapeutic target in the context of tau accumulation.
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30. Insight into the cross-seeding molecular mechanism between the islet amyloid polypeptide and other amyloid forming proteins.

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Human islet amyloid polypeptide (hIAPP) is a highly amyloidogenic peptide found in pancreatic islets of type 2 diabetes mellitus patients (T2DM). Epidemiologic data show that T2DM patients have a higher risk to develop neurodegenerative diseases, such as Alzheimer's (AD) and Parkinson's (PD) diseases than the rest of the population. In this context, we performed biophysical studies of hIAPP in the absence and in the presence of seeds composed of other amyloid forming proteins such as the amyloid β and α-synuclein, involved in AD and PD respectively.

Our fluorescence data indicate that the kinetics of hIAPP fibril formation is accelerated by the addition of the seeds of Aβ42 and α-synuclein while the kinetics is reduced by the seeds of Aβ40 and H18R-IAPP (a weakly fibrillogenic mutated form of hIAPP). Both, electron and atomic force microscopy images reveal no difference between hIAPP fibrils and hetero-fibrils morphology. These results suggest that while the final state, the hIAPP fibrils as well as the hetero cross-fibrils have the same morphology, the mechanism of cross-fibril formation is somehow different. We thus used kinetic analysis to elucidate the aggregation mechanism of hIAPP in the presence of the seeds. Depending on the nature of the seeds (IAPP, Aβ or α-synuclein), the results converge to models in which co-aggregation is dominated either by secondary nucleation, fragmentation or saturating elongation. These results suggest that the formation of cross-fibrils involves different residues and may have different stacking shapes.

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31. Protein Aggregation: Key Challenges and Research Approaches

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The abnormal self-assembly of proteins underlies many devastating diseases, including Alzheimer's, Parkinson's, and Huntington's. Hence, understanding the molecular mechanisms of aggregation is crucial to developing effective therapies. However, gaining molecular-level insights into this process has been difficult due to the transient and diverse nature of aggregation species. High-resolution techniques, such as cryo-electron microscopy, x-ray diffraction, nuclear magnetic resonance (NMR) spectroscopy, and advanced fluorescence microscopy, have provided valuable insights into the structure and dynamics of protein aggregates. However, a significant challenge is that as the resolution of these techniques increases, so does the time and complexity required for sample preparation and data collection. Additionally, many biophysical methods rely on simplified in vitro models, and validating these in-vitro findings within complex cellular systems can be difficult. This talk will review the advantages and limitations of classical high-resolution methods used to study protein aggregation, including cryo-EM, x-ray diffraction, and fluorescence techniques. Emerging approaches to overcome current technical challenges, such as hybrid methods that integrate multiple techniques for a more comprehensive view of the aggregation process, will also be discussed.

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32. Self-assembly of amyloid plaques from monomers versus isolated fibrils

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Amyloidosis refers to a class of diseases that are associated with the build-up of amyloid plaques in disease-specific tissues. These plaques are formed by amyloid fibrils which are characterized by a cross-β sheet structure. Although the role of plaques in neurodegenerative diseases remains contentious, toxicity of huntington fibrils was shown to be noticeably reduced upon assembly into plaques. In contrast, amyloid

plaques are believed to underly the symptoms in many non-neuropathic amyloidosis. While there has been extensive work on the self-assembly of monomers into individual fibrils, little is known about the process of plaque assembly from monomers or isolated fibrils. Here we look at the salt-dependence of the super-structures formed during amyloid grown from monomers versus their self-assembly from preformed fibrils. We monitored the kinetics of fibrillar super-assemblies with Thioflavin-T (ThT) using hen egg white lysozyme (HEWL) monomers, as well as preformed and isolated HEWL fibrils. We found that increased salt concentration promoted self-assembly of isolated fibrils into plaques and correlated with an increase in ThT fluorescence. This suggests that lateral fibril assembly results in the formation of additional ThT binding sites. Alternatively, already occupied ThT binding sites become covered during lateral fibril assembly, thereby unquenching ThT fluorescence. In either case, ThT kinetics during fibril growth likely contain contributions from plaque formation in addition to those from individual fibril growth. Intriguingly, plaques formed during growth from monomers were significantly more fluorescent than those assembled from isolated fibrils at equivalent concentrations. Our data imply that secondary nucleation during amyloid growth from monomers promotes plaque formation well beyond the inherent propensity of isolated fibrils towards lateral assembly into plaques.

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33. Using Amyloid β 42 Growth Kinetics to Identify Oligomer-Selective Dyes

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Alzheimer's Disease (AD) is a neurodegenerative disease caused by the aggregation of the proteolytic protein fragment amyloid-β (Aβ). Of the two common Aβ fragments, Aβ-42 is the more aggregation-prone and disease-relevant variant. One major recent milestone was the FDA-approval of the first two drugs (aducanumab and lecanemab) for the treatment, instead of just symptomatic relief, of AD. Both drugs are believed to attack preferentially Aβ-42 oligomers. These oligomers are small, typically spherical assemblies of Aβ formed well before the emergence of amyloid fibrils. However, while there are positron-emission tomography (PET) probes for the detection of amyloid plaques in vivo, no oligomer-selective PET probes are available to monitor oligomer populations. The development of oligomer-selective assays, and PET probes in particular, is hampered by the difficulties in generating, isolating and stabilizing amyloid oligomers as binding targets.

Our lab has developed a kinetic assay for identifying fluorescent dyes selectively binding to oli-gomer vs. fibrils or plaques. Specifically, we have shown that Thioflavin-T (ThT) fluorescence traces undergo a transition from purely sigmoidal to biphasic kinetics upon the emergence of amyloid oligomers. By searching for fluorescent dyes only responsive to the initial oligomer-related phase of biphasic ThT kinetics, we have identified promising candidates for the detection of Aβ oligomers over fibrils. This kinetic assay also sidesteps the need to isolate oligomers as they are the dominant amyloid species in a well-defined time window during this assay. In our search for oligomer-selective dyes, we focused on commercially available lipophilic, DNA/ RNA binding, triphenylmethane, traditionally amyloid binding, and other environmentally sensitive dyes. Here we present a series of candidates for oligomer-selective dyes in vitro, some of which we have further evaluated for their selectivity in animal models and human brain tissues of AD.

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34. Elucidation of the role of fatty acids in aggregation of amyloidogenic proteins

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In our body, amyloidogenic proteins interact with a large number of molecules including lipids. Fatty acids (FAs) are very important components of cell metabolism and therefore, they are broadly utilized as food supplements. Using thioflavin T, we examine the effects of FAs on the kinetics of protein aggregation, while atomic force microscopy (AFM) and nano-IR spectroscopy were used to determine the topology and the secondary structure of amyloid aggregates formed in the presence of FAs. Finally, neurons were used to investigate the extent to which lipids alter the toxicity of transthyretin, amyloid β peptide, insulin and alpha-synuclein fibrils. Our results indicate that FAs exert drastically different effects on the aggregation properties of transthyretin, amyloid β peptide, insulin and alpha-synuclein.

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35. Clearance of intracellular tau protein pathology through novel immunotherapy approaches

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Pathological tau aggregates cause cognitive decline in neurodegenerative tauopathies, including Alzheimer's disease (AD). These aggregates are prevalent within intracellular compartments. Current tau immunotherapies have shown limited efficacy in clearing intracellular tau aggregates and improving cognition in clinical trials. In this study, we developed toxic tau conformation-specific monoclonal antibody-2 (TTCM2) that selectively recognized pathological tau aggregates in brain tissues from patients with AD, dementia with Lewy bodies (DLB), and progressive supranuclear palsy (PSP). TTCM2 potently inhibited tau-seeding activity, an essential mechanism underlying tauopathy progression. To effectively target intracellular tau aggregates and ensure rapid delivery to the brain, TTCM2 was loaded in micelles (TTCM2-ms) and administered through the intranasal route. We found that intranasally administered TTCM2-ms efficiently entered the brain in hTau-tauopathy mice, targeting pathological tau in intracellular compartments. Moreover, a single intranasal dose of TTCM2-ms effectively cleared pathological tau, elevated synaptic proteins levels, and improved cognitive functions in aged tauopathy mice. Mechanistic studies revealed that TTCM2-ms cleared intracellular, synaptic, and seed-competent tau aggregates through tripartite motif-containing 21 (TRIM21), an intracellular antibody receptor and E3 ubiquitin ligase known to facilitate proteasomal degradation of cytosolic antibody-bound proteins. TRIM21 was found to be essential for TTCM2-ms-mediated clearance of tau pathology. Our study collectively provides evidence of the effectiveness of nasal tau immunotherapy in targeting and clearing intracellular tau pathology through TRIM21 and enhancing cognition in aged tauopathy mice. This study could be helpful in designing effective tau immunotherapies for AD and other tauopathies.

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36. Intrinsic Disorder in p53 and its Mutants Causes Aggregation and Inactivation of DNA Repair

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It has been reported that there is excessive DNA damage, and a limited DNA damage response (DDR) in early Alzheimer's disease (AD)1. A crucial protein in the response to DDR is p53, known colloquially as 'the guardian of the genome'2.

p53 forms oligomers and fibrils, similarly to Aβ and tau1. The aggregates have been observed in cancer, along with multiple mutations that cause a loss of function and changes of conformation3. They lose function, and can gain toxic functions in vitro, and it has been shown by our lab that they are present in AD patients1.

We hypothesize that p53 aggregates are more easily formed by specific p53 mutants, and that all aggregates have a low DNA binding capacity, which, in turn, limits their ability to effectively reduce damage to the cell.

- Recombinant wild type and naturally-occurring mutant p53 proteins were made and aggregated.
- DNA probes were used to find the p53 proteins' DNA binding capacity via the use of AFM and DNA-ELISA.
- The ability of the p53 proteins to inhibit cell damage was determined by the use of comet assays in cell cultures exposed to toxic tau oligomers.

Recombinant wild type and mutant proteins were used, both with aggregates and without, in DNA binding assays. There is clear evidence of changes to DNA binding capacity, dependent upon the type of p53 protein. Moreover, mutant p53 showed a distinct decrease in its ability to prevent cell death compared to the wild type protein in comet assays.

The mutant p53 proteins, both in aggregated and non-aggregated form, show clear signs of failing to bind to DNA as effectively as the wild type. Moreover, in the comet assays, this is clearly demonstrated when they fail to preserve the cells as well as the wild type materials.

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37. Prion dynamics and the consequences of coexisting multiple prion conformations

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Prion pathology is based on the autonomous propagation of structural information, leading to single or multiple protein conformational changes. Over the past decade, the concept of prions—referring to the transmission of structural information—has been extended to various regulatory systems and pathologies, including Alzheimer’s and Parkinson’s diseases. The unified theory of prion replication involves the transfer of structural information from a prion to a non-prion conformer through a mechanism often inaccurately described, from a biophysical standpoint, as the “seeding” phenomenon. Recently, we reported that prion replication is intrinsically a source of structural diversification. The coexistence of multiple prion assemblies with distinct structures and replication propensities raises questions about how these assemblies are maintained within the same environment and how they avoid selection by the most efficient replicators. Through analysis of the dynamics of prion assembly quaternary structure, we demonstrated the existence of an exchange process within structurally diverse prion assemblies at the single-assembly level, using nanoscale infrared spectroscopy (NanoIR) and time-resolved atomic-force microscopy. This exchange process results in

damped oscillations in the kinetics of prion replication. Data assimilation and kinetic modelling led us to propose a kinetic scheme in which structurally diverse prion assemblies catalytically exchange material. According to this scheme, catalytic depolymerization competes with catalytic conformational changes. The observed exchange process within structurally diverse prion assemblies causes the system to behave as a complex, interacting network. Additionally, the competition between catalytic depolymerization and conformational change suggests a balance that could modulate the
fragmentation process, potentially playing a significant role in tissue spread and neuroinvasion.
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38. Quantifying amyloid polymorphism with microscopy and spectroscopy

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Amyloid polymorphism is important in neurodegenerative and metabolic disease. Understanding fibril molecular polymorphism is critical to understanding why certain protein aggregates made from the same protein are pathogenic while others are benign. Amyloid polymorphism is typically studied with electron microscopy and NMR. Here, we introduce super resolution far-field microscopy of amyloids using Thioflavin T, a common amyloid probe, which allows nanoscale imaging of amyloid fibrils in situ. Combined with vibrational spectroscopy and functional experiments, we characterize two polymorphs of insulin, as model system, induced by a thermal change in the nucleation (lag) phase. Both amyloid strains predominantly exhibit β-sheet structures; however, the cold-induced fibrils (formed under a 2-hour cold (4 °C) shock) display a more heterogeneous structure compared to the more uniform content in the conventional warm fibrils (formed at 37 °C the whole time). These structural variations highlight the complexity of the nucleation and growth mechanisms, with cold fibrils potentially trapped in non-equilibrium states due to a higher nucleation barrier. We find that the functional activity of cold / warm fibrils is unique in in vitro seeding / amyloid propagation, stability against inhibition, and cellular cytotoxicity. Our results show how amyloid polymorphs could differentially modulate pathogenicity. Moreover, the importance of environmental conditions on the molecular features of amyloid nucleation may be critical for design and stability of future protein biologics beyond therapeutic insulin.
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39. Tau selectively aggregates on membranes and induces membrane damage

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Tau is an amyloid protein implicated in various diseases collectively known as tauopathies, including Alzheimer's disease and frontotemporal dementia. In pathological conditions, tau can disassemble from microtubules and accumulate in the cytosol of neuronal cells, leading to the formation of amyloid fibers. The precise mechanism underlying tau pathogenicity remains elusive.
Previous investigations have highlighted critical aspects: (i) tau's tendency to aggregate into fibers [1] or bind [2] when interacting with negatively charged lipids, (ii) its ability to form structured species upon contact with anionic membranes [3], and (iii) the potential disruption of the membrane upon tau binding [4].
In this wook, we examine the disease-associated P301L mutation of the 2N4R isoform of Tau and its effects on phosphatidylcholine (PC) and phosphatidylserine (PS) lipid bilayers mimicking the inner neuronal membrane. To address this, we have combined polarized ATR-FTIR (Fourier-transform infrared in attenuated total reflection), plasmon waveguide resonance (PWR) and atomic force microscopy (AFM) real time imaging to characterize tau-membrane interactions.
Our findings reveal that the Tau protein can induce damage to both PC and PS lipid bilayers, albeit through seemingly distinct mechanisms. Tau exhibits a robust interaction with anionic lipid membranes, resulting in bilayer disruption followed by the accumulation of protein in various aggregates, from flat "carpet"-like patches to fibrillary structures reminiscent of amyloids [5]. In contrast, Tau's interaction with zwitterionic

bilayers is influenced by their fluidity. This study deepens our understanding of Tau's multifaceted interactions with lipids, shedding light on its role in tauopathies and the potential mechanisms underlying its membrane-related toxicity.

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40. Short- and Long-Range 2D ^{13}C-^{13}C, 13C-15N, ^{15}N-^{15}N, 1H-1H NMR Correlations in Peptide Groups Using $^{13}C/^{15}N$-Labeled and Naturally Abundant Samples

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We employ advanced solid-state NMR techniques to obtain two-dimensional (2D) ^{13}C-^{13}C, ^{13}C-^{15}N, ^{15}N-^{15}N, and ^{1}H-^{1}H dipolar correlations within peptide groups, utilizing $^{13}C/^{15}N$-labeled samples to explore biomolecular structure. For samples with natural isotopic abundance (i.e., without ^{13}C or ^{15}N labeling), we use dynamic nuclear polarization (DNP) to enhance ^{13}C and ^{15}N NMR signals, significantly increasing sensitivity. Central to our approach is the innovative dipolar recoupling scheme, Adiabatic Linearly FREquency Swept reCOupling (AL FRESCO), which enables homonuclear ^{13}C-^{13}C, ^{15}N-^{15}N, and ^{1}H-^{1}H correlations over extended dipolar distances at arbitrary magic-angle-spinning (MAS) rates. We demonstrate the effectiveness of these techniques using the model protein GB1 labeled with $^{13}C/^{15}N$, as well as unlabeled bone tissue samples and fungal cell walls from Aspergillus and Candida species.

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41. Protein-incorporating self-assembling peptides for injured brain regeneration

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We are developing peptide materials that promote regeneration and functional recovery of the brain, a tissue with poor regenerative capacity. Cerebral infarction is a disease that prevents patients from returning to society due to aftereffects such as paralysis of limbs. Currently, thrombolytic therapy and thrombus retrieval therapy are available for cerebral infarction, but they must be started within 4 hours and 8 hours, respectively, and the number of patients eligible for these therapies is limited. We conceived the idea of developing a material that would enable single-dose treatment of subacute cerebral infarction at one week after onset, when no treatment has yet been established. We designed a self-assembling material that slowly releases vascular endothelial growth factor (VEGF) in the in vivo environment, based on the idea that vascular regeneration is effective for functional recovery from cerebral infarction. A supramolecular gel was constructed using self-assembling peptide JigSAP, which has no chemical cross-links and is composed entirely of natural amino acids, and VEGF was immobilized inside via non-covalent bonds. The self-assembling peptide JigSAP forms a fibrous structure with cell-adhesive properties and functions as an artificial extracellular matrix. VEGF immobilized inside the gel was released from the gel over a week, and administration of the VEGF-immobilized JigSAP gel into the brain of mice one week after the onset of cerebral infarction was shown to promote angiogenesis and inhibit neuronal cell death in the brain injury periphery, as well as improve gait function in a mouse model of subacute cerebral infarction. We further demonstrated that the administration of the drug in the brain of mice after 1 week of infarct onset promoted angiogenesis and inhibited neuronal cell death in the brain injury periphery. We also found that administration of self-assembling peptide material

presenting cadherin, a cell adhesion factor that promotes migration of newborn neurons, restored neurological function. We will present a self-assembling peptide material that functions as a scaffold to enable regeneration and functional recovery of neural tissue through sustained release of growth factors and fixation of cell adhesion factors.
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42. Single molecule observation of tau aggregation

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Amyloidogenic proteins associated with various diseases form aggregates and fibers with diverse three-dimensional structures, even within the same protein species. Fiber structures differ according to the type of disease and its symptoms. It has also been shown that the introduction of aggregates of different three-dimensional structures into animals leads to different symptoms. Amyloid aggregation is correlated with structure and disease. Therefore, understanding the molecular mechanisms underlying the aggregation process is essential for elucidating pathology. However, many amyloidogenic proteins are intrinsically disordered, and different types of aggregating molecular species coexist, making it difficult to elucidate the structural dynamics of the aggregation process. In this study, we used high-speed atomic force microscopy (high-speed AFM) to analyze the structural dynamics of tau aggregation. High-speed AFM can observe structural changes in individual aggregate molecules, including intrinsically disordered regions (IDR), in real time, even when there is a mixture of various types of aggregates. We observed the dynamics of the IDR of tau and the formation of aggregates. We found that tau monomers are string-like IDRs with a partially folded region. We also found that amyloid fibrils have a dense distribution of IDR (fuzzy coat) around the folded fibril core. When the IDR was removed, the fibers bound together to form large aggregates. This indicates that IDR prevents fibers from binding together. We observed the elongation process of the fibers after the addition of monomers and found that elongation was faster at one end of the fibers, indicating that there was polarity in the elongation of the fibers.
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43. Leveraging AI-Driven Molecular Glue Design for Targeted Protein Degradation of Amyloid-β in Alzheimer's Disease Therapy.

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A persistent challenge in drug development is the limited accessibility of deep binding pockets on many targets, rendering them "undruggable" by traditional small-molecule inhibitors. Amyloid-β (Aβ), whose aggregation is implicated in Alzheimer's disease (AD), exemplifies such "undruggable" targets. Eliminating Aβ aggregation is crucial to potentially slow or halt AD progression. One innovative approach addressing this challenge is "targeted protein degradation," which aims to completely eliminate these pathogenic proteins. This strategy leverages the proteasome, the cell's intrinsic degradation machinery, to recognize and degrade tagged proteins, effectively recycling them within the cell.
During ubiquitination, E3 ubiquitin ligase labels a target protein with ubiquitin, marking it for proteasomal degradation. However, not all proteins naturally interact with E3 ligases, complicating this essential degradation pathway. To overcome this limitation, we are developing small-molecule drugs known as "molecular glues" to facilitate and stabilize the interaction between proteins and enzymes. Molecular glues act as connectors between two proteins that would not normally interact.

While discovering and developing molecular glues presents unique challenges, this modality holds significant potential. We are utilizing artificial intelligence-driven tools to design molecular glues that induce productive interactions between changes proteins. Specifically, molecular glues must bind one protein and induce conformational that enable its association with another protein, such as E3 ubiquitin ligase.
In this project, we propose the use of an AI model based on a deep neural network to identify potential molecular glues that can promote the interaction between Aβ protein and E3 ligase, altering the fate or function of Aβ. This approach will allow us to predict binding affinities and select E3 ligases with optimal efficacy in degrading Aβ targets, which could offer a promising avenue for AD treatment. Additionally, we aim to build AI-generated molecular glue libraries targeting Aβ, prioritizing E3 ligases through insights gained from structural biology and high-throughput screening data.
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44. My quarter-century of fameFAIM: from apoptosis to proteostasis

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Many neurodegenerative diseases are associated with accumulation of mutant, misfolded proteins that form aggregated, insoluble deposits. It has been suggested that an agent capable of preventing protein aggregation and/or disassembling protein aggregates might ameliorate these diseases. We identified FAIM as such an agent. FAIM is a 20kDa highly evolutionarily conserved protein that is widely expressed and was originally cloned as an inhibitor of Fas apoptosis (Fas Apoptosis Inhibitory Molecule) [1]. An alternatively spliced longer form is expressed primarily in the brain [2]. Beyond apoptosis, FAIM protects cells and mice from stress-induced loss of viability accompanied by reduction of intracellular protein aggregates [3]. We extended this in in vitro studies where we showed FAIM inhibits de novo protein aggregation, and disassembles preformed aggregates, of aggregation-prone proteins including Amyloid-β-42, SOD1-G93A, and ï¡-synuclein-A53T [4]. FAIM manifests a unique, double clamshell structure that contains no known functional motifs and ATP is not required for activity. To determine the mechanism by which FAIM opposes protein aggregation, mutational analysis to correlate structure and function is being carried out. However, renewed focus on the toxicity of oligomers has injected a note of caution toward agents capable of disaggregating fibrils. To address this, we obtained HEK cells modified to read-out aggregation of endogenous tau monomers. We then transfected these cells with the products of preformed tau fibrils disaggregated by FAIM. It was reported that disaggregation of tau fibrils with HSP70 machinery plus ATP yields products that, like fibrils themselves, induce aggregation of monomeric tau in HEK cells [5]. In preliminary experiments, we found disaggregation of tau fibrils with FAIM (FAIM-L>FAIM-S) yielded products that did not induce tau aggregation in HEK cells. Thus, FAIM appears capable of reducing tau fibrils in vitro without producing pro-aggregation byproducts. This supports further study of FAIM mechanism through structure/function analysis.

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45. Designer Peptides to Study the Redox-Controlled phase separation of Biomolecular condensates

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Biomolecular condensates (BC) are phase-separated hubs predominant with proteins and nucleic acids. Due to their increasing prevalence in biological functions, the mechanisms underlying the formation of BC have recently attracted a lot of attention. BCs are predominantly formed by liquid-liquid phase separation (LLPS), in which proteins demix from the bulk solution to form dense and dilute phases. This reversible phase separation process underlies the formation of membraneless organelles in cells. LLPS is best described by a "stickers and spacers" model in which weak multivalent interactions between the stickers separated by the disordered spacers drive phase separation. Although significant progress has been made over the past decade, the specific contribution of cysteine residues to the formation and regulation of biomolecular condensates remains unclear. In this study, we explored the role of disulfide bonds and their redox responsiveness in the behavior of biomolecular condensates. We investigated this phenomenon with designed phase-separating peptides containing cysteine residues at different positions. Our biophysical analysis revealed that cysteine residues facilitate LLPS under oxidizing conditions through disulfide crosslinks. These crosslinks can be modulated via redox reactions, offering tunable control over condensate behavior. We found that rather than acting as spacers or stickers, cysteines work as covalent nodes to prevent system-spanning percolation networks and reduce the effective concentrations for sticker interactions. This research aims to address the gap by investigating the influence of cysteine-mediated interactions on condensate dynamics, particularly in oxidative stress and other cellular environmental changes, and to develop tunable biomaterials. We believe this work provides insights into the fundamental understanding of the role of cysteine on the mechanisms underlying the formation and regulation of condensates relevant to both cellular biology and biomaterials science.

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46. Heterotypic amyloid formation between alpha Synuclein and TDP-43

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In recent years, significant clinical and pathological overlaps have been observed in many neurodegenerative disorders like frontotemporal lobar degeneration (FTLD), Parkinson's disease (PD), Lewy body dementia (LBD), and Limbic age-related TDP_43 proteinopathies (LATE). Specifically, co-amyloid deposits of proteins α-synuclein (αS), andTDP-43 and its pathologic prion-like c-terminal fragment (PrLD), among others. Our research explores the interplay between these proteins, focusing on heterotypic aggregation and liquid-liquid phase separation (LLPS). We demonstrated that αS and TDP-43PrLD synergistically promote aggregation of each other and form heterotypic fibrils containing stoichiometric proportions of the two proteins. Such hybrid fibrils show enhanced neurotoxicity and synaptic dysfunction than their homotypic counterparts. By a multi-

disciplinary approach involving biophysical, biochemical, structural biological, and cell biological methods, we uncover interactions to propose αS-TDP-43PrLD hybrid fibril as possible distinct entities in a multitude of pathologies. The distinct aggregation pathways observed in heterotypic vs. homotypic systems also suggest potential therapeutic targets for modulating protein interactions, offering new hope for the future of neurodegenerative disorder treatments.
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47. Immuno-metabolic regulation of brain region-specific organoids with isogenic microglia-like cells

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Brain organoids are self-assembled three-dimensional aggregates generated from induced pluripotent stem cells (iPSCs) with cell types and cytoarchitectures that resemble the embryonic human brain. However, brain organoids originate from ectoderm lineage, they lack cell types originate from other derm lineage, such as the mesoderm-derived microglia. Microglia are resident immune cells in the central nervous system. Like brain organoids, microglia can be differentiated from iPSCs, and they are named microglia-like cells (MGCs). In this study, MGCs were co-cultured with induced forebrain cortical organoids (iFCo) and induced hindbrain cerebellar organoids (iHCo). MGCs expressed microglial markers IBA1 and P2RY12, and they responded to Aβ42 and dexamethasone stimulation by regulating carbon metabolism and inflammation. The cell tracker red staining of MGCs showed that MGCs covered and penetrated into brain organoids from day 1 to day 7. The 7-day co-culture expressed neuronal markers β-tubulin III, glutamate, GABA, and synaptic markers SYN1 and PSD95, indicating a functional signal transduction. Gene expression results showed that organoids promoted IBA1 level in MGCs, and MGCs promoted forebrain marker FOXG1 level in iFCo. MGCs and co-cultures were treated with extracellular vesicles (EVs) from spent media of Alzheimer's disease (AD) patients' cells, and the existence of organoids in the co-culture showed resistance to AD by reducing Aβ and Tau pathology related gene CD2AP and inhibiting microglial activating gene TREM2, as well as mitigating pro-inflammatory genes IL-6, IL-12β, iNOS, and TNFα. This study paves the way for understanding the role of microglia and brain organoids in AD pathology and the therapeutic interventions.
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48. Human brain organoid-derived extracellular vesicle therapeutics for combating neuronal cell type associated senescence

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Extracellular vesicles (EVs) are small phospholipid bound particles derived from cells used for intercommunication with each other through the delivery of various cargo such as proteins, nucleic acid, lipids, and growth factors. EVs derived from induced pluripotent stem cells (iPSCs)) have been shown to possess anti-senescent properties due to the cargo they possess. Cell senescence occurs when cells stop dividing and undergo irreversible growth suspension. Age has been shown to play a large factor in cell senescence along with other variables such as DNA damage, telomere shortening, and stress. As cell senescence matures, changes in gene expression and EV cargo can be observed; It is thought that these changes can impact neighboring cell activity. These changes can cascade into tissue aging and age-related

diseases such as Alzheimer's and Blood Brain Barrier degradation. This study focuses on the effects senescence has on blood brain barrier cell types and the potential therapeutic effects of EVs from healthy cells. Not much is known about how differentiation of iPSCs into neural cell type affects the anti-aging properties of the EVs secreted. To induce senescence, D-galactose was utilized on multiple neural cell types differentiated from iPSCs. iPSC-derived pericytes (iPC), neural progenitor cells (iNPC) and blood vessel organoids (iBVO) had senescence induced via D-galactose at various concentrations. The EVs of Alzheimer's disease patient-derived brain organoids were also evaluated. The influences of various types of EVs on oxidative stress, inflammation, and mitochondrial disfunction were investigated in this study. This study showed a concentration dependence on D-galactose to influence common senescent genes Sirt-1 and Sirt-3 activity, reactive oxidative stress, metabolic activity, inflammation and apoptosis. This study also tests the anti-aging ability of the respective EVs produced from multiple neural differentiations from iPSCs relative to undifferentiated iPSC-EVs. The results show that differentiation path, EV dosage and cargo all serve as key factors in the anti-aging effects of differentiated iPSC-EVs. This study has significance in understanding key markers that affect aging and neurogenerative diseases that can occur as a by-product as well as development of potential non-invasive EV therapeutics.

49. Elucidating the mechanism of recognition and binding of heparin to amyloid fibrils of serum amyloid A

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Amyloid diseases feature pathologic deposition of normally soluble proteins and peptides as insoluble fibrils in vital organs, which co-deposit with various non-fibrillar components including heparan sulfate (HS), a glycosaminoglycan that promotes amyloid formation in vitro for many unrelated proteins. We exploit the high-resolution cryo-EM structures of the highly homologous ex vivo murine (PDBID: 6DSO) and human (PDBID: 6MST) SAA fibrils in a computational study employing molecular docking, Brownian dynamics simulations, and molecular dynamics simulations to elucidate how heparin, a highly sulfated HS mimetic, recognizes and binds to amyloid protein fibrils. Our results demonstrate that heparin binds to linear arrays of uncompensated positively charged basic residues along the spines of amyloid fibrils facilitated by electrostatic steering. This work has been submitted and is currently under review in *Biochemistry*.

50. Investigating FAIM's Role in Insulin Aggregation: Mechanisms of Inhibition in Protein Aggregation Disorders

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Protein aggregation is a hallmark of several metabolic and neurodegenerative diseases, including type 2 diabetes mellitus, where insulin aggregation can disrupt cellular function. Recently, the Fas Apoptotic Inhibitory Molecule (FAIM) has emerged as a potential modulator of protein aggregation, yet its effects on insulin fibril formation remain unclear. This study investigates the role of FAIM in insulin aggregation, hypothesizing that FAIM inhibits insulin fibrillation through mechanisms that stabilize insulin's native conformation and prevent misfolding.

Experiments were conducted to monitor insulin aggregation with and without FAIM over 48 hours. Using Thioflavin T (ThT) fluorescence assays, transmission electron microscopy (TEM), circular dichroism (CD), Fourier-transform infrared spectroscopy (FTIR), and solution-state NMR, we evaluated FAIM's impact on the kinetics and structural characteristics of insulin aggregates. Results from ThT assays indicated a significant reduction in fluorescence intensity in FAIM-treated samples compared to controls, suggesting an inhibitory effect on fibril formation. TEM analysis further revealed morphological differences, with FAIM-treated samples displaying fewer and shorter fibrils. CD and FTIR spectra supported these findings, showing structural integrity in FAIM-treated samples, while controls exhibited secondary structure transitions typical of amyloid formation. NMR analysis of 1D ^1H spectra provided additional insights into

molecular interactions between FAIM and insulin. These findings suggest that FAIM plays a protective role against insulin aggregation, offering insights into a potential therapeutic strategy for disorders characterized by protein aggregation. Further investigation will focus on optimizing FAIM's efficacy and exploring its potential in other aggregation-prone proteins.

51. Non-micellar lipids influence insulin aggregation pathways

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Protein aggregation, culminating in amyloid fibril formation, is a hallmark of proteinopathies such as Type 2 Diabetes (T2D), Parkinson's Disease (PD), Alzheimer's Disease (AD), and Huntington's Disease. Insulin, a peptide hormone essential for glucose uptake and blood sugar regulation, is susceptible to aggregation under various environmental conditions. In T2D, decreased cellular responsiveness to insulin often leads to its overproduction, which can trigger aggregation. Since insulin is biologically active only in its correctly folded monomeric form, aggregation reduces its bioavailability. Additionally, insulin aggregation poses challenges for T2D patients undergoing insulin therapy, occurring both at injection sites and in the bloodstream post-administration. Previous in vitro studies have shown that factors like low pH, high temperatures, hydrophobic interfaces, and mechanical agitation promote insulin aggregation. This study investigates how non-micellar anionic phospholipids, specifically DMPG, affect insulin aggregation. Thioflavin-T assays revealed distinct aggregation pathways and intermediates in the presence of negatively charged lipids. Dynamic light scattering (DLS) and transmission electron microscopy (TEM) indicated that DMPG promotes the formation of low-molecular-weight soluble intermediates, followed by larger fibrils. Solid-state NMR analysis showed that fibrils formed with or without lipids exhibit similar rigidity and minimal mobile regions. Overall, this study highlights the role of the chemical and physical properties of non-micellar lipids in modulating insulin aggregation pathways and intermediates. These insights may inform strategies to improve insulin stability and efficacy in therapeutic applications.

52. Modulation of the aggregation and toxicity of islet amyloid polypeptide by ganglioside lipids

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While amyloid formation by the islet amyloid polypeptide (IAPP) has been associated with the development of Type 2 Diabetes (T2D), a mechanism of amyloid-induced cytotoxicity is unclear. IAPP is known to bind to and disrupt anionic phospholipid membranes, so it has been proposed that the disruption of cellular membranes might contribute. However, despite ganglioside lipids being the primary anionic lipid component of the outer leaflet of mammalian plasma membranes, their interaction has not been characterized. Here, we describe a biophysical investigation of the effects of three ganglioside lipids - GM1, GM3, and GD3 - on the aggregation, conformation, and toxicity of IAPP. We found that the three lipids have similar effects, determined by the lipid:peptide ratio. At low lipid:peptide, the gangliosides catalyzed IAPP aggregation, bound to the IAPP fibrils, and increased toxicity to cells. GD3, the most negatively charged ganglioside, catalyzed IAPP aggregation most efficiently. In contrast, high lipid:peptide inhibits aggregation and stabilizes a helical conformation of IAPP. This inhibitory effect increased in potency with the size of the lipid headgroup. IAPP also altered the structure of ganglioside aggregates, promoting the formation of spherical micelles over larger worm-like micelles. In the context of these results, we speculate that gangliosides might facilitate membrane disruption and cell toxicity during T2D by IAPP via detergent-like or curvature strain mechanisms.