5th International Symposium on Pathomechanisms of Amyloid Diseases

Bordeaux, sept. 5-7th 2023

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12:40 - 14:05	lunch - 85 min	12:30 - 14:30	lunch + poster session 2		
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Ubiquitin, metal ions, and amyloids: neutral observers or malicious conspirators?	Milardi Danilo
Role of zinc and insulin on IAPP's amyloid aggregation	Ramamoorthy Ayyalusamy
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Virtual histology of Alzheimer's Disease through synchrotron-based X-ray phase-contrast imaging	Chauveau Fabien
Keggin-type polyoxometalate to guide the self-assembly of Abeta peptide	De Cremoux Lucie
AFM-IR characterization of tau fibrils and aggregates obtained with different cofactors	Feuillie Cecile
Chaperone regulation of the protein condensation and aggregation continuum in Alzheimer's disease	Gomes Claudio
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Early diagnosis and treatment of Alzheimer's disease by targeting toxic soluble Abeta oligomers	Rahimipour Shai
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P21	Amyloid aggregation of the tau protein involved in neuro-degenerative diseases	Piersson Clara
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P24	Domain-domain interactions and dimerization of the human λ-III immunoglobulin light chain FOR005 investigated by NMR spectroscopy	Sieluzycka Olga
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Invited speaker abstracts

in alphabetical order

Causes and consequences of IAPP amyloid formation in diabetes and new therapeutic approaches

Ping Cao, Annette Plesner and Andisheh Abedini

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Pancreatic islet amyloidosis by human islet amyloid polypeptide (hIAPP, also known as amylin) contributes to the dysfunction and death of insulin-producing islet beta-cells in diabetes and plays a role in the failure of islet transplants. However, whether islet amyloidosis develops in the pre-diabetic state or after the development of hyperglycemia and full-blown type 2 diabetes has been unclear. The mechanism(s) of hIAPP induced beta-cell toxicity are incompletely understood. Using mouse models, we demonstrated that islet amyloidosis begins before the development of hyperglycemia in the pre-diabetic state. Pre-diabetes is characterized by beta-cell dysregulation and impaired glucose tolerance (not beta-cell apoptosis and hyperglycemia which defines diabetes). We next critically tested the hypothesis that hIAPP-induced beta-cell dysfunction correlates with species formed in the lag phase of amyloid formation (before the accumulation of amyloid fibrils), by determining whether altering the length of the lag phase affects the duration of cellular dysfunction. We examined the rate of amyloid formation, and the onset, duration and endpoints of toxicity by wild-type and mutants of hIAPP, including the naturally occurring S20G mutation, under a range of conditions. Our data unequivocally revealed a linear correlation between the length of the lag phase of amyloid formation and the duration of toxicity towards insulin-secreting beta-cells. We showed that islet amyloidosis increases expression of the pattern recognition receptor RAGE in *in vitro* and *in vivo* models of pre-diabetes and human type 2 diabetes. We showed that binding of hIAPP oligomers to RAGE leads to beta-cell oxidative stress, inflammation, dysfunction and apoptosis. Inhibition of hIAPP-RAGE interactions significantly protected cultured cells and pancreatic islets, and transgenic mice from hIAPP toxicity. RAGE transduces signals via interaction with Diaph1 and knockout of Diaph1 protects islets from the toxic effects of hIAPP. Collectively the data argues that activation of RAGE signaling of pre-fibrillar forms of hIAPP leads to beta-cell toxicity in the pre-diabetic and diabetic state.

Single nanopore sensing to investigate protein aggregation and to develop diagnosis tool

Sébastien Balme

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Amyloid fibrils are formed through the assembly of proteins into highly ordered β-sheet structures. They play a role in various neurodegenerative conditions, including Alzheimer's and Parkinson's diseases. The aggregation mechanism, particularly during the lag phase, is not fully understood due to the lack of a method that enables continuous measurement of amyloid fibril formation and maturation through amyloid shape analysis. To address this, nanopores with low aspect ratios and high aspect ratios, specifically those with a conical shape, have been considered. The detection of protein aggregates is based on resistive pulse sensing, which involves recording the current perturbation induced by the passage of protein aggregates through the nanopore. A silicon nitride (SiN) nanopore with a low aspect ratio is suitable for detecting and sizing small oligomers (n-mers) of aβ42 and α-synuclein[1]. Polymer nanopores offer stability and a suitable asymmetrical geometry for detecting protofibers[2,3]. However, their resolution and the difficulty of obtaining several single nanopores with the same diameter limit their application as a characterization method. Furthermore, functionalized nanopipettes with diameters ranging from 10 to 50 nm are more suitable for analyzing larger oligomers and protofibrils. The use of a geometric model allows for the estimation of the oligomer volume based on the current perturbation. However, for fibers, the diameter rather than the length becomes the most discriminant parameter for lengths above 50-75 nm. Another advantage of using nanopipettes is the ability to investigate the kinetics of aggregation by directly inserting monomers inside the nanopipette. By employing nanopores with different diameters, the size distribution of transient and polymorph species can be determined as a function of the incubation time, with a particular focus on the lag phase. This approach has been used for the direct characterization of pro-aggregating agents such as metal ions and pollutants, as well as the addition of preformed seeds. In the latter case, the simultaneous detection of single-molecule fluorescence bursts specific to large structures rich in β-sheet structure and the nanopore-based analysis specific to the volume of small oligomers can provide insights into the mechanism of secondary nucleation. Finally, in addition to its relevance for fundamental research, nanopipettes have been utilized in the development of Real-Time Fast Amyloid Seeding and Translocation (RT-FAST). This method enables the semi-quantitative detection of preformed amyloid seeds in a sample, opening up new possibilities for early diagnosis of neurodegenerative diseases[4].

References

(1) Abrao-Nemeir, I.; Bentin, J.; Meyer, N.; Janot, J.-M.; Torrent, J.; Picaud, F.; Balme, S. Investigation of α-Synuclein and Amyloid-β(42)-E22Δ Oligomers Using SiN Nanopore Functionalized with L-Dopa. *Chemistry, an Asian journal* **2022**, *17* (20), e202200726. DOI: 10.1002/asia.202200726. Published Online: Sep. 15, 2022.

(2) Meyer, N.; Arroyo, N.; Janot, J.-M.; Lepoitevin, M.; Stevenson, A.; Nemeir, I. A.; Perrier, V.; Bougard, D.; Belondrade, M.; Cot, D.*; et al.* Detection of Amyloid-β Fibrils Using Track-Etched Nanopores: Effect of Geometry and Crowding. *ACS sensors* **2021**, *6* (10), 3733–3743. DOI: 10.1021/acssensors.1c01523. Published Online: Sep. 23, 2021.

(3) Giamblanco, N.; Fichou, Y.; Janot, J.-M.; Balanzat, E.; Han, S.; Balme, S. Mechanisms of Heparin-Induced Tau Aggregation Revealed by a Single Nanopore. *ACS sensors* **2020**, *5* (4), 1158–1167. DOI: 10.1021/acssensors.0c00193.

(4) Meyer, N.; Janot, J.-M.; Torrent, J.; Balme, S. Real-Time Fast Amyloid Seeding and Translocation of α-Synuclein with a Nanopipette. *ACS central science* **2022**, *8* (4), 441–448. DOI: 10.1021/acscentsci.1c01404. Published Online: Feb. 23, 2022.

Insights into alpha- synuclein aggregation and inhibition mechanisms

Jean Baum

Rutgers university, USA

Towards Modelling Proteinopathies in Non-Human Primates

Erwan Bezard

IMN, University of Bordeaux, France

Proteinopathies are a group of neurodegenerative diseases characterised by the abnormal accumulation of misfolded proteins in the brain or other tissues. Examples of proteinopathies include Alzheimer's disease, Parkinson's disease, and Huntington's disease. Exposing non-human primates to the pathological landmarks of each of these diseases (Senile plaques, Tau tangles, Lewy bodies, Huntington Aggregates, etc...) leads to the (partial) reproduction of the pathology and behavioural manifestations in a disease-of-origin specific manner in wild-type non-human primates. These primates exhibit highly specific responses supporting the concept of protein strain for a given protein aggregate to lead to a specific disease. The added value of the similarities to humans, the possibility of long disease progression with complex longitudinal behavioural and imaging investigations, and the translational value of these non-human primates models will be discussed.

From Tau seeding and spreading among tauopathies: which anti-tau therapeutic strategies?

Luc Buée

Lille Neuroscience and cognition, INSERM, France

From Discovery to Design: Toward early detection of Alzheimer's disease

Valerie Daggett

University of Washington, Seattle, USA

We have been involved in the development and use of realistic atomistic computer simulations of proteins for over 30 years. Some time ago we expanded to Dynameomics, an effort to simulate the native state and unfolding process of representatives of all known protein folds, which in turn led to development of Big Data methods and databases. In parallel we have been performing simulations to characterize the conformational changes associated with amyloid formation. In so doing we discovered a novel structure adopted by amyloidogenic proteins, but not 'normal' proteins, and we proposed that it defines the toxic soluble oligomers formed en route to the nontoxic mature fibrils. As such, this structure, which we call a-sheet, represents a new target for amyloid therapeutics and diagnostics. We have designed, synthesized, and tested compounds to be complementary to this 'toxic' structure and they inhibit amyloid formation in multiple amyloid systems by specifically binding the toxic oligomers, which in turn neutralizes the toxic species, and they are being used for early diagnostics.

Deciphering amyloid formation and degradation mechanisms: Where mathematical models help

Marie Doumic

INRIA and Ecole Polytechnique, France

How amyloid aggregate or break down ? Faced with the complexity of possible mechanisms, this presentation will show how the confrontation between mathematical models and experimental data can provide answers - quantitative in some cases, qualitative in others. In particular, we focus at depolymerization experiments carried out by Human Rezaei's team, and fragmentation experiments by Wei-Feng Xue's team.

Towards a Tool for Real-Time Detection of Reactive Oxygen Species Produced by Cu Bound to Amyloid-β

Yelisetty Venkata Suseela[1], Sabyasachi Mandal [2], Thimmiah Govindaraju, [2] Peter Faller[1]

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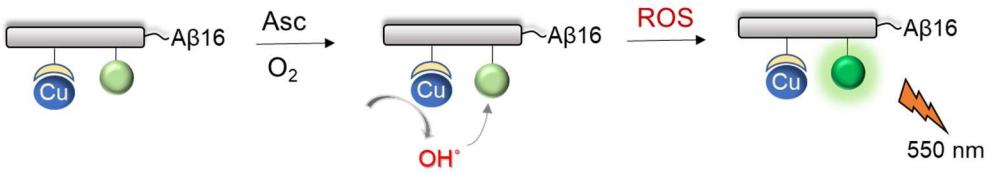
In Alzheimer's disease Cu is found accumulated in amyloid plaques mostly bound to the Amyloid-β peptides (Aβ), its main constituent. In vitro, Cu bound to Aβ can catalysis the production of ROS, such as $O_2^{\bullet-}$, H_2O_2, and $HO^{\bullet}$, in the presence of the physiological relevant reducing agent ascorbate and as O_2. It has been proposed that Cu-Aβ catalyzed ROS could contribute to the oxidative stress and neuronal cell loss observed in Alzheimer's disease. However, the direct generation of ROS mediated by Cu bound to Aβ and its contribution to the Aβ toxicity *in vivo* has not been directly demonstrated. Among ROS, $HO^{\bullet}$ radicals are of the most reactive and react rapidly with a wide variety of biomolecules and they are hence very short-lived.

The present project aims to establish a fluorescence tool to monitor the $HO^{\bullet}$ produced by the Cu bound to Aβ, but not from other potential sources. The strategy was to attach a $HO^{\bullet}$ sensor close enough to the Cu-site in Aβ, and to identify a suited fluorophore that has a turn-on response and reacts selectively with $HO^{\bullet}$ (among all the ROS). The first results with the truncated model peptide Aβ1-16 showed the proof of principle. Naphthalene monoimide (NMI) was identified as a fluorophore that is capable of probing $HO^{\bullet}$ quite selectively via a turn-on mechanism.



Scheme 1: Detection of hydroxyl radical generated catalytically by Cu bound to Aβ1-16 via a closed-by fluorophore. Reaction with the hydroxyl radical leads to a turn-on signal of the fluorophore. The proximity to the Cu-site of the fluorophore enable a quite selective detection of the short-lived hydroxyl radical produced by Cu-Aβ.

Aggregation propensity hidden in the conformation of the IDP tau

Yann Fichou

CBMN, IECB, University of Bordeaux, France

Amyloid aggregation of the intrinsically disordered protein (IDP) tau is involved a class of disease called tauopathies. Modifications in tau, such as phosphorylation and mutations, act as strong modulators of its aggregation. In particular, multiples mutations are associated with frontotemporal dementia and parkinsonism linked to chromosome 17Q (FTDP-17). We have explored the mechanisms through which diseases-associated single point mutations promote amyloid formation. We combined biochemical characterization and small angle X-ray scattering (SAXS) to study different FTDP-17 derived mutations. We found that the mutations promote aggregation to different degrees and can modulate tau conformational ensembles, intermolecular interactions and liquid-liquid phase separation propensity. In particular, we found a direct correlation between the aggregation lag time of the mutants and their radius of gyration. We show that mutations disfavor intramolecular protein-protein interactions which in turn favor extended conformations and promote amyloid aggregation. This work proposes a new connection between the structural features of tau monomers and their propensity to aggregate, providing a novel assay to evaluate aggregation propensity of tau variants.

The Role of the Metabolostasis Network in the Pathomechanisms of Amyloid Diseases

Ehud Gazit

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It was found by our groups and others that various metabolites, such as amino acids, nucleobases, vitamins, lipids, and other metabolic intermediates could form ordered assemblies with biological, chemical and physical properties very similar to amyloid made of proteins and polypeptides. This includes the similar morphology, dye-binding specificity, electron diffraction pattern and the induction of late apoptosis as protein amyloids as well as membrane binding, mechanical and optical properties. This discovery paved the way for understanding the pathophysiology of the inborn error of metabolism disorders from a new perspective, relating them to amyloid-associated diseases. Metabolites have a vital role across all kingdoms of life and their involvement in various disorders has been investigated for many decades. Many metabolites are poorly soluble in water or in physiological buffers which is consistent with their tendency to form amyloidal supramolecular aggregates. On the other hand, in the cell, they should be preserved in a pool and be readily available for the execution of biochemical functions. We thus propose that a quality-control network, termed 'metabolostasis', has evolved to regulate the storage, retrieval, and recycling of aggregation-prone metabolites. Such a system should control metabolite concentration, subcellular localization, supramolecular arrangement, and interaction in dynamic environments, thus enabling normal cellular physiology, healthy development, and preventing disease onset. Similar to proteostasis, various diseases may be related to metabolostasis impairment. The paradigm-shifting concept of metabolostasis calls for a reevaluation of the traditional view of metabolite storage and dynamics in physiology and pathology and proposes unprecedented directions for therapeutic targets under conditions where metabolostasis is imbalanced.

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 α-synuclein and Aβ 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. In addition, we determined the structure of lipidic Aβ fibrils in the absence (2) and presence of anle138b (3) and will compare with the structures determined for α -synuclein in the past. (4). 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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Recruiting α-synuclein regions to support inhibition of fibril nucleation and elongation

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Progression of Parkinson's disease and other neurodegenerative diseases is associated with the propagation of amyloid fibrils of the protein α-synuclein (αS) in the affected brain. The region comprising residues 36-56 is an interaction hotspot. We have previously shown that A) binding of this region by the engineered binding protein β-wrapin AS69 results in suppression of fibril nucleation and B) introduction of a specific intramolecular disulfide bond into this region yields the variant αSynCC that blocks fibril elongation. Here, we performed protein engineering, chemical kinetics, and AFM to determine the αS regions that contribute to these two types of substoichiometric inhibition. We generated a set of αS fusion constructs that expose different intrinsically disordered regions (IDRs) of αS. Testing the fusion constructs' effects on lipid-induced primary nucleation, secondary nucleation, and fibril elongation of αS showed that the inhibitory efficiency depends on the presence of intrinsically disordered regions of αS adjacent to the interaction hotspot. Our results provide insight into interactions at fibril ends and lateral surfaces, into fibril elongation and secondary nucleation.

Inorganic modulators of Aβ self-assembly

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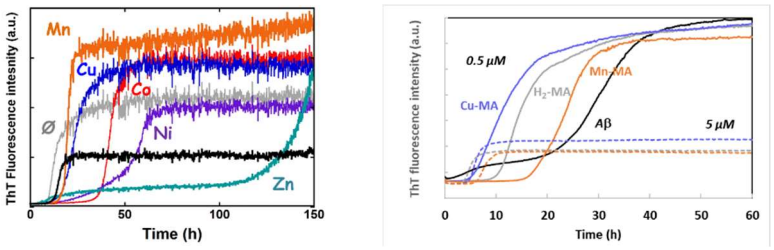
Alzheimer's disease (AD) is characterized by the extracellular deposits made of supramolecular assemblies of the amyloid-β (Aβ) peptides, in which up to mM level of metal ions, mainly copper and zinc has been found. Two Aβ-related events are involved in the etiology of AD: (i) the production of reactive oxygen species by Cu(Aβ) species [1] and (ii) the self-assembly of the Aβ peptide leading to aggregates, including amyloids.[2]

One therapeutic option is to modulate the self-assembly of Aβ towards less toxic aggregates and hence the search for modulators, beyond inhibitors, is a strategy we aim at working on.

During the talk, the effect of two synthetic inorganic modulators on Aβ self-assembly will be described and discussed. PolyOxoMetallates (POMs)[3] and cationic or anionic porphyrins,[4] including metal-substituted POM and porphyrins, have been studied. The molecular interactions that take place at an atomic level between the peptides and the polyions, which are responsible for such modulatory effects, have been deduced from spectroscopic studies including NMR, UV-Vis and fluorescence. The morphologies of the formed aggregates and their interactions with the polyions will also be proposed from imaging microscopy and indirect titration methods.



Self-assembly of 20 μM Aβ (black line) and in presence of various stoichiometric ratio of metal-substituted Keggin POM (left) and of various porphyrins (right, MA= tetraguanidinium middle arm porphyrins, plain line: 0.5 μM, dotted line: 5 μM)

ANR-21-CE06-0030-03 and ANR-22-CE44-0002-02 are acknowledged for financial supports.

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The AD/ADRD Interdisciplinary Research Network on Biologically Active Tau Aggregate Polymorphs from Alzheimer's Disease and Related Dementias

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Aims

Tauopathies, including Alzheimer's disease (AD), are characterized by intracellular lesions composed of aggregated and post-translationally modified tau proteins. Methods for preparation and biochemical characterization of stable filaments from various tauopathies are well established. In contrast, methods for preparation of soluble oligomeric tau aggregates have not been standardized to the same degree. Additionally, the structures of oligomers which associate most closely with tau toxicity, are not fully established. The AD/ADRD Resource Network aims to isolate structurally vetted tau filaments and oligomers from human tauopathy cases and distribute them to the research community. In addition, it seeks to bank and distribute detection reagents and associated protocols. The overall mission of the network is to support impactful tauopathy research. Therefore at this meeting we aim to present our progress/plans and get feedback and suggestions from our amyloid research community to make this network as useful as possible to the filed.

Methods

Tau oligomers and filaments are isolated from human brain samples using biochemical methods. Oligomeric aggregates are interrogated by mass spectrometry methods whereas filaments are vetted for isoform composition, post-translational modification and mass per unit length. Interaction of aggregates with molecular probes is assessed using Fluorescent Amyloid Multi Emission Spectra (FLAMES) and radioligand binding assays. Biological activity is evaluated through toxicity, seeding and electrophysiological measurements.

Results

Protocols for simultaneous isolation of oligomeric and filamentous tau aggregates have been developed along with analytical and quantitative methods to estimate sample heterogenicity and stability. Resource Network members are establishing cross-laboratory analytical benchmarks for experiments to ensure rigor and reproducibility of results.

Conclusions

The AD/ADRD Research Network banks rigorously validated preparations of structurally vetted tau aggregates along with directed protocols and reagents. Dissemination of these reagents is expected to maximize rigor and reproducibility of basic research involving tau protein aggregates.

Modelling the lipid-chaperone hypothesis: a kinetic and thermodynamic approach to elucidate the role of lipids in regulating ion-channel pore formation in membrane

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The lipid-chaperone hypothesis[1] is a recent hypothesis in the field of amyloid research that proposes that lipids, specifically phospholipids, play a critical role in regulating the formation and toxicity of amyloid fibrils. Amyloid fibrils are long, insoluble fibers that are a hallmark of several neurodegenerative diseases, including, type 2 diabetes, Alzheimer's and Parkinson diseases. These fibrils are composed of misfolded proteins that aggregate together and form these long fibers. The accumulation of amyloid fibrils is thought to be one of the key factors that contribute to the development and progression of these diseases. The lipid-chaperone hypothesis suggests that phospholipids, which are the main building blocks of cell membranes, can interact with amyloid fibrils and regulate their formation and toxicity. Specifically, it is proposed that phospholipids can act as chaperones, guiding the formation of fibrils and ion-channel-like pores. Furthermore, the hypothesis proposes that changes in the lipid composition of cell membranes can influence the formation and toxicity of amyloid fibrils. For example, changes in the levels of certain phospholipids, such as phosphatidylcholine or sphingomyelin, have been shown to affect the aggregation of amyloid-beta, the protein that forms the amyloid fibrils in Alzheimer's disease.

Overall, the lipid-chaperone hypothesis provides a new perspective on the role of lipids in the development and progression of neurodegenerative diseases and suggests that targeting the lipid composition of cell membranes may be a promising strategy for developing new treatments for these diseases.

The lipid-chaperone hypothesis was tested experimentally by using phospholipids with different critical micellar concentrations.

Presented here is a kinetic model that starting from the differential equations describing the process of interaction between the protein and the phospholipid membrane allows calculation of the distribution of species involved in the process of pore formation in the membrane. Note the distribution of species pertinent to the entire process, the free energy of formation of the protein-lipid complex was calculated.

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Tau molecular structures and related strategies for disease-modifying intervention

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Some intrinsically disordered proteins are implicated in the development of proteinopathies by their susceptibility to aggregation in pathological conditions, which might involve post-translational modification. Tau protein is such an intrinsically disordered protein whose aggregation in neuronal cells is at the core of a family of neurodegenerative diseases collectively named tauopathies, including the most common Alzheimer's disease. The ensemble of conformations adopted by the Tau protein in its soluble state,[1] as well as its three-dimensional structure when aggregated into fibres,[2] have been well-documented. However, the path that leads from the highly soluble Tau to the very organised ß-sheets present in the fibres, as revealed by single-particle cryo-electron microscopy, remains poorly understood. The multiple phosphorylation that is reported in Tau pathological states appears in an order manner following the disease stages contributing to Tau aggregation in a poorly defined manner concerning the role of phosphorylation in the aggregation process. We address this question by investigating the effect of specific disease-relevant phosphorylation on Tau conformational ensemble and local elements of structure using nuclear magnetic resonance and chemical biology tools.[3,4] Finally, we conclude by discussing the promises of using single domain antibody fragments as disease-modifying biomolecules in tauopathies.[5]

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Interplay Between Liquid-liquid Phase Separation and Amyloid Formation of TDP-43 C-terminal domain

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Liquid-liquid phase-separation (LLPS) is a biological phenomenon wherein a metastable, concentrated droplet phase of biomolecules spontaneously forms. A link may exist between LLPS of proteins and the disease-related process of amyloid fibril formation; however, this connection is not fully understood. Here, we investigated the relationship between LLPS and aggregation of the C-terminal domain (CTD) of the transactive response DNA binding protein of 43 kD (TDP-43) by monitoring conformational changes during droplet aging using Raman spectral imaging. The C-terminal domain of TDP-43 (TDP-43_{CTD}) is biologically relevant as it is accumulated in cytoplasmic inclusions associated with neurodegenerative diseases including amyotrophic lateral sclerosis (ALS) and frontotemporal lobar dementia (FTLD). Moreover, in vitro experiments have shown that both LLPS and amyloid formation of TDP-43 are driven by TDP-43_{CTD}. To identify site-specific differences between fluid droplets, aged droplets, and amyloid fibrils formed by TDP-43_{CTD}, we have used 4-ethynylphenylalanine (F_{CC}) substituted at single aromatic sites throughout the sequence *via* Amber codon suppression. F_{CC}—an aromatic unnatural amino acid with a terminal alkyne on the aryl ring—exhibits a distinctive Raman band arising from the stretching mode of the alkyne bond (C≡C). Raman spectra were acquired from within nascent and aged droplets as well as amyloid fibrils as function of time. The amide-I band was analyzed to interrogate the secondary structural characteristics of these species and the C≡C band was used as a reporter of sidechain-specific interactions. Although TDP-43_{CTD} droplet aging is associated with development of amyloid-like β-sheet structure, these structures are distinct from fibrillar amyloid aggregates as shifts in the C≡C band indicate local environmental differences between the two species. The coexistence of solidified droplets and amyloid fibrils indicates that while these droplets are amyloid-like, they do no to propagate. This suggests that LLPS is not necessarily on-pathway for TDP-43_{CTD} amyloid formation, but rather that different β-sheet structures or polymorphs are stabilized within the phase-separated state.

Modeling of brain function and pathology

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Insights into aSyn pathology in human brain using correlative light and electron microscopy

Amanda Lewis

EPFL and UNIL, Switzerland

Chemical Strategies to Study Multiple Facets in Alzheimer's Disease

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Alzheimer's disease (AD), associated with degeneration of neurons and synapses in the brain, leads to motor impairment and eventual fatality. Neurodegeneration could be related to various interconnected features, including (i) plaque formation from amyloid-β (Aβ) peptide fragments, (ii) metal ion dyshomeostasis and miscompartmentalization, as well as (iii) inflammation and increased oxidative stress due to overproduction of reactive oxygen species (ROS). The inter-relations between some of these pathological factors have been investigated. Metals are found entangled in the Aβ plaque and likely contribute to Aβ neurotoxicity and oxidative stress. ROS have been shown to increase the rate of Aβ plaque formation. Our understanding of the correlation between these elements and AD neuropathogenesis has been very limited, however. There is currently no cure for AD; therapies are focused on symptomatic relief targeting the decrease in the levels of acetylcholine, only one of the multiple factors causing the disease.[1-3] To find a cure for AD, we require a better understanding of the relationship between various causative factors of this devastating disease. Towards this goal, we have been developing suitable chemical tools capable of targeting and regulating multiple underlying factors or identifying the pathogenic networks composed of their direct interactions and reactivities.[4-11]

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Ubiquitin, metal ions, and amyloids: neutral observers or malicious conspirators?

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There is compelling evidence indicating that individuals with Alzheimer's Disease (AD) experience dysregulation of metal homeostasis and impairment of the Ubiquitin Proteasome System (UPS). Ubiquitin (Ub)-encoded signals play a critical role in managing the physiological clearance of Aβ, as evidenced by the presence of Ub-containing amyloid aggregates in the AD brain. Furthermore, metal ions such as Cu(II) and Zn(II) have been found to accumulate in pathological amyloid deposits and inhibit UPS function. Despite these evident correlations, our understanding of the interplay between metal dyshomeostasis and UPS failure in AD pathogenesis remains limited. To address this issue, our objective was to investigate whether metal ions and Aβ peptide could bind Ub and affect Ub-regulated amyloid clearance pathways. For the past decade, our laboratory has been studying the properties of metal/Ub species at neutral pH. We conducted cell-free assays to determine the effects of metal ions on polyubiquitination reactions. Through our research, we discovered that metals have an impact on Lys63- and Lys48-PolyUb chain synthesis. This effect is likely due to metal anchoring to His68, which results in Ub being less available for polyubiquitination. Furthermore, our findings suggest that the interaction between Aβ and Ub may play a significant role in regulating the initial stages of UPS. Considering that aging is associated with increasing levels of Ub and metal imbalance in neurons, this presents an intriguing hypothesis that could explain why the brain is more susceptible to developing neurodegenerative diseases like AD as we age.

Role of zinc and insulin on IAPP's amyloid aggregation

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Dynamic of prion assemblies and the consequences of the coexistence of multiple prion conformations

Human Rezaei

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The prion pathology is based on autonomous structural information propagation towards single or multiple protein conformational changes. Since this last decade the prion concept referring to the transmission of structural information has been extended to several regulation systems and pathologies including Alzheimer and Parkinson's diseases. The unified theory in Prion replication implies structural information transference from the prion to a non-prion conformer through a mechanism also called improperly, with regards to biophysical considerations "seeding" phenomenon. Recently we reported that prion replication is intrinsically source of structural diversification. The coexistence of multiple prion assemblies with different structural and replication propensity questions how they are maintained within the same media and how they escape to best replicator selection.

The analysis of the quaternary structure dynamic of prion assemblies revealed the existence of an exchange process within the structurally diverse prion assemblies. This exchange process has for consequence the apparition of dumped-oscillation. Data assimilation and kinetical modelling conduced us to propose a kinetical scheme in which structurally diverse prion assemblies catalytically exchange material. According to this kinetic scheme a catalytical depolymerization competes with a catalytic conformational change.

Secondary Nucleation Mechanism at near Atomic Resolution

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Heterotypic amyloid interactions and their impact on amyloid assembly

Frederic Rousseau

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Sparking protein misfolding and neurodegeneration in Alzheimer's disease

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Amyloid beta and Tau deposition pathological hallmarks of Alzheimer's disease while possession of ApoE4 is the major genetic risk factor for developing AD, providing a genetic background which leads to susceptibility to developing disease. Amyloid beta rapidly self-assembles and oligomeric species have been previously shown to affect neuronal health. We have studied the uptake and effects on organelles including lysosomes and synaptic vesicles to dissect mechanisms that lead to neuronal dysfunction and cell death. We reveal damage to specific organelles of the cell which are accompanied by impaired synaptic vesicle release and reuptake Tau is a natively unfolded protein which, unlike Amyloid-beta, does not readily self-assemble. We have developed a model fragment, tau297-391, which self-assembles to form paired helical filaments in vitro which we have used to examine cellular mechanisms of transmission and toxicity. tau297-391 self-assembles to form PHF under agitating conditions but that under quiescent conditions is able to associate with RNA in via complementary charge-charge interactions to form coacervates. This work provides insights into the potential mechanisms of initiation of misfolding of tau in AD.

We consider the underlying mechanisms that may consolidate these findings and explore potential therapies.

Mechanisms of IAPP aggregation and toxicity *in vitro* and *in vivo*

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Type II diabetes is associated with the loss of pancreatic beta-cells, which has been linked to the fibrillar aggregation of islet amyloid polypeptide (IAPP). IAPP is a 37-residue peptide hormone that is co-secreted with insulin from the beta-cells. Previously, it had been shown that IAPP aggregation is greatly accelerated by membranes containing anionic lipids. The aggregation mechanism and the link to membrane damage were however incompletely understood. Using quantitative analysis of the aggregation kinetics of IAPP in the presence of model membranes, we demonstrated that anionic lipids accelerate both primary and secondary nucleation events on the membrane surface [1]. Vesicle leakage is associated specifically with fibril elongation, whereas oligomeric species generated by nucleation do not significantly contribute to membrane damage under these conditions [2]. Living cells and organims are evidently more complex and it remains to be determined to what extent the same mechanisms of aggregation and toxicity apply. Currently, we are establishing *C. elegans* models to enable such studies as well as screening for small molecule modulators.

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Decorated vesicles- Tipping points in between α-synuclein - lipid co-assembly

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α-Synuclein is a small neuronal protein that associates with lipid membranes. The membrane interactions are believed to be crucial to both its healthy and disease functions. The healthy functions are associated with neurotransmitter release by regulating synaptic vesicle trafficking. Alterations in the membrane interaction may trigger pathological aggregation of α-Synuclein. There is growing evidence suggesting that the intracellular inclusions characteristic for Parkinson's disease and related disorders also contain membrane lipids. Taken together, α-Synuclein may switch from a vesicle-bound state to lipid-containing fibrils, whereas the threshold conditions for the transition between those states is still not unravelled.

In this presentation, I will focus on how α-synuclein associates with lipid membranes, and on the consequences of this association. The protein has a non-uniform charge distribution, and the binding is controlled by anisotropic (patchy) electrostatic interactions.[1] We further show strong cooperativity of α-synuclein binding to lipid membranes,[2] meaning that the affinity of the protein to a membrane is higher where there is already protein bound compared to a bare membrane. This leads to regions at the membrane with high protein density, which also induces membrane deformations.[3] In cases where there is excess free protein in solution, the vesicles decorated with bound protein may trigger the formation of α-synuclein amyloid aggerates that also contain lipids.[4,5]

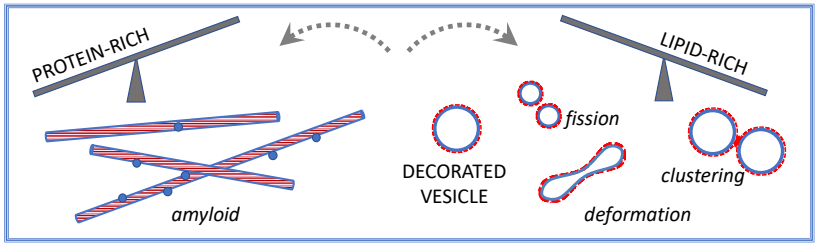

Figure 1: Tipping point in lipid-protein co-assembly: transition between lipid-rich and protein-rich assemblies

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Inhibition of human IAPP aggregation and interaction with mitochondrial membranes by small-molecule compounds

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Type-2 diabetes mellitus (T2DM) is a complex metabolic illness, impacting tens of millions worldwide. T2DM is associated with the aberrant misfolding, oligomerisation and deposition of the amyloid-forming human islet amyloid polypeptide (hIAPP) in the pancreas of over 90% of such individuals. Importantly, toxicity of hIAPP oligomers has been linked to mitochondrial dysfunction and impaired insulin release. Here, we investigated a group of natural polyphenol and synthetic diphenylpyrazole (DPP) drug-like compounds for their efficacy in inhibiting hIAPP aggregation and damage to mitochondrial membranes, using model mito-mimetic membranes and isolated mitochondria. Notably, the DPP-derived compound sery166a potently inhibited hIAPP aggregation, disaggregated hIAPP fibrils, effectively protected mitochondrial membranes against disruption, and preserved mitochondrial integrity. We intend to exploit these initial findings to further test the best performing compounds for the preservation of mitochondrial function, and hence potentially advance therapeutic application of these compounds in T2DM patients.

Targeting Protein Aggregation in Neurodegenerative Diseases

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The phenomenon of protein misfolding and aggregation is associated with a wide range of human disorders, including Alzheimer's and Parkinson's diseases. A central role in these conditions is played by protein misfolded oligomers, which are among the most cytotoxic species resulting from the process of protein aggregation. It has been very challenging, however, to target these oligomers with therapeutic compounds, because of their dynamic and transient nature. To overcome this problem, I will first describe a thermodynamic-based approach based on the stabilization of the native states of proteins. I will then discuss a kinetic-based approach, which enables the discovery and systematic optimization of compounds that reduce the number of oligomers produced during an aggregation reaction. I will illustrate these strategies for the amyloid beta peptide, which is closely linked to Alzheimer's disease. As these strategies are general, they can be applied in drug discovery programs targeting any aggregating protein.

Computational study on the aggregation of amyloid proteins and its inhibition by polyphenols

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Protein aggregation, which involves the formation of oligomers, protofibrils, and mature fibrils, is closely linked to the pathogenesis of several neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. Due to the transient nature of low molecular weight aggregates and the polymorphisms of amyloid fibrils, the atomistic mechanisms of protein aggregation are not well understood and there is no cure yet for those neurodegenerative diseases. Inhibiting protein aggregation and disaggregating preformed protofibrils (the intermediates for fibril growth) are considered as promising strategies for the treatment of neurodegenerative diseases. Molecular dynamic simulations play a crucial role by providing atomic-level information on the conformational ensemble of oligomers as well as the interactions between protofibrils and potential inhibitors. In this talk, I'll present our simulation studies on the oligomeric structures of amyloid core peptides of different proteins and the impacts of mutations/phosphorylations, as well as the mechanisms by which polyphenols disrupt the protofibrils with different polymorphs. Our results provide mechanistic insights into protein aggregation and its inhibition by polyphenols, which might be helpful for the design of new therapeutic strategies against the pathological aggregation of amyloid proteins.

Oral presentations

New studies on the cross-talk between α-Synuclein and Tau protein

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The progressive loss of neuronal cells, as well as the decline of cognitive and motor functions are common features of several neurodegenerative disorders, such as Parkinson's disease (PD) and α-synucleinopathies[1]. Other key factors in the development of these disorders should be oxidative stress, dyshomeostasis of metal ions and α-synuclein (αSyn)[2]. Moreover, the abnormal aggregation process of αSyn is considered a crucial event in the pathogenesis of α-synucleinopathies.

Several Post-Translational Modifications play an important role of the physiological and pathological routes of αSyn: the copper-protein interaction regulates αSyn intracellular localization and cytotoxicity[2]; lipoxidation and carbonylation somehow affect the aggregation process of αSyn[3]. For this reason, the interplay between acrolein (a reactive carbonyl species), copper, and αSyn has been properly investigated[4].

Mounting evidence confirms amyloid proteins cross-talk each other. For example, αSyn has a biological link with Tau protein because neurofibrillary tangles have been found in PD[5]. A53T mutant of αSyn also induce Tau phosphorylation. Moreover, NMR studies suggest that this non-covalent interaction involves the C-terminal region of αSyn and the microtubule binding domain of Tau[6]. Based on these data, we have investigated the interaction of some Tau peptide fragments with αSyn, using the limited proteolysis approach, Native Mass Spectrometry and amyloid-type aggregation assay.

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Tip-enhanced Raman spectroscopy for chemical and structural characterization of amyloid fibrils

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Tip-enhanced Raman spectroscopy (TERS) has emerged as a powerful technique for chemical and structural characterization with nanoscale (and even sub-nanoscale) spatial resolution. TERS combines the chemical specificity of Raman spectroscopy and the high spatial lateral resolution of scanning probe microscopies (such as AFM). So far, TERS allowed the nanoscale investigation of many biomolecules and biosystems such as nucleic acids, proteins/peptides, lipid membranes, viruses and cells.[1]

Here, we present our studies related to the characterization of amyloid fibrils and oligomers implicated in neurodegenerative diseases. We show that spectral regions assigned to amide bands can be exploited to distinguish amyloid-β fibrils from more toxic oligomers,[2] and determine changes in the secondary structure of Tau filaments as a function of the aggregation cofactor.[3] Furthermore, TERS allowed the presence of phospholipid to be revealed inside the structure of Tau fibrils formed from a mixture of phosphatidylinositol (PIP_2) and phosphatidylcholine (POPC) as cofactor.[3] Using a home-made TERS system in total internal reflection,[4,5] we were able to perform TERS imaging with nanoscale spatial resolution of a Tau fibril grown in the presence of heparin sodium, and show nanoscale regions with different amino acid contributions and secondary structures.[5] More recently, the same TERS system was used to probe Tau fibrils formed using polyadenine as cofactor, and try to correlate the protein secondary structure and the amino acid content with the presence or absence of polyadenine so as to better understand Tau protein – polyadenine interactions at the origin of the fibril formation. These first studies are promising to better understand molecular mechanisms involved in amyloid aggregation processes.

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Chemical tools to probe the mechanistic complexity of amyloid formation and associated cytotoxicity

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The aggregation and tissue deposition of polypeptides in the form of amyloids are the hallmark of many diseases, including the Alzheimer's disease and systemic amyloidoses. These protein misfolding disorders differ on the identity of the protein that misassembles into aggregates, including those with a cross-β-sheet quaternary structure. While substantial pharmacological evidence supports the amyloid hypothesis, the elucidation of the mechanisms of self-assembly under physiological conditions as well as the identification of the proteotoxic species remain challenging, ultimately precluding the development of therapeutic interventions. In this context, by using the islet amyloid polypeptide (IAPP) whose deposition in the pancreatic islets is associated with type II diabetes, we are engineering chemical tools and biophysical approaches. First, by combining the fluorogenic probe fluorescein arsenical hairpin with positional scanning of the split-tetracysteine motif, we developed a detection method to kinetically investigate the conformational ensemble of the proteospecies assembled throughout the amyloidogenic pathway. This approach is well-suited to detect thioflavin T-negative assemblies and to screen inhibitors of amyloid formation. Secondly, to address the limitations of synthetic lipid models to study plasma membrane perturbation, we implemented a simple and potentially high-throughput assay based on giant plasma membrane vesicles (GPMVs) derived from mammalian cells. By labelling the GPMVs with either red, or green, lipophilic tracers, the perturbation of GPMVs induced by IAPP could be kinetically detected by Förster resonance energy transfer. Thirdly, by modulating the conformational ensemble of IAPP through macrocyclization and residue-specific modifications, we revealed that helical species are off-pathway to amyloid formation and that the α-helix stabilization prevents cell degeneration. Overall, these novel chemical strategies expand the toolbox available to study amyloid-associated disorders, ultimately bridging the gap between *in vitro* models and the pathomechanisms of these diseases.

Anti-amyloid and Anti-oxidative effects of Moringa Oleifera extracts: a phyto-therapeutic strategy for Alzheimer disease

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Alzheimer's disease (AD) is a chronic neurodegenerative disease representing the most common form of dementia in the elderly. The pathology is characterized by the accumulation of extracellular amyloid plaques composed by well-ordered, β-sheet rich, fibers of the Amyloid β peptides (Aβ). Although Aβ fibers are the hallmark of AD, consistent evidences rather indicate that oligomers of Aβ act as toxins, responsible for oxidative stress and mitochondrial dysfunction, causing the neurodegenerative damage. Many efforts have been focused therefore on the individuation of molecules capable to inhibit the pathological aggregation and/or reduce ROS production. An increasing number of studies, conducted under in vitro and in vivo conditions, highlights that natural polyphenols can work both as amyloid inhibitors and antioxidants. This double action makes it these molecules very attractive for the development of effective phyto-therapeutic strategies.

In this work anti-amyloid and anti-oxidative effects of aqueous extracts of Moringa Oleifera leaves were tested. Extracts have been characterized for their polyphenolic content and their inhibitor effect was studied on the in vitro aggregation kinetics of $A\beta_{1-42}$ peptide. Results show a strong anti-amyloid effect at very low concentration of aqueous extracts, as seen by ThT assay, circular dichroism and atomic force microscopy. Interestingly, in the range explored, the lag time of fibrillation was inversely correlated with polyphenolic concentration. Polyphenols on their own present a tendency to aggregate. These results suggest that after an initial shielding of the peptides, later on polyphenols/peptides aggregates can nucleate an amyloid-like aggregation, while overall maintaining a reduction of fibrillation. Finally, retinoic acid-differentiated SH-SY5Y cells, challenged with glyceraldehyde (GA), were used as an AD cell model to test the effects of Moringa Oleifera extracts. The extracts antioxidant action and their efficacy to inhibit the production of $A\beta_{1-42}$, stimulated by GA, were tested. Treated cells showed an increase on cell viability in comparison with the controls. Moreover, mitochondrial ROS and GA-stimulated $A\beta_{1-42}$ production were reduced in cells exposed to Moringa Oleifera extracts.

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Virtual histology of Alzheimer's Disease through synchrotron-based X-ray phase-contrast imaging

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While numerous transgenic mouse strains have been produced to model the formation of Aβ plaques in the brain, efficient methods for whole-brain 3D analysis of Aβ deposits have to be validated and standardized. Moreover, routine immunohistochemistry performed on brain slices precludes any shape analysis of Aβ plaques, or require complex procedures for serial acquisition and reconstruction.[1] We here introduce and show the potential of X-ray phase-contrast tomography (XPCT), performed under coherent synchrotron light source, to visualize Aβ deposits in intact, fixed brains of transgenic animals. Performed in several Alzheimer mouse strains, the proposed workflow enabled hippocampus-wide detection and 3D morphometric analysis of Aβ plaques at an isotropic pixel size of 6.5 µm (Fig. 1).[2] Furthermore, using complementary synchrotron techniques, namely i) Fourier-transform infrared microspectroscopy (µFTIR) to quantify the proportion of β-sheets in the plaques, and ii) X-ray fluorescence microspectroscopy (µXRF) to assess metal quantities in the plaques, we show that XPCT contrast is mainly driven by the level and spatial distribution of metal entrapment.[3]

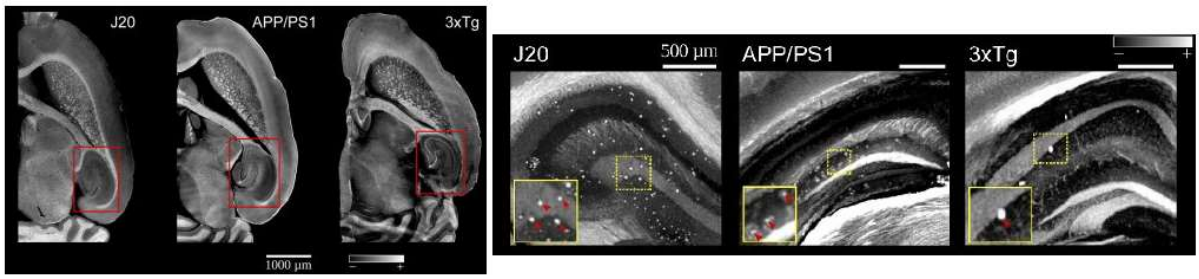

Figure 1. (right) Whole-brain anatomy of ethanol-dehydrated brains from three mouse strains. (left) XPCT zoom-in at the level of the dorsal hippocampus, showing the density and appearance of Aβ plaques in the three mouse strains. From [2].

XPCT virtual histology could thus become instrumental in quantifying the 3D spreading and the morphological impact of seeding when studying prion-like properties of Aβ aggregates in animal models of Alzheimer's disease.

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Keggin-type polyoxometalate to guide the self-assembly of Aβ peptide

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Alzheimer's disease is one of the most common and studied neurodegenerative disease worldwide.[1,2] It is characterised by the production and the accumulation of the amyloid-β (Aβ) peptide. Its ability to self-assemble leading to toxic oligomers and then, to stable fibrils rich in β-sheets, forming senile plaques is regarded as a key early event in the development of the disease. [2,3,4] Recent studies have reported the capacity of different polyoxometalates (POMs) to modulate the self-assembly of the Aβ peptide [5,6,7,8] but also to improve the cognitive capacity in AD mice model in direct link with the self-assembly results,[6,7,8] while they were shown to cross the blood-brain barrier.[6] Here, we studied the effect of a lacunary spherical Keggin polyoxometalate α-$[SiW_{11}O_{39}]^{8-}$ (also called K^{Si}_{L}) on the Aβ peptide self-assembly. The addition of K^{Si}_{L} was studied at different concentration on Aβ and its impact on the kinetics of the peptide self-assembly and on the obtained fibrils morphology were evaluated. We found out that the addition of K^{Si}_{L} on Aβ induce an increase of the self-assembly kinetics at high ratio and an enhancement of the fluorescence intensity by forming more and longer fibrils. These studies are currently completed by investigating the binding site of K^{Si}_{L} on Aβ by nuclear magnetic resonance studies to gain better insights into its impact on the peptide self-assembly. Several other factors will be further considered such as metallic substitution of the lacuna in the host POM, the nature of the POM (Dawson for instance) and of the central cation that will alter the overall charge.

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Acknowledgments

The ANR-21-CE06-0030 SUPRAMY is thanked for its financial support. The integrative electron microscopy platform METi is also warmly acknowledged for its support and their advices.

AFM-IR characterization of tau fibrils and aggregates obtained with different cofactors

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Aggregates of the tau protein are involved in several neurodegenerative diseases, called tauopathies, including Alzheimer's disease. Cryo-EM structures of brain-extracted tau filaments have revealed distinct conformations depending on the tauopathy but homogeneous ones within each disease (*1*). Recent findings suggest aggregation cofactors, and notably membrane lipids, might dictate tau aggregates properties and pathological activity. Central questions about tau aggregation remain unanswered. In particular, are the cofactors incorporated in the fibrils? Does the secondary structure of an aggregate depend on the cofactor used for aggregation? A previous study used TERS (tip enhanced Raman spectroscopy) to show that tau construct K18 aggregated in the presence of PIP_2 lipids suggested lipid recruitment inside the fibers (*2*).

In this study, we use AFM-IR (atomic force microscopy coupled to infrared spectroscopy) to obtain local IR spectra and chemical mapping of tau aggregation products with nanoscale resolution (~20 nm). In addition to morphological characterization, this method therefore allows for chemical characterization of single amyloid species, allowing us to assess structural and chemical heterogeneity of a sample at the nanoscale (*3*). After a thorough optimization of the AFM-IR signal on heparin-induced tau fibers and aggregates, we notably focused on POPS- and arachidonic acid-driven aggregation of tau. We compare obtained morphologies, chemical signatures and structural heterogeneities.

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Chaperone regulation of the protein condensation and aggregation continuum in Alzheimer's disease

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Protein aggregation is central in AD where aggregation of Aβ and tau proceeds during prodromal stages, implying a heightened proteostasis burden along the disease continuum. While the extracellular space is the primary site for Aβ aggregation, it is known that intracellular tau is promptly secreted and spreads pathology to nearby cells in the form of toxic oligomers. Therefore, intra and extracellular proteostasis modulators are key to define molecular events in the diseased brain. Here I will present recent findings linking proteins of the S100 family to novel neuroprotective functions as chaperones that modulate tau condensation and aggregation phenomena. S100 proteins are Ca-binding proteins abundant in the brain with intra and extracellular functions, both trophic and pro-inflammatory as a response to proteotoxicity [1]. We established that S100 proteins co-localize with Aβ protein aggregates [2], and that S100B operates as a chaperone inhibiting the aggregation, toxicity and proteopathic seeding of both Aβ42 and Tau [3-7]. Recently, we uncovered that Tau liquid–liquid phase separation is modulated by the S100B chaperone, which incorporates into droplets readily reversing tau demixing [8]. I will also discuss the underlying mechanisms of action of the S100-type chaperones, including latest unpublished findings, and elaborate on the hypothesis that S100 proteins are important proteostasis regulators acting on protein aggregation and condensation phenomena across the neurodegeneration continuum. Acknowledgments: Collaborators, research team and funders are gratefully acknowledged. Funded by FCT/MCTES (BioISI) and the European Union (TWIN2PIPSA, GA 101079147).

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Fibril Structure of Islet Amyloid Polypeptide (IAPP) and Structural Basis for the Inhibition of IAPP Fibril Formation by the Co-Chaperonin Prefoldin

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Deposits of the 37 aa peptide hormone islet amyloid polypeptide (IAPP), also known as amylin, are found as amyloid aggregates surrounding islet β-cells in the pancreas in 90% of type II diabetes cases and are a hall mark of diabetes-type II. It is suggested that IAPP aggregation is most likely one main cause for cell death of insulin producing β-cells, which ultimately lead to diabetes-type II.

Here, we aimed i) to solve the fibril structures of *in vitro* aggregated IAPP at atomic level by cryo-EM and ii) studied at atomic molecular level on how aggregated fibrillar IAPP is recognized and attacked by a cellular chaperonin system, namely by the HSP60 type II co-chaperonin prefoldin (PFD).

The near atomic cryo-EM structure of *in vitro* obtained IAPP fibrils[1] is characterised by a dimeric cross-β structure with S-shaped monomers containing a flexible N-terminus. Importantly, it revealed structural similarities to fibrillar amyloid-β, the main component of extracellular plaques in brains of Alzheimer Disease (AD) patients[1] and may explain the cross-seeding activities of IAPP and amyloid-β, which may cause the observed predisposition of diabetes-type II affected humans towards increased AD susceptibility.

We further report the mechanistic study of IAPP fibrillation inhibition by PFD[2]. Homologs of this co-chaperonin are found in the cytosol of archaeal and eukaryotic cells. The heterohexameric PFD is a holdase with a characteristic jellyfish architecture, consisting of a β-barrel body and coiled-coil α-helix tentacles. PFD binds substrates and delivers it to HSP60 for refolding. PFD has previously been shown to interact with different disease-relevant amyloidogenic substrates and to inhibit their aggregation, however no structural or mechanistic insights in this process have been described until now.

We integrated kinetic investigations with structural studies using AFM, EM and NMR spectroscopy to elucidate the different inhibition pathways and to provide a structural understanding of the prefoldin-amyloid interaction[2]. The highly dynamic complex between the 90 kDa-PFD and intrinsically disordered 4 kDa-IAPP enabled us to obtain insights into the chaperoning process at sub-molecular level.

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C-terminal region in the Sm-like Hfq forms an amyloid-like β-rich motif

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Hfq is a pleitropic actor, serving as stress response and virulence factor in the bacterial cell. It executes his functions mostly by binding to small noncoding RNAs, conferring annealing with mRNAs [1]. To fulfil these functions, Hfq assembles into a symmetric torus-shaped hexamer, called the Sm-core, with two concave structurally distinct surfaces and a convex rim. As extension outward from this core, Hfq contains an intrinsically disordered C-terminal region when in solution [2]. Many aspects of the elongated C-terminal segment of around 40 residues remain unclear. For instance, assembly into amyloid-like filament has been reported for the C-terminal region in isolation [3]. However, the structure of full-length protein including C-terminal extension and Sm core and how the structures arrange in the assembled state remain unclear. Based on x-ray diffraction and magic-angle spinning solid-state NMR, we show that a minimal 11-residue motif C-terminal of Hfq assembles into amyloid fibers. Our data further suggest that the full-length Hfq in its filamentous state contains a motif with comparable spectral fingerprint than that of the short β-strand peptide and that the unique structure of the Sm-core is not affected by the formation of the filament. Hfq proteins might thus adapt two conformations *in vivo*, either as soluble hexamers or as assembled hexamer-containing filaments, which form through amyloid-like interactions, possibly self-regulating cellular functions.

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Synthetic α-Synuclein fibrils capable of seeding Glial Cytoplasmic Inclusions in mice share their amyloid fold with Multiple System Atrophy filaments extracted from patients: structure/function considerations

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Besides the presence of a prototypical Greek-key motif, atomic-scale characterization of α-Synuclein (α-Syn) filaments extracted from Multiple System Atrophy (MSA) brains using sarkosyl has revealed a complexity of the amyloid core[1] that was never observed before in any of the synthetic α-Syn fibrils assembled *in vitro*[2]. In addition, demonstration of the participation of a small molecule component at the protofilament interface has casted doubts on the possible protein-only mechanism of propagation of these extracted fibrils[1]. This notion has been reinforced by the message that MSA fibrils do not constitute a template for fibril elongation *in vitro* but merely cause secondary nucleations giving rise to structurally unrelated fibrils[3]. While these notions seem to question the biological relevance of the α-Syn fibrils assembled *in vitro*, it should however be noted that none of the MSA fibrils that were structurally characterized thus far using cryo-electron microscopy (cryoEM) have been proven to be capable of seeding a MSA-like α-Syn pathology *in vivo*, in wild-type animals.

Taking the problem from the other end, we isolated and selected the synthetic α-Syn fibril strain 1B using an array of spectroscopic methods and bioassays and found, after intracerebral injections in non-transgenic mice, that it was capable of seeding the formation of cytopathological α-Syn inclusions that are selectively observed in MSA and not in Parkinson's disease nor in Dementia with Lewy Bodies: neuronal intranuclear inclusions (NIIs) and glial cytoplasmic inclusions (GCIs)[4-5]. This prompted us to put these fibrils under scrutiny using CryoEM in order to identify a possible structural "common denominator" to the 1B and the MSA fibrils. Our results indicate that 1B fibrils present a prototypical supramolecular architecture with 2 intertwined protofilaments and axial symmetry. The protofilament interface is reminiscent of the classical type 1a synthetic fibrils, however the amyloid fold of each protofilament is almost identical to one of the MSA protofilaments described by the group of Scheres and Goedert[1,3].

These observations allow us to for the first time to make structure/function considerations and in particular to define the active, disease-relevant fold of MSA filaments in terms of capability to seed MSA pathology (GCIs and NIIs).

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Chaperones, inhibitors of protein aggregation – when and how?

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Numerous proteins, called chaperones [1], small organic molecules [2], and metal ions present in the cellular microenvironment have been implicated as inhibitors or drivers of aggregation of amyloid-forming proteins and peptides [3]. The complexity of protein folding and misfolding trajectories is vastly overwhelming for the current biophysical techniques, and the process itself depends on numerous factors that cannot be observed in isolation or simple in vitro two-component systems. Keeping that in mind, we examined the Zinc (Zn^{2+}) ions reported to directly interact with α-synuclein (AS), a causative agent of Parkinson's disease and other neurodegenerative diseases and promote its aggregation. AS is a small intrinsically disordered protein (IDP), i.e., understanding molecular factors that drive its misfolding and aggregation has been challenging since methods used routinely to study protein structure are not effective for IDPs. Here, we report the atomic details of Zn^{2+} binding to AS at physiologically relevant conditions using proton-less NMR techniques that can be applied to highly dynamic systems like IDPs. We also examined how human serum albumin (HSA), the most abundant protein in human blood, binds to AS and whether Zn^{2+} and/or ionic strength affects this. We conclude that Zn^{2+} enhances the anti-aggregation chaperoning role of HSA that relies on protecting the hydrophobic N-terminal and NAC regions of AS rather than the polar negatively charged C-terminus. This suggested a previously undocumented role of Zn^{2+} in HSA function and AS aggregation.[1] Similarly, combining the novel spectroscopic techniques operating with more complex systems at physiologically relevant conditions, we study the inhibitory properties of common flavonoids, e.g., EGCG, TB3, from tea, to understand the inhibitory mechanisms, that pave the way towards more efficient anti-aggregation therapeutics. Here, we want to emphasize the need to combine state-of-the-art technology development and expansion towards more physiologically relevant systems better to reveal the aggregation modus operandi of disease-related proteins.

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What and why can alter the peptide aggregation? Molecular insights.

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Amylin, which is a 37 Amino Acids (AA) long peptide, whose aggregation is characteristic for the diabetes type II, what is a disease affecting young people, because it is connected with changing lifestyle in recent decades. It is worth mentioning that the most common cause of this health disorder is a combination of excessive body weight and insufficient exercise. Diabetes Type II makes up 90% of cases for diabetes worldwide. Recently, the detailed characterization of the interactions between amylin, its non-toxic analogs with very much limited ability to form aggregates (rat amylin and synthetic pramlintide) and biologically important metal ions, like Zn(II), Cu(II) has been performed at the atomic level. Interestingly, some metals, like Cu(II) ions at some concentrations can inhibit and at some can facilitate the amylin aggregation process. Surprisingly, a review of the currently available scientific literature showed that this topic has not yet been profoundly studied. Moreover, the molecular basis of the interaction of the Cu(II) ions with human amylin analogs and the influence of these ions on their aggregation have not yet been studied at all. This neglected question had been approached not only with the NMR spectroscopy, but also with the EPR, potentiometric as well as ThT fluoresce methods. The outcome has shown that both peptides form stable complexes with Cu(II) with similar affinities at a 1:1 ratio. The *N*-termini of both peptides are involved in Cu(II) binding; Histidine 18 (His18) imidazole is an equally attractive binding site in the case of pramlintide. Our results show for the first time that Cu(II) ions influence the aggregation of pramlintide, but not that of rat amylin.

Unsaturated Fatty Acids Uniquely Alter Aggregation Rate of α-Synuclein and Insulin and Modify Sec-ondary Structure and Toxicity of Amyloid Aggregates Formed in Their Presence

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Docosahexaenoic (DHA) and arachidonic acids (ARA) are omega-3 and omega-6 long-chain polyunsaturated fatty acids (LCPUFAs). These molecules constitute substantial portion of phospholipids in plasma membranes. Therefore, both DHA and ARA are essential diet components. Once consumed, DHA and ARA can interact with a large variety of biomolecules, including proteins such as insulin and α-synuclein (α-Syn). Under pathological conditions known as injection amyloidosis and Parkinson disease, these proteins aggregate forming amyloid oligomers and fibrils, toxic species that exert high cell toxicity. In this talk, I will present the most recent findings that are focused on the elucidation of the role of DHA and ARA in the aggregation properties of α-Syn and insulin. We found that presence of both DHA and ARA at the equimolar concentrations strongly accelerated aggregation rates of α-Syn and insulin.1 Furthermore, LCPUFAs substantially altered the secondary structure of protein aggregates, whereas no noticeable changes in the fibril morphology were observed. Nanoscale Infrared analysis of α-Syn and insulin fibrils grown in the presence of both DHA and ARA revealed presence of LCPUFAs in these aggregates. We also found that such LCPUFAs-rich α-Syn and insulin fibrils exerted significantly greater toxicities compared to the aggregates grown in the LCPUFAs-free environment. These findings show that interactions between amyloid-associated proteins and LCPUFAs can be the underlying molecular cause of neurodegenerative diseases.

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Molecular assembly of functional amyloids involved in bacterial regulated cell-death

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We will present the structural characterization of functional amyloid sequences found in intracellular Nod-like receptors (NLRs). These high-order protein assemblies control innate immunity in plants, mammals and fungi, and sometimes contain amyloid motifs involved in complex signal transduction mechanisms leading to the activation of cell-death inducing proteins. Here we uncovered the structural architecture at atomic resolution and the assembly mechanism of such amyloid sequences in multicellular bacteria. We employed high-resolution magic-angle spinning NMR to solve the high resolution structure of the so-called BASS motif (Bacterial Amyloid Signaling Sequence) in its amyloid state and compare its architecture in isolation and in the context of full-length protein fibrillar assembly. In addition, we used X-ray crystallography to solve the structure of the globular domain attached to the amyloid domain and compare its molecular organization to known amyloid-associated NLRs found in fungi. Our work reveals the presence of new amyloid folds in bacterial NLRs and provides the first step toward the structure-function understanding of functional amyloid sequences in the execution of regulated cell-death in bacteria.

Advancing Insights into Protein Oligomer Structure and Dynamics through Protein Nanopore Engineering and Sensing

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Protein oligomers, ubiquitous in nature, assume pivotal roles in both physiological and pathological contexts. The intricate multimeric composition and variable conformations of these protein assemblies pose a challenge for comprehensive comprehension of their architecture and role. Employing a specialized biological nanopore engineering and sensing approach, we aim to discern and manipulate protein oligomer formation.

To begin, we have designed an Alzheimer's Aβ42-oligomer mimic by incorporating the extracellular domain of the pore-forming protein α-hemolysin (αHL), closely resembling Alzheimer's Aβ42 oligomers. Cryo-EM analysis confirms its stable stoichiometric structure, while single-channel electrical recordings establish functional equivalences. This engineered mimic lays a robust foundation for elucidating oligomeric structures and devising conformation-specific antibodies. Furthermore, we delve into Parkinson's α-Syn's lipid-binding kinetics within an αHL nanopore. By utilizing voltage-driven forces, we selectively capture a tailored α-Syn sequence. Pertinent conformational alterations are observed at the interface of the pore and membrane, enhancing our analytical insights. We also investigate the impact of disease-associated metal ions and peptidomimetics on α-Syn dynamics within membranes. Lastly, the dynamics and antimicrobial influences of LL-37 fragments remain elusive due to their modest size and feeble interactions. Our approach integrates an αHL nanopore, mass spectrometry, and simulations to unveil the oligomeric states of these fragments. The nanopore captures, validated by MS and MD, unveil stability and shed light on the nexus between AMP oligomer dynamics and antimicrobial actions.

Anticipating the future, our nanopore engineering and single nanopore sensing techniques hold promise in advancing our grasp of oligomers' structure and dynamics in lipid membranes. This, in turn, is expected to yield fresh insights into the intricate molecular mechanisms governing oligomer formation, toxicity, and functionality.

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Application of β2,2-amino acid based peptidomimetic foldamers as chemical model system for studying the mechanism of tau misfolding

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Tauopathies are a group of neurodegenerative diseases characterized by the deposition of abnormal aggregates of tau protein into the brain, forming neurofibrillary tangles (NFTs) inside the neuronal cells. Tau is a microtubule (MT) associated protein, whose physiological role is related to the modulation of microtubule dynamics and the correct axonal transport in neurons. In tauopathies, tau toggles from a physiological to a pathological shape, with a mechanism still being unclear. It is widely accepted that conformational or post-translational changes in the protein must initiate the autocatalytic aggregation, and that this self-assembly, associated with abnormal phosphorylation, and induces the detachment from the MT and the formation of the intracellular aggregates. Two hexapeptide sequences, named PHF6 and PHF6*, which are located in the R3 and R2 repetition domains, respectively, seem to be crucial in triggering the aggregation.1 In the native state, the hydrophobic residues of PHF6 are protected by a β-hairpin-like structure, where PHF6 can directly interact with PHF6*.2 Contrary to that, the misfolded protein exposes hydrophobic residues which can form hydrophobic interactions and drive the self-assembly process. Chemical model systems that are simpler and easy accessible than full-length amyloid proteins, but inspired by their sequences, can be designed to behave in controlled fashions to form well-defined conformations. These model systems can be used to provide insights into the structures and modes of folding of amyloid proteins. Peptidomimetic foldamers are synthetic molecules that mimic the structure of proteins. Several examples of peptidomimetic foldamers can be found in literature in the frame of a wide panel of applications, such as catalysis, medicinal chemistry, and materials science. To our knowledge, only one example of foldamer application in chemical biology, as chemical model system for the understanding of amyloid aggregation, exists.3 Here, we present the application of modular peptidomimetic foldamers as chemical model system for studying the mechanism of tau misfolding. Thanks to a rational design approach employing a new non-natural β2,2-amino acid,4 our aim is to provide, through the interchange between β-hairpin-like and extended conformations, a possible explanation of the mechanism of tau misfolding. By performing FDAP experiments, we demonstrated that the exposure of the hydrophobic residues of PHF6, due to a conversion towards an extended conformation, driving away PHF6* from PHF6, is the triggering event leading to the tau self-assembly process and to the tau microtubule dissociation.

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Funding from the European Union's Horizon 2020 Grant Agreemnt No: 860070-TubInTrain

Early diagnosis and treatment of Alzheimer's disease by targeting toxic soluble Aβ oligomers

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Objectives. The main aim of this study was to develop novel self-assembled cyclic D,L-α-peptide nanotubes as theranostic agents to diagnose early Aβ oligomers in pre-symptomatic stage of AD and to diminish memory and cognition decline.

Methods. Kinetic Thioflavin T, electron microscopy, NMR and CD spectroscopy as well as immunochemical and biochemical methods were used to study the effect of aza-glycine insertion on cyclic D,L-α-peptide self-assembly, Aβ oligomer disruption, and toxicity. The *In vivo* PET imaging studies and therapeutic activity were performed in AD transgenic mice and *Caenorhabditis elegans.*

Results. Introducing an aza-glycine residue with extra hydrogen-bond donor to tune nanotube assembly and amyloid engagement, cyclic azapeptide **1** interacted with early Aβ oligomers (1-3 mers) and inhibited Aβ aggregation and toxicity at sub-stoichiometric concentrations. NMR studies revealed dynamic interactions between **1** and Aβ42 residues F19 and F20, which are pivotal for early dimerization and aggregation. In an AD mouse model, brain PET imaging using stable ^{64}Cu-labeled azapeptide **1** gave unprecedented early amyloid detection in 44-day pre-symptomatic 5xFAD mice better than ^{11}C-PIB. No tracer accumulation was detected in the cortex and hippocampus of treated AD mice; instead, intense PET signal was observed in the thalamus, from where Aβ oligomers may spread to other brain parts with disease progression. Effectively crossing the BBB, the cyclic (aza)peptides reduced Aβ oligomer levels, prolonged lifespan of AD transgenic *Caenorhabditis elegans*, and abated memory and behavioral deficits in AD mice.

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αS modulates TDP-43 phase transitions to form distinct heterotypic amyloids.

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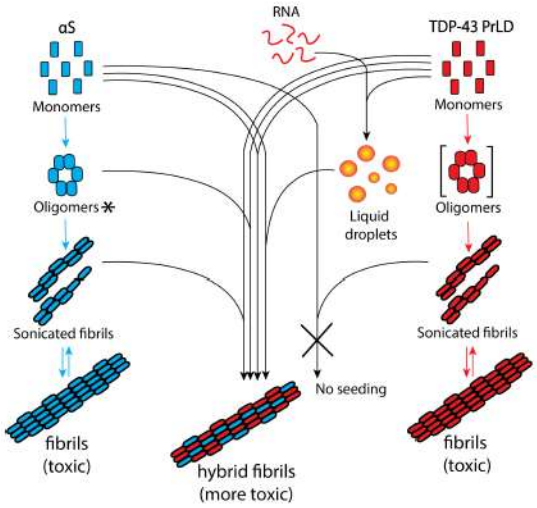
Emerging new phenotypes and clinical presentations in neurodegenerative diseases challenge the current paradigm of homotypic (single-protein) amyloid aggregates as the underlying cause for the onset and propagation of the disease. Our incomplete understanding is apparent when considering an increasing number of pathologies that exhibit distinct phenotypes and clinical presentations correlating better with colocalized cytoplasmic amyloid inclusions of both a-synuclein (αS) and TDP-43 proteins. Among these pathologies are limbic predominant age-related TDP43 encephalopathy (LATE), and multiple system atrophy (MSA). Aberrant aggregates of the two proteins also form the hallmarks of sporadic and familial proteinopathies, including frontotemporal lobar degeneration (FTLD), amyotrophic lateral sclerosis (ALS), and Lewy body dementia (LBD). We recently demonstrated that αS and TDP43 synergistically interact with each other to form distinct and toxic heterotypic aggregates and suggest a molecular basis for the observed colocalization of the two proteins and the pathological phenotypes[1,2]. Our data specifically show that: *a*) αS and prion-like c-terminal domain (PrLD) of TDP43 monomers synergistically co-aggregate toward hybrid fibrils containing both proteins, *b*) αS fibrils selectively cross-seed PrLD monomers, *c*) αS modulates the liquid droplets of TDP43-RNA coacervates to promote amyloid aggregates, and *d*) heterotypic aggregates show greater neurotoxicity and cause synaptic dysfunction than their homotypic counterparts. Together these data suggest heterotypic amyloids as underlying molecular entities for distinct phenotype emergence and comorbidities in some neurodegenerative diseases.



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Variation in stability of amyloid-beta fibrils: relation with clinical diversity and course of Alzheimer's disease?

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An intriguing feature of Alzheimer's disease (AD) is its remarkable clinical and neuropathological heterogeneity. The molecular factors underlying the clinicopathological diversity of AD are poorly understood. Structural polymorphism of amyloid-beta (Ab) fibrils abundantly found in AD patients' brains has been proposed as a molecular factor potentially underlying this diversity. Here, we present NMR data on pressure stability of various Ab40 fibrils and demonstrate how familial AD-related mutations D23N, E22G and DE22 differentially alter the kinetic and thermodynamic stability of Ab fibrils. The MD simulation data on the wild-type and mutated Ab40 fibrils provide mechanistic insights on potential structural and dynamical basis of the observed stability variation. Considering the role of fibril stability in prion-like spreading of amyloid aggregation pathology in AD brains, we propose "stability variation" as an additional molecular factor potentially contributing to clinical diversity of familial and sporadic AD.[1] Furthermore, we demonstrate through high-pressure NMR how the kinetic and thermodynamic stability of amyloid-beta fibrils is enhanced during the so-called maturation of Ab40 fibrils. In line with previous 2D IR spectroscopy data,[2] the maturation-dependent alterations in pressure stability of Ab40 fibrils suggest the higher compaction of "aged" versus freshly prepared Ab40 fibrils. We argue that this observation has implications regarding rational design of molecular imaging probes for amyloid fibrils in AD and other neurodegenerative diseases.

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Modulation of Aβ40 fibrils polymorphism by alpha-B-crystallin

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Differences in the structure of fibrils formed from beta-amyloid (Aβ) peptides might be one of the reasons for variations in Alzheimer's disease (AD).[1,2] The Small heat-shock protein alpha-B-crystallin (αB) is found at high levels in the Alzheimer's disease brains colocalizing with Aβ peptides in the amyloid plagues.[3] There are conflicting investigations that report that αB can either increase of reduced the toxicity of Aβ in the cell.[4,5] Although it was shown that αB can inhibit alpha-synuclein and Aβ fibril elongation by binding along the mature fibrils the detailed mechanism of the chaperone action and the impact on the fibril formation and structure remains unclear.[6,7,8]

In the present work, we show that αB does not only inhibit Aβ40 aggregation both in presence and absence of seeds, but also changes the aggregation pathway leading to the formation of a new fibril polymorph. We employed MAS solid-state NMR, various biophysical methods and MTT viability assay for functional and structural characterization of both obtained polymorphs. We found that both polymorphs show toxic effects on PC12 cells and have a similar amyloid core structure although they differ in the flexibility of the N-terminus. In addition, we studied the interaction of αB with Aβ40 using high-resolution fluorescent imaging. We propose a mechanism that allows to explain the modulation of the Aβ40 amyloid fibril structure and the inhibition of aggregation by αB.

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Amyloid-like fibrils of the GlcCer sphingolipid occur in Gaucher disease patient cells and cross-seed αSyn aggregation, suggesting a mechanism linking of Parkinson's and Gaucher disease

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Self-assembly and formation of amyloid fibrils has traditionally been described for proteins/peptides. Recently, it has been shown that certain metabolites, which accumulate in various hereditary Inborn Error of Metabolism (IEM) diseases, can form amyloid-like fibrils, and may contribute to disease pathology. Gaucher Disease (GD) is a lysosomal storage disease caused by mutations that cause loss-of-function of Glucosylcerebrosidase, resulting in abnormal accumulation of the sphingolipid glucosylceramide (GlcCer). GD manifests as neuropathic and non-neuropathic symptoms, and present treatment involves routine enzyme replacement therapy which is very costly. Interestingly, various epidemiological observations indicate a correlation of GD with Parkinson's disease, yet the underlining mechanism for this association is not known. Using a series of biophysical approaches, we found that GlcCer can self-assemble *in vitro* into amyloid-like fibrils which are highly reminiscent of fibrils of proteinaceous amyloids and are cytotoxic. Notably, at near lysosomal pH the GlcCer aggregates induced α-Syn aggregation *in vitro* and stabilized its nascent oligomers, comparable to the phenomenon of 'cross-seeding' of heterologous proteinaceous amyloids. This may suggest a novel mechanism to account for the prevalence of Parkinson's among GD patients. Furthermore, we found that small molecules, which are bona-fide inhibitors of aggregation of proteinaceous amyloids, can likewise mitigate amyloid-like fibril formation by GlcCer [1]. Recently we have validated these results *in vivo*: SH-SY5Y cells treated with an inhibitor of Glucosylcerebrosidase exhibited abundant GlcCer puncta which stained also with amyloid-specific dyes. Moreover, in cells conditionally expressing αSyn these GlcCer aggregates colocalized with αSyn puncta. Notably, similar observations were made in iPSCs programmed to neurons, derived from PD-GD patients indicating relevance to disease pathology.

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Tau protein induces membrane damage

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Tau, a microtubule associated protein, is an intrinsically disordered protein involved in various diseases called tauopathies, such as frontotemporal dementia and Alzheimer's disease. Indeed, in pathological conditions, tau can disassemble and accumulate in the cytosol of neuronal cells, leading the formation of amyloid fibers. The pathogenic mechanism of tau remains poorly understood.

Previous studies have shown (i) the ability of tau to form fibers upon binding with negatively charged cofactors such as heparin, nucleic acids or lipids, and (ii) the preferential disruption of membrane composed of negatively charged lipids after incubation with a tau construct called K18[1].

Here, we first focused on the disease-associated mutation P301L of the full length Tau and its impact on POPC and POPS, lipids present in the inner membrane of neurons. To address this, we have combined polarized ATR-FTIR (Fourier-transform infrared in attenuated total reflection) and AFM (atomic force microscopy) to characterize tau-membrane interactions.

Our results reveal that Tau protein can induce damage to both lipid bilayers, independently of the lipid nature, but following a seemingly different mechanism. A detergent like effect is observed on POPC membranes. On POPS membranes, protein accumulation is observed at the bilayer surface, possibly promoting tau aggregation.

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Infrared nanospectroscopy: an emerging tool to study Aβ aggregation

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Alzheimer's disease (AD) is the most prevalent form of dementia and characterized by fibrillar amyloid deposits in extra neuronal spaces. These amyloid plaques are composed essentially of the amyloid β peptide (Aβ). Aβ is also associated to early onset AD and cerebral amyloid angiopathy due to point mutations in the peptide. This polymorphism of Aβ is apparently reflected in the adopted structures, intrinsically related to its toxicity. Therefore, a better knowledge of aggregated structures of the peptide and its variants is really important for the understanding of AD and the associated pathologies.

The commonly used techniques to perform structural analysis with a high-resolution (X-ray diffraction or Nuclear Magnetic Resonnance) on those aggregated forms are not suitable, due to their transitory and/or insoluble states. Infrared spectroscopy is therefore an exquisite tool to study aggregated species, because it is possible to measure quickly protein structure even the insoluble part. Nevertheless, in attenuated total reflection Fourier transform infrared (ATR-FTIR), it is quite difficult to discriminate the different aggregated structures present in complex mixture during aggregation. The recent coupling of infrared spectroscopy with atomic force microscopy (called AFM-IR) [1] overcomes the weak spatial resolution of the usual infrared micro-spectroscopy and achieve a resolution around ten nanometers, which fits well with the size of amyloid fibrils [2]. The AFM-IR allows us recording spectrum on the different aggregated amyloid species (oligomers, isolated fibrils or amorphous aggregates) localized thanks to the height image (morphological description) obtained during AFM measurements [3]. In the future, this capability to investigate the structure and the shape of aggregated species may improve the detection of biomarkers characteristic of Alzheimer's disease and lead to a better understanding of the polymorphism of amyloids proteins.

Description of the AFM-IR measurements will be given for amyloid fibrils and results on different isolated amyloid fibrils with a diameter of < 10 nm will be shown and discussed based on their structures.

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Poster presentations

P1 - Structural characterization of PGRP-LC amyloid fibrils using solid-state NMR

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Many proteins that function as monomers can undergo conformational transitions and self-assemble into supramolecular assemblies to serve different purposes. Amyloid formation is associated with several neurodegenerative diseases, and also has been implicated in performing specific biological functions in fungi, mammals, and bacteria.

In our study, we use a combination of electron microscopy, atomic force microscopy, X-ray diffraction, and solid-state NMR spectroscopy to investigate and obtain high-resolution structural information on Peptidoglycan Receptor Protein-LC (PGRP-LC) amyloid protein. Programmed cell death (PCD) plays a vital role in the development, homeostasis, and both control and progression of a multitude of diseases. Apoptosis and necroptosis are examples of PCD processes.[1] RIP homotypic interaction motif (RHIM)-like amyloid motifs were recently identified in the *Imd* pathway controlling anti-bacterial defence pathways in insects.[2] Our goal is to understand the molecular mechanism of the *Imd* pathway from *Drosophila melanogaster* via the formation of amyloid structures.[3] We use the recombinant bacterial expression in *E. coli* to overexpress and purify amyloid motifs. Then, dipolar coupling-based NMR experiments are used to get information about the rigid part of the protein and J-coupling-based NMR experiments to obtain information on the dynamic part of the proteins. We aim to provide a high-resolution structural characterization of *Drosophila* amyloid structures formed by the protein PGRP-LC. Our results provide preliminary structural insights into the fibrillar assembly of PGRP-LC amyloid fibrils and also the characterization of the structural evolutionary diversification of amyloid motifs across different kingdoms.

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P2 - **Elucidating the molecular mechanisms of amyloid self-assembly using conformationally constrained derivatives of amylin**

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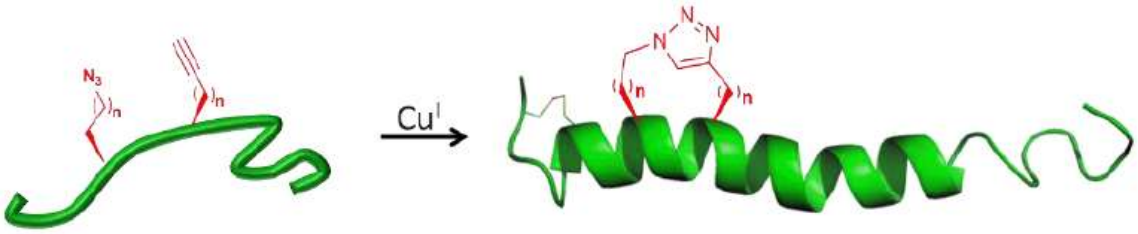
The islet amyloid polypeptide (IAPP), or amylin, is a 37-residue peptide hormone playing a key role in glucose homeostasis [1]. Nonetheless, IAPP is particularly known as the main component of amyloid deposits found in the pancreatic islets of type 2 diabetes patients [2]. The conformational modulation of this natively disordered peptide finely regulates its physiological role by affording high affinity to receptors, or its pathological implication through the formation of toxic oligomeric intermediates and eventually, insoluble cross-β-sheet amyloid fibrils. Increasing evidence suggests the presence of helical intermediates during conformational rearrangements preceding aggregation [3]. However, the highly dynamic and heterogeneous nature of oligomers constitutes a major challenge for their structural and biological characterization. In this context, the objective of this study is to design helically stabilized derivatives of IAPP through sidechain stapling to investigate the role of transient helical conformations and to modulate the function/cytotoxicity duality of IAPP. Stabilization of the helical conformation was carried out between two non-charged residues in the central domain and located on the same face of the helix (*i*, *i*+4) by azide-alkyne click chemistry, conferring structural rigidity. The stabilized helical structure strongly altered the propensity of IAPP to self-assemble into amyloid fibrils, to perturb synthetic lipid vesicles, and to induce death of β-pancreatic cells. Overall, this study constitutes the first example of macrocyclization as a chemical tool applied to an amyloidogenic peptide, allowing a better understanding of the molecular basis of protein misfolding diseases, as well as being a promising route to identify potent and stable AMY receptor agonists.



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P3 - **Investigation of Amyloid-β aggregation processes induced by light-triggered nitrosylation**

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Several studies have highlighted the presence of nitrosylation damage following neuroinflammation in Alzheimer's disease. However, published researches exhibit discordant results and observations about the possible consequences of Amyloid-β (Aβ) peptide nitrosylation, regarding both the rate and speed of the aggregation process, and the neurotoxicity of the nitrosylated product.[1][2][3]

Here, we show new data obtained by the use of a light-inducible peroxynitrite-releasing compound – the molecular hybrid benzophenothiazine–NO (BPT-NO)[4] – as a paraphysiological tool for Aβ nitrosylation. The photoactivation of BPT-NO was conducted in DMEM-F12 culture medium at the wavelength of 620-630 nm, in the presence of $A\beta_{1-42}$ peptide. The nitrosylated Aβ product was analyzed for aggregation through Western Blot analysis, Thioflavin-T fluorescence assay, High-Resolution Mass Spectrometry and tested for its activity on cellular cultures. *In vitro* studies involved the investigation of the effects of photo-nitrosylated Aβ on differentiated SH-SY5Y neuroblastoma cells[5], and microglial C57BL/6 BV2 cells.[6]

Overall, data indicate the potential of the molecular hybrid BPT-NO, irradiated at the non-toxic wavelength of 620-630 nm, as a novel tool for *in vitro* studies of Aβ nitrosylation processes.

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P4 - **The downstream effects of soluble Core Tau (297-391) on the proteome of human neuronal cells.**

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Tau is implicated in a large number of neurodegenerative diseases but its involvement in neuronal damage remains unclear. Recently, we have developed a model fragment of tau which self-assembles in the absence of additives to form Alzheimer's disease like-paired helical filaments (Al-Hilaly et al., 2017). The fragment, tau297-391 is named dGAE is internalised and can be used to examine the downstream effects of tau addition to model transmission in cells (Pollack et al., 2020). Here, we have utilised mass-spectrometry to assess the proteomic response to administration of soluble dGAE to human differentiated neuroblastoma cells (SH-SY5Y). After 24 hours incubation with soluble dGAE the cellular proteome was largely unchanged, however, there were significant alterations to the Tau interactome. Comparison of the interactome data with existing proteomic data from Human AD brains reveals that dGAE initiates cellular changes that have been attributed to early events in AD. In particular, we highlight the association of Tau with nuclear proteins, in particular proteins that are associated with both histones and the nucleolus. These observations support the hypothesis that Tau, in addition to its role in microtubule binding, has a nuclear role affecting RNA metabolism in response to cellular stress.

P5 - Architecture of a bacterial signalosome revealed by magic-angle spinning NMR-based integrative structural biology

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Signalosomes are large protein machineries associated with innate immunity functions in plants, bacteria, fungi and mammals. To fulfill their functions, several families of signalosomes rely on a complex signaling process that is achieved through the formation of functional amyloids associated with prion-like properties. The signal transduction often leads to protein relocalisation at the membrane to control a highly regulated cell death mechanism.

Here we aim at deciphering the molecular mechanism of bacterial signalosome formation and how functional amyloids are used to achieve efficient signal transduction. Using a combination of magic-angle spinning NMR combined with solution NMR, X-ray cristallography and electron microscopy, we investigate the molecular architecture of the signalosome from *Streptomyces olivochromogenes*. Taking advantage of a divide-and-conquer strategy, we first solved the high resolution structure of the globular effector domain called BELL and compared its architecture to reported fungal signalosome effectors to reveal structural divergence. Next, we showed that a domain of the signalosome can self-assemble to form homogenous filaments. X-ray diffraction was employed to determine the cross-beta nature of the filament fold, and chemical shift analysis using magic-angle spinning NMR revealed the secondary structure of this functional amyloid domain in isolation. We show no structural homology to known amyloid fold reported by solid-state NMR for signalosomes in fungi and mammals. Magic-angle spinning NMR was then used to study filamentous assemblies formed by a protein construct encompassing the globular effector and the functional amyloid domain, to explore the scaffold role of the amyloid fold in bacterial signalosomes. Taken together, our study paves the way to provide a transkingdom comparison of functional amyloid domains in signalosome machineries involved in regulated cell-death processes.

P6 - **S100B chaperone multimers suppress Aβ42 aggregation and oligomer formation**

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Aggregation of the amyloid-β 1-42 peptide (Aβ42) into fibrils and toxic oligomers is a major hallmark of Alzheimer's disease (AD) and understanding how molecular chaperones harness such conformers is critical to establish the mechanisms of neuronal proteostasis. This includes S100B, an astrocytic EF-hand Ca^{2+}-binding protein whose Ca^{2+}-switched chaperone activity counteracting Aβ42 aggregation and toxicity we recently unveiled[1]. S100B occurs predominantly as a homodimer but it is also found in the human brain as higher order multimers such as octamers, hexamers and mainly tetramers[2], whose chaperone activities remain however uncharacterized.

Towards this goal, we first set to investigate the chaperone activity of tetrameric S100B combining biophysical, kinetic and computational approaches. Resorting to ThT-monitored Aβ42 aggregation kinetics we determined that, unlike the dimer, tetrameric S100B inhibits Aβ42 aggregation at sub/equimolar ratios, an effect that persists even in the absence of Ca^{2+} binding. Structural analysis revealed that this enhanced catalytic efficiency results from a secondary Ca^{2+}-independent binding site formed by tetramerization of S100B, to which monomeric and fibrillar Aβ42 bind, as corroborated by molecular docking calculations, fluorescence and CD analysis[3].

Next, we investigated the influence of S100 multimers on the generation of Aβ oligomers (AβO) formed during Aβ42 aggregation. Mechanistic analysis revealed that both dimeric and tetrameric S100B preferentially inhibit Aβ42 surface-catalysed secondary nucleation, decreasing the reactive influx towards neurotoxic oligomers (AβO) down to <10%. To verify this mechanism-derived prediction, we established a fluorescence-based approach to independently evaluate the formation of AβO using a combination of thioflavin-T and X-34, a Congo red derivative which detects early thioflavin-negative Aβ42 conformers[4]. Altogether, our findings establish different S100B multimers as inhibitors of Aβ42 oligomerization and aggregation, further underpinning their neuroprotective role in the AD brain.

Acknowledgments: Funded by EU (TWIN2PIPSA/GA101079147), LabCollector Award (Agilebio) and FCT (Portugal) through fellowship BD/06393/2021 (AJF) and grant UID/MULTI/04046/2020 (BioISI).

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P7 - **Amyloid effect on Hsp60 abundance and distribution: implications for Alzheimer's disease therapy**

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by morphologic and molecular changes in the brain that irreversibly lead to neuronal loss and dementia. Accumulation of Amyloid-β into extracellular plaques is a neuropathological hallmark of AD. Several works have indicated that the self-association of Aβ monomers into soluble oligomers is crucial for the development of neurotoxicity [1]. Our previous studies have demonstrated that Aβ monomers are devoid of intrinsic toxicity, showing, instead, to be neuroprotective [2] and involved in glucose metabolism by acting as positive allosteric modulators of type-1 insulin-like growth factor receptors (IGF-IR) [3]. Impaired insulin-mediated signaling and decreased brain glucose consumption represent early pre-symptomatic signatures of AD development [4]. Herein we present data about Hsp60 modulation in response to the toxic stimulus of Amyloid-β exposure, and its effect on IGF-IR in primary cortical neurons. We used pre-formed oligomers to mimic AD-like conditions in vitro and a high concentration of insulin to promote brain insulin resistance as observed in AD post-mortem brains [5]. Using Western Blot analysis from total or cellular fractions, MTT assay, and flow cytometry analysis we investigated Hsp60 level and distribution, as well as its potential correlation with IGF-IR abundance. To confirm in vitro data, we also analyzed brain homogenates from APP/PS1 mice, fed or not with a high-fat diet to induce insulin resistance. Taken together, our data shed light on the mechanisms underlying the reduced glucose metabolism and indicate Hsp60 as an interesting target to be considered in Alzheimer's disease therapy.

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P8 - **Unveiling the Impact of Ubiquitin Oxidation on Ub-Aβ42 Interaction. Possible implications in AD pathogenesis**

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Protein misfolding processes play a pivotal role in the development of neurodegenerative diseases, as extensively supported by consolidated evidence [1]. However, many pathogenetic mechanisms underlying these diseases remain elusive. The accumulation of misfolded proteins, resulting from the failure of proteolytic degradation systems such as the ubiquitin-proteasome system (UPS) and autophagy, significantly contributes to the disruption of proteostasis within diseased cells.

The UPS and autophagy-lysosomal pathway rely heavily on the functionality of ubiquitin (Ub), a highly conserved and abundant protein constituting approximately 5% of the total cellular protein content. Ubiquitin aptly earns its name due to its omnipresence and involvement in a multitude of cellular functions. Remarkably, the presence of Ub-positive protein aggregates within the senile plaques of Alzheimer's disease (AD) patients suggests that UPS dysfunction observed in AD may be primarily associated with upstream components, particularly ubiquitination.

Moreover, recent studies have reported that Aβ1-40, a key peptide implicated in AD pathology, competitively binds to Ub. Notably, the binding of Ub to Aβ has been shown to reduce the propensity of Aβ to aggregate into amyloid-like fibrils [3]. To further explore the effects of oxidative stress commonly observed in senescent tissues, we investigated the functional implications of Ub oxidation and its subsequent impact on its ability to interact with Aβ1-42, another significant peptide involved in AD pathogenesis.

Preliminary results evidenced that UbOx delays the amyloid-type aggregation of Aβ in a dose dependent manner influencing the amyloid-fibril formation. These findings open new perspectives about the current understanding of the role of ubiquitin-dependent processes in the progressive loss of neurons.

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P9 - **Protein unfolding and aggregation at all stages as seen by NMR-spectroscopy**

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Protein misfolding and aggregation is closely linked to many neurodegenerative diseases like Alzheimer's or Parkinson's disease, and understanding the underlying processes like protein (un)folding, aggregation and fibril formation is a rewarding challenge. Here we demonstrate the enormous potential of NMR-Spectroscopy at all time-scales ranging from solution NMR, HR-MAS via room-temperature solid-state NMR to DNP enhanced low-temperature NMR in frozen solutions for the study of protein folding, unfolding and aggregation.

We apply a multidisciplinary approach combining solution-NMR, in-situ MAS NMR-spectroscopy and DNP-enhanced NMR solid-state NMR-spectroscopy of frozen solutions to follow and characterize the process of unfolding, oligomerization and protein aggregation of the model protein PI3K SH3 in real time and with high resolution. Structural ensembles of backbone and side-chain conformations seen in frozen solution are linked to aggregation kinetics and products at different conditions.

The protein unfolding and aggregation kinetics at different pH values and temperatures is followed by fluorescence spectroscopy as well as solution and in-situ solid-state NMR-spectroscopy at ambient temperature. Complementarily, conformational ensembles of the protein at different pH values are determined by DNP-enhanced solid-state NMR-Spectroscopy of protein in frozen solution.

Stepwise lowering the pH leads to reversible unfolding of the protein, particularly at high temperatures. Lowering the pH also enhances the conformational space sampled by the protein, thus leading to line broadening of DNP-enhanced spectra in frozen solution. At the same time, the dispersion of chemical shifts, which in solution NMR represent the ensemble average, reduces upon protein unfolding. Under conditions, which favor protein unfolding, aggregation into aggregates and protein fibrils is observed.

With concerted high-resolution and solid-state NMR spectroscopy at different temperatures we have obtained valuable insight into the concerted unfolding/aggregation process, which also plays a role in many degenerative diseases associated with protein misfolding.

P10 - **Designed Peptides as Potent Inhibitors of α-Synuclein Amyloid Self-Assembly and its Cross-Seeding by IAPP fibrils**

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Aberrant amyloid self-assembly is linked to numerous devastating cell- and neurodegenerative diseases including Parkinson's disease (PD) and type 2 diabetes (T2D). The key amyloid proteins of PD and T2D are α-synuclein (αSyn) and islet amyloid polypeptide (IAPP), respectively. Emerging evidence suggests that T2D is a risk factor for PD[1-2]. Thereby, cross-amyloid interactions between IAPP and αSyn have been suggested to be a molecular link between the two diseases[3-5]. In this context, IAPP fibrils (fIAPP) were found to cross-seed αSyn amyloid self-assembly *in vitro*[3]. Underlying mechanisms and implications of IAPP/αSyn cross-interactions for the pathogenesis of the two diseases are unclear yet. Nevertheless, the above findings suggest that inhibition of cross-seeding of αSyn by fIAPP could be a reasonable approach to suppress amyloid self-assembly and related cell damage in PD.

Here we will present biophysical and biochemical studies on designed peptides which are able to bind αSyn with nanomolar affinity and to suppress amyloid self-assembly and related cell toxicity of both self- and fIAPP-cross-seeded αSyn with nanomolar IC_{50}s. Based on their favourable properties, these peptides should be valuable tools for understanding the molecular mechanism of potentially pathogenic αSyn/IAPP cross-seeding interactions and promising leads for the development of anti-amyloid drugs in PD.

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P11 - **Mechanistic insights into the interactions between *S. aureus* amyloids and cell membranes**

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The virulence of *S. aureus*, a multi-drug resistant pathogen, depends on the expression of a plethora of toxins, among which PSMα3, secreted as either formylated (f-) or deformylated (df-) peptides. PSMα3 is able to self-assemble into amyloid-like fibrils, and, depending on its capping and conformation, can participate in diverse physiological functions, *e.g.* cytotoxicity, immune stimulation or biofilm structuration. The specific interactions with cell membranes underlying such activities remain so far elusive. We thus aim at unveiling the structure-function relationship of PSMα3, in light of its dynamic interactions with biomimetic membranes. We first showed that f- and df-PSMα3 behaves similarly in terms of fibrillation kinetics and structural content, with thin twisted cross-α fibrils, revealed by nanoinfrared spectroscopy. Atomic force microscopy additionally demonstrated that while df-PSMα3 tends to accumulate and elongate at any membrane interface, f-PSMα3 has a much higher affinity for eukaryotic membranes, especially for fluidic phases, on which it fibrillates while partially inducing morphological and mechanical disruption. Our data support the idea that f-PSMα3 oligomers might be the membrane-active entities and that local lipid disorganization could, in turn, triggers further aggregation. Besides, unlike df-PSMα3, f-PSMα3 does not deposit on bacterial membranes but has gained a bactericidal activity. Such discrepancies in the behabvior of f- and df-PSMα3 might be explained by the change in hydrophobic and electrostatic interactions imposed by the presence of a formyl group at the N-terminus or alternatively by differences in their intrinsic structures, that would require further work *e.g.* by solid state NMR and cryo-EM.

P12 - **Peptidomimetics adopting β-hairpin and helix conformation based on chaperone proteins inhibit the aggregation of amyloid proteins**

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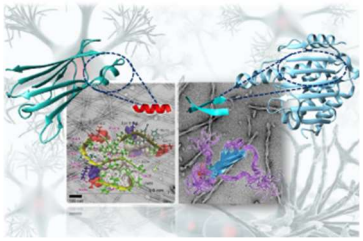
The process of amyloid deposition formation is implicated in cell degeneration and the pathogenesis of diseases such as Alzheimer's disease, Parkinson's disease and type 2 diabetes. Numerous anti-amyloid molecules have been reported over the past 25 years and most of them belong to the class of small molecules or antibodies. However, so far only one of the anti-amyloid drug candidates, Tafamidis, which inhibits transthyretin (TTR) amyloidogenesis, and two antibodies, Aducanumab and Lecanemab, which target β-peptide aggregates amyloid (Aβ1-42), have reached the clinic. Peptides represent an attractive alternative to small molecules and antibodies as anti-amyloid drugs, thanks to their improved efficacy, selectivity or specificity and potency. However, very few of them have reached the (pre)clinical stages. Peptidomimetic foldamers, bioinspired by the secondary structures of proteins, offer a promising alternative to peptides because they retain the specific side chains of a peptide sequence while having new and improved structural, biological and pharmacokinetic properties. Here I will present our new strategy to modulate amyloid protein aggregation, based on the design of peptidomimetic foldamers adopting β-hairpin and helical structures inspired by peptide sequences of chaperone proteins. [1,2] Indeed, in healthy cells, protein misfolding and aggregation are counteracted by molecular chaperones. Age-related decline renders chaperones unable to perform this function effectively. These synthetic chaperones are able to imitate the function of natural chaperones to inhibit the aggregation of amyloid proteins and allow to participate in the understanding of the role and the mechanism of natural chaperones.



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Funding: This research has received funding from the European Union's Horizon 2020 research and innovation program Marie Skłodowska-Curie Innovative Training Networks (European Joint Doctorate)—Grant Agreement No: 860070—TubInTrain (PhD funding of D. Di Lorenzo and N. Bisi). The Chinese scholarship council (CSC) is thanked for the PhD funding of C. Shi.

P13 - **ROLE OF ALPHA-SYNUCLEIN IN SONG LEARNING IN THE ZEBRA FINCH**

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Alpha-synuclein (α-Syn) is a small intrinsically disordered protein of 14 kD with a central role in the pathogenesis of neurodegenerative diseases called α-synucleinopathies, characterized by the misfolding and the accumulation of α-syn amyloids. However, its normal function is still poorly known and studied, when better understanding its role could help with research on its pathological involvement.

α-Syn is typically enriched at presynaptic terminals and has a lipid binding ability which directly influences the regulation of neuronal activity, through the modulation of vesicle clustering. This step, contributing to the control of the synaptic activity, could be crucial in learning and memory process.

During juvenile song acquisition or adult seasonal plasticity in birds, the modulation of α-Syn expression in the main telencephalic nuclei of the avian song-control circuit suggests a contribution in neural plasticity and circuit reorganization. Indeed, there is an early overexpression of α-Syn in the LMAN (lateral magnocellular nucleus of the anterior nidopallium), followed by an overall under-expression in the LMAN, the RA (robust nucleus of the arcopallium) and the HVC. Furthermore, a study in 2015 from Tanaka and al., used lentiviral vectorization on the bird song to study the clinical impact of huntingtin aggregation.

Combined with the strong involvement of aSyn in the song circuitry, our hypothesis is that aSyn aggregation spread into the song-learning brain circuitry would results in strong disturbances of the song structure. In the end, a better understanding of this protein could help with research on its pathological involvement and lead to the development of a preclinical model of synucleinopathies based on a function readout.

P14 - **Prediction of amyloid cross-interactions**

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Amyloid proteins are generally associated with diseases, such as Alzheimer's, Parkinson's, and many others. However, there is a wide class of functional amyloids that are beneficially utilized by many organisms in physiological functions, such as the formation of microbial biofilms or the storage of hormones. Recent studies showed that an amyloid fibril can affect the aggregation of another protein, even from another species, which may result in amplification or attenuation of the fibril build-up. Such cross-interactions may be crucial for understanding some of the amyloid diseases or the influence of microbial amyloids on human amyloidogenic proteins. However, due to the demanding experimental protocols, understanding of interaction phenomena is still limited. Here, we present PACT (Prediction of Amyloid Cross-interaction by Threading) – the first computational method for prediction of amyloid cross-interactions. The method is based on modeling of a heterogenous fibril formed by two amyloidogenic peptides. The resulting structure is assessed by using the statistical potential that approximates the plausibility of the model and its energetic stability. PACT was first evaluated on data collected in the AmyloGraph database and achieved high values of Area Under ROC (AUC=0.88) and F1 (0.82). We then applied PACT to study the interactions of CsgA, a bacterial biofilm protein of several bacterial species, which inhabits the human intestines, and the human Alpha-synuclein protein, which is involved in the onset of Parkinson's disease. CsgA proteins, similarly as other functional amyloids, were not used in development or general testing of our method. PACT predicted cross-interactions between these proteins. Furthermore, the method indicated the importance of specific regions in both proteins, which were shown to play a central role in the interactions. We validated this novel result experimentally by performing the experiments on these fragments.
In conclusion, we state that the new method opens a possibility of high-throughput studies of amyloid interactions. Importantly, the method can work with long protein fragments and, as a purely physicochemical approach, it relies very little on scarce training data.

The tool is available as a web server at: https://pact.e-science.pl/pact/. The local version can be downloaded from: https://github.com/KubaWojciechowski/PACT.

P15 - **Proteasome activators in neurodegenerative diseases: a new perspective of "old" drugs.**

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The therapeutic potential of compounds that enhance the activity of the 20S proteasome in treating Alzheimer's disease (AD) and other neurodegenerative disorders has been already established [1]. Activation of 20S proteasome activity can be achieved through various mechanisms utilizing small molecules that are already in the market as drugs. Our research focus in recent years has been on the repurposing of "old" molecules, specifically anti-inflammatory drugs called pyrazolones [2], and two natural compounds known as sylibins [3]. We conducted experiments using a small library of pyrazolones to assess their ability to boost proteasome activity and protect neuronal cells from amyloid toxicity. Notably, aminopyrine demonstrated promising results as a proteasome activator for the treatment of AD. Through the use of an "open gate" mutant (α3ΔN) proteasome, we were able to demonstrate that aminopyrine activates the proteasome by binding to the surfaces of the α-ring and influencing gating dynamics. Furthermore, ESI-MS studies confirmed that aminopyrine dose-dependently enhances the degradation of Aβ by the proteasome. In the case of the two natural diastereoisomers, Sil A and Sil B isolated form silybin, we evaluated their potential to increase proteasome activity. It was observed that Sil A exhibited a higher affinity and more efficient activation of human 20S proteasome (h20S). Experimental data supported by computational studies revealed that the taxifolin group present in both diastereoisomers plays a crucial role in anchoring them to the α5/α6 groove of the outer α-ring. These findings highlight the significance of stereospecific interactions in guiding the binding of small molecules to the 20S proteasome. These findings may support future rational design of proteasome enhancers.

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P16 - **Investigation of damage around aggregates of A□1-42 in brain tissue by vibrational microscopies**

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Alzheimer's disease (AD) is the most common neurodegenerative disorder and cause of dementia. The disease is pathophysiologically characterized by aggregated amyloid protein, as Aβ (Aβ). The disease is identified pathologically by amyloid plaques composed of aggregated amyloid peptide, neurofibrillary tangles composed of aggregated, hyperphosphorylated tau protein and neuron loss.
The objective of our work was to determine what damage is created within the tissue in the vicinity of the amyloid plaques of the Aβ1-42 peptide. To probe this damage, we chose to study brain sections of mice and humans with severe Alzheimer's disease by vibrational microscopy methods. These methods require no labeling and are non-destructive. Fourier transform infrared and Raman imaging on Alzheimer's diseased mice and human brain tissue were performed. Our finding suggests the accumulation of hemes in the senile plaques of both murine and human samples. We compared the Raman signature of the plaques to the ones of various hemeoproteins and to the hemin-Aβ-42 complex. The detected Raman signature of the plaques does not allow identifying the type of heme accumulating in the plaques [1]. With the same approach, by FTIR and Raman imaging, we evidenced a reorganization of phospholipids in brain tissue from AD diseased tissues of mice with severe AD [2].

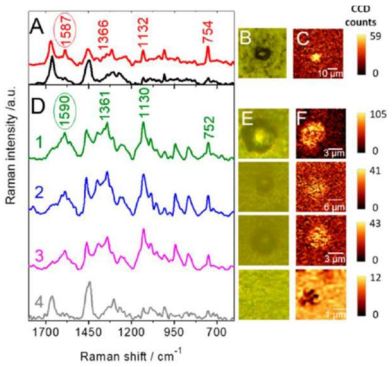

Figure 1. Raman mapping of the murine and human samples. Panel A shows the average spectra of the murine plaques (red trace) and its surrounding (black trace), the corresponding white light (B) and Raman image at 1587cm^{-1}(C). Average spectra of human plaques (D). Traces 1−3 three human AD samples and trace 4 a non-AD control.

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P17 - **The chaperone DNAJB6 forms hot-spots that delineate the neuronal α-Synuclein inclusions seeded by exogenous fibrils in primary cultures and in vivo in non-transgenic mice.**

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Parkinsons's disease, Dementia with Lewy Bodies and Multi-System Atrophy are all α-Synucleinopathies in which neurodegeneration is concomitant with the appearance and the progressive intracerebral spread of abnormal intracellular aggregates, populated by self-replicating amyloid fibrils made of α-Synuclein (α-Syn). Recent studies have focused on the stability of the α-Syn fibrils, and the possibility that the fragmentation of those fibrils could lead to the creation of small aggregates or "seeds" that could then propagate the aggregation phenomenon to neighboring cells. Molecular chaperones are enzymes that help fold newly synthetized proteins. In synucleinopathies, a chaperone system containing, among others, DNAJB6, is thought to modulate the stability and cause the fragmentation of a-syn fibrils by attempting to break them down back into monomers.

This hypothesis has been tested by inducing the aggregation of endogenous α-Syn with human pre-formed fibrils (PFFs) in vivo by intrastriatal injections and in vitro by challenging primary cultures of cortical neurons. In both experimental systems, the presence of the chaperone DNAJB6 and the neoformed a-syn aggregates were simultaneously imaged using double immunofluorescence detection. We found that DNAJB6 is upregulated in neurons in which neoformed aggregates can be observed, and that it gets concentrated in granules delineating the α-Syn inclusions. This type of association is observable both in cytoplasmic and in intranuclear α-Syn inclusions. These granules do not seem to be in a strong interaction with the a-syn aggregates, as they do not co-sediment with the aggregates after sarkosyl extraction and ultracentrifugation.

P18 - **Lipid-templated fibrillation versus off-pathways oligomers interaction with lipid membranes**

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The relationship between prion propagation and the generation of neurotoxic species and clinical onset remains unclear. Several converging lines of evidence suggest that interactions with lipids promote various precursors to form aggregation-prone states that are involved in amyloid fibrils [1]. In case of prion protein (PrP), the helical H2H3 domain is suggested to be the minimal region involved in the oligomerization process. *In vitro* experiments showed that negatively charged lipids accelerate the PrP aggregation [2]. In the current work, we examined the role of the off-pathway oligomers from a murine sequence, which has the advantage to be isolated and purified and is more stable than the corresponding oligomers generated with the ovine sequence, which is more prone to aggregation [3]. It is thus important to compare the membrane effect of the off-pathway oligomers produced in solution to the distinct intermediates, if any, generated by the lipid-templated fibrillation. Here, the biophysical characterization of the stable 12-mers from the full-length moPrP$^{23\text{-}231}$ sequence and from the truncated moPrP$^{164\text{-}231}$ are compared to decipher the effect of the H2H3 polymerization domain on their ability to bind to model membranes. Changes in the secondary structure of the proteins in contact with the planar lipid membrane were concomitantly compared to the free state in solution. The conformational changes were also studied in the presence of the lipid vesicles by circular dichroism. Static light scattering (SLS)-ThT binding assays of the different oligomeric constructs interacting with negatively charged vesicles were compared to the corresponding monomers to determine the ThT-positive lipid-bound state of the different constructs. The membrane impairment was determined by dye-release assays and single molecule capacitance experiments, and the results are discussed in relation to neuronal toxicity of the off-pathways oligomers [4].

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P19 - **The involvement of disulphide bonding in truncated tau 297-391 self-assembly and seeding capability**

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Tau protein is a natively unfolded protein involved in microtubule polymerisation and stabilisation. However, in a group of neurodegenerative diseases, termed tauopathies, tau undergoes aberrant self-assembly to form insoluble amyloid fibril aggregates, which are strongly associated with neurodegeneration [1]. With the use of recent cryo-electron microscopy, it has been shown that this single natively unfolded protein can form structurally distinct filaments in the different tauopathies [1]. Understanding the process of tau misfolding and assembly is an important step in understanding tauopathies and producing potential therapies. We use a truncated form of tau, corresponding to 297-391 of full-length tau (termed dGAE) as an *in vitro* model, which has been shown to form filaments that are structurally identical to AD filaments [2]. Disulphide bonding has previously been thought to be essential for tau self-assembly using other models of tau aggregation, such as K18/K19 and T40 with heparin [3,4]. Here we show that inhibiting disulphide bonding during dGAE self-assembly, either with reducing conditions or dGAE-C322A cysteine variant, enhances self-assembly through a reduction in the lag phase. However, we see no difference in global assembly mechanisms buy using the global fitting application in Amylofit analysis software [5]. We then show that dGAE assembled fibrils formed via inhibiting disulphide bonding are more capable of seeding endogenous tau in FRET Biosensor cells and seeding dGAE *in vitro*, as well as exhibiting a higher resistance to protease activity. This suggests that disulphide bonding is not an essential step in the self-assembly of tau filaments found in AD and further characterises the use of dGAE as a model for tau self-assembly and pathology.

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P20 - **Cryo-EM Structures of Amyloid-β Fibrils from Alzheimer's Disease Mouse Models**

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The development of novel drugs for Alzheimer's disease has proven difficult, with a high failure rate in clinical trials. Typically, transgenic mice displaying amyloid-β peptide brain pathology are used to develop therapeutic options and to test their efficacy in preclinical studies. However, the properties of Aβ in such mice have not been systematically compared to Aβ from the patient brains. Here, we determined the structures of nine *ex vivo* Aβ fibrils from six different mouse models by cryo-EM. We found novel Aβ fibril structures in the APP/PS1, ARTE10, and tg-SwDI models, whereas the human familial type II fibril fold was found in the ARTE10, tg-APP_{Swe}, and APP23 models. The tg-APP_{ArcSwe} mice showed an Aβ fibril whose structure resembles the human sporadic type I fibril. These structural elucidations are key to the selection of adequate mouse models for the development of novel plaque-targeting therapeutics and PET imaging tracers.

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P21 - **Amyloid aggregation of the tau protein involved in neurodegenerative diseases**

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Tau is a protein found in the brain which plays a crucial role in regulating microtubules. It is an intrinsically disordered protein which means that it does not have a unique three-dimensional structure. Tau can form aggregates ordered in a cross-β structure that are directly involved in several neurodegenerative diseases, called tauopathies (eg Alzheimer's disease). The formation of Tau aggregates in vitro often relies on the use of cofactors, such as Heparin, RNA and lipids. Recent research has shown that Tau protein can also interact with the neuronal membrane. It has been found that Tau can bind to phospholipids, which are major components of the neuronal membrane. Over the past few years, advancements in cryo-electron microscopy have enabled the tau aggregate structure extracted from human brains, demonstrating a structure-pathology relationship. These studies prompt us to evaluate the structure and properties of tau aggregates formed with model membranes. Here, we present biochemical and biophysical characterization of tau amyloid filament formed with different lipids. We show that anionic lipids promote the aggregation and a minimum density of charges is necessary. Together, our results demonstrate that interaction of tau with membrane might be a key modulator of tau amyloid formation.

P22 - **Overcoming a-syn antibody conformational biases for the extraction, quantification and bioactivity assessment of a-syn aggregates in biological samples.**

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Background: Synucleinopathies, such as Parkinson's disease (PD), Dementia with Lewy bodies (DLB) or Multiple System Atrophy (MSA) are neurodegenerative diseases characterized by the accumulation of alpha-synuclein (α-syn) amyloids in neurons or glial cells. α-syn is an intrinsically disordered protein, and can undergo conformational changes, leading to its aggregation. The study of these pathological aggregates of α-syn often requires the use of antibodies directed against the protein. However, the aforementioned transition between normal and amyloid α-syn implying structural changes, it can lead to the masking of some antibody recognition sites. Antibodies must then be chosen carefully by taking into account which α-syn species are studied. Unfortunately, several commonly used assays are overlooking these structural changes, which leads to biased analyses of α-syn aggregates amounts and properties in biological samples.

Methods and results: With this in mind, we thoroughly characterized α-syn antibodies, which allowed us to develop an ELISA enabling quantification of all α-syn species in a sample. In parallel, we showed that several commercially available ELISA assays were not capable of detecting amyloid α-syn. Knowing that antibodies can be specific of different α-syn species - normal, amyloid, or total - can also be useful to develop a separation technique of α-syn species in biological samples. We are currently developing an α-syn immunocapture with specific antibodies allowing to specifically separate amyloid α-syn from the pool of α-syn in samples. It has the advantage to use none or very little detergent, thus allowing us to assay amyloid α-syn bioactivity, for example in neuronal cultures.

Conclusions: Commonly used assays to analyze amyloid α-syn are flawed due to the overlooking of the amyloid structure, and as a result don't give accurate read-outs of amount of α-syn species. We were able to develop methods to determine the absolute α-syn amyloid quantity in biological samples. Furthermore, we are currently developing a novel separation method to specifically obtain amyloid α-syn from brain samples readily usable in bioactivity assays.

P23 - **Probing the physiological relevance of the "Lipid Chaperone Hypothesis"**

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An abnormal interaction of intrinsically disordered proteins (IDP) with lipids is believed to be a key player in the pathogenesis of amyloid diseases. Therefore, it is urged to unveil the molecular mechanisms at the root of lipid-protein interactions to define safe and effective drug targets. The "lipid chaperone hypothesis" [1] is a unifying theory describing the early steps of protein-membrane interaction phenomena. According to this hypothesis, phospholipids that exist in solution at their critical micellar concentration - and which are typically vesicle-free - can bind misfolded proteins such as Aβ peptide, IAPP, or α-synuclein. This binding enhances the proteins'ability to penetrate cellular membranes and modulates their pathophysiological mechanisms. In fact, free phospholipids belong to the metabolites that are considered prognostic of AD [2] and also they are released from "activated" platelets and associated with endothelial and insulin-producing beta cells damage, in diabetes [3].

Although previous works have confirmed the formation of these lipid-peptide complexes via 2D NMR, circular dichroism (CD) spectroscopy, molecular dynamics (MD) simulations, and isothermal titration calorimetry (ITC) [1], it remains unclear whether they occur in a physiologically relevant environment. To address this critical knowledge gap, we treated insulinoma cell cultures (INS 1) with fluorescent hIAPP-FAM and SHSY5Y neuroblastoma cell cultures with fluorescent Ab1-40FAM in the presence of free phosphatidylcholine at its CMC. We then used flow cytometry - a cutting-edge technique for quantifying the presence of localized fluorophores in cells - to analyze the samples. The team's findings provided evidence that vesicle-free lipids can indeed facilitate hIAPP internalization in INS 1 cells. Further studies are already underway to confirm the lipid chaperone hypothesis for Aβ peptide and other amyloidogenic proteins.

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P24 - **Domain-domain interactions and dimerization of the human λ-III immunoglobulin light chain FOR005 investigated by NMR spectroscopy**

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Misfolding of light chain (LC) immunoglobulin and deposition of amyloid fibrils gives rise to systematic amyloidosis. This study aims to characterize variable (VL) and constant (CL) domain-domain interactions in full length LC protein to better understand initial unfolding events.
We aim to find out whether specific mutations influence domain-domain interactions in light protein. To address this question the structure and misfolding of both patient and germline full length light chains are analysed via solution-state Nuclear MagneticResonance (NMR) spectroscopy.
Patient mutations are found both in the backbone and the linker region.

All proteins were expressed using 15N and 13C isotopically enriched media, and purified via anion exchange chromatography. In addition to the patient sequence, protein coding for the germline sequence as well as individual point mutations was prepared. We also prepared fibrils using ex-vivo or in-vitro patient seeds and characterize it using MAS (Magic angle spinning) solid state NMR.

We obtained high quality solution-state NMR spectra of LC, VL and CL protein. Backbone assignment experiments have been performed for both LC and CL protein. To probe dimerization, concentration dependent HSQC spectra were recorded for LC and CL protein. We established a relationship between the concentration dependence of the NMR chemical shift and the oligomeric states of the respective protein. By comparison of the chemical shifts of VL, CL, and LC protein, we were able to identify the residues that are involved in domain interactions. To find out whether full length LC protein is able to form fibrils, we performed seeding experiments using VL fibril seeds and examined the resulting samples using MAS solid-state NMR. To probe fibril formation kinetics, we carried out Thioflavin T assays as a function of the protein concentration, and in presence and absence of fibril seed.

Using NMR we were able to get molecular insight into the role of the mutations G136V and C214S for LC aggregation. We find that the LC protein is less likely to form aggregates on its own, but requires VL seeds. We hypothesize that protein unfolding is required for LC fibril formation.

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P25 - **hIAPP Fibrils by Proton-Detected Solid-State NMR**

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Deposition of the islet amyloid polypeptide (hIAPP) plays a major role in β-cell death in type II diabetes. Recently, several cryo-EM structures of hIAPP fibrils have been solved.[1,2,3] In these structures, the N-terminal part of the peptide, which contains a disulfide bond between residues C2 and C7, is not resolved. This part of the peptide is known to be important for seeding, fibril assembly and interactions with chaperones, consistent with the notion that the "fuzzy coat" on the fibril surface catalyzes these processes. Using MAS solid-state, we were able to observe the N-terminus of hIAPP in the fibril state. We find that the N-terminus is rigid, but heterogeneous. To better understand the influence of this heterogeneous region on seeding and fibril formation, we investigated wt- hIAPP and hIAPPC2S,C7S fibrils. Both peptide fibrils adopt a very similar fibril core structure.

Formation of the disulfide bond in wt-hIAPP fibrils yields a cyclic structure, which induces some strain in the amyloid fibril structure. As a consequence, the N-terminal β-strand of the oxidized peptide is shorter and only starts at residue 8, while a continuous β-strand involving residues 3-11 is observed for the peptide fibrils that lack the disulfide bond. In both peptide fibrils, His18 is solvent exposed, but protected from exchange with solvent by a hydrogen bond involving Ser-20. We find that disulfide bond formation directly affects the side chain orientation within the first β-strand. Arg11 represents a switch residue that points into the core in the hIAPPC2S,C7S fibril, while it is solvent exposed in the wt-hIAPP fibril. As a consequence, the orientation of the N-terminus changes and shields the fibril surface in the wt-hIAPP fibril. The three positively charged residues K1, R11 and the N-terminus electrostatically impede the association of monomeric peptide, and thus decrease the rate of secondary nucleation. By contrast, we observe an extended β-strand conformation for $hIAPP_{C2S,C7S}$ fibrils, facilitating nucleation processes at the fibril surface.

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P26 - **The syntetic heptapeptide SEMAX protects from Aβ cytotoxicity by reducing CU(II) catalysed ROS production and by stimulating the mitochondrial function.**

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Oxidative stress is recognized as being involved in the development of numerous illnesses, notably in Alzheimer's disease (AD)[1]. Among the acknowledged pathological hallmarks of AD are i) the presence of plaques composed of the amyloid-beta peptide (Aβ) in aggregated form along with metal-ions, and the presence of dysfunctional mitochondria. Aβ conformation, aggregation, and interaction with biological membranes are strongly influenced by the chemical modifications occurring when transition metal ions come across Aβ. In particular, the generation of reactive oxygen species (ROS) in Fenton-like reaction connected with Cu(II)/Cu(I) redox cycling of the Cu(II)- Aβ complex can play a key role in the molecular mechanism of neurotoxicity in AD)[2,3,]. The resulting ROS production may contribute to oxidative damage on both the Aβ peptide itself and on several surrounding molecule and subcellular structures such as mitochondria. However, cells can activate many strategies to counteract ROS thus improving the wellbeing of cells. Several of these strategies are coordinated by active and heathy mitochondria [4]. Semax is a synthetic analogue of the adrenocorticotropic hormone fragment ACTH, whose neuro-regenerative and cognitive activities are well known [5,6]. We have previously reported that i) Semax possesses a high affinity for Cu(II) ions [7] and affects copper-induced aβ aggregation and amyloid formation in artificial membrane model [8]. Herein, we show that Semax is able to rescue neuroblastoma cells from oxidative stress by two complementary mechanisms: i) Semax is able to extract Cu(II) from Cu(II)-Aβ species as well as influence the redox cycling of the Cu(II)- Aβ complex, decreasing the level of associated ROS production, and finally conferring cytoprotection against oxidative stress induced by copper catalyzed oxidation of Aβ peptide. ii) in the presence of Semax, SHSY5Y show improved mitochondrial functions, increased ATP levels and resistance to Aβ. We demonstrate, that Semax sustains mitochondria, through the activation of the MC4R and p-CREB signaling pathways. These findings have important implications for the Semax repositioning potential as a neuroprotective molecule.

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P27 - **New insight into the activity of the 8-20 fragment of Amyloid-β 1-42**

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Alzheimer's disease (AD) is a neurodegenerative disorder associated with cognitive impairments and progressive memory loss. In recent years, besides symptomatic treatments, new potential medications are emerging to slow down the disease and interfere with its progression. The most promising drugs, approved so far, are designed to remove soluble Aβ aggregates which represent the responsible species of amyloid toxicity [1]. Targeting specifically Aβ toxic aggregates over monomeric Aβ forms, known to cover physiologic functions [2,3], seems to be a successful approach for the treatment of AD. Several studies have been focusing on peptide fragments encompassing the primary sequence of Aβ to exploit any eventual recognition abilities toward the full-length Aβ parent peptide [4].
We have recently reported data on the Aβ 8-20 fragment which contains the self-recognizing Lys-Leu-Val-Phe-Phe sequence and lacks key points involved in the aggregation pathway and stabilization of the fibrillary structure of Aβ [5]. We showed that Aβ 8-20: i) keeps a stable random coil conformation and is not able to form amyloid aggregates by itself, ii) suppresses the Aβ1-40 and Aβ1-42 random coil to β-sheet conformational transition and reduces the dimension of Aβ1-40 soluble aggregates, iii) hampers the formation of Aβ1-42 oligomeric species, probably by interacting with the N-terminal region of the Aβ1-42, iiii) protects neuronal-like cells from Aβ1-42-toxicity. These promising results along with the observed increase in CREB phosphorylation following Aβ 8-20 treatment in differentiated neuroblastoma cultures, lead us to also investigate its potential ability to induce BDNF release by ELISA assay. Finally, the stability of Aβ 8-20 in mouse brain homogenates will be tested by HPLC-MS analysis. Overall, the herein reported data indicate Aβ 8-20 as an interesting candidate for the treatment of AD, showing to combine the required anti-aggregating properties of β-sheet breakers with the neuroprotective features of Aβ monomers.

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P28 - **Abnormalities in lysosome trafficking and ultrastructure revealed by whole-cell analysis of amyloid-b treated hippocampal neurons**

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Damage to proteostatic mechanisms that are essential for cellular health have been implicated in neurodegenerative diseases such as Alzheimer's disease (AD). The endosomal-lysosomal pathway is one such key pathway where material from extracellular sources is taken up by cells, degraded and recycled. Misfolding and self-assembly of amyloid beta proteins is characteristic of AD, but how this process is linked with the impairment of correct proteostatic functioning is not clear. Using a model of AD neurodegeneration where toxic amyloid beta oligomers are applied exogenously to primary hippocampal neurons, we have established that oligomers are endocytosed and trafficked to lysosomes resulting in a stalling of further endocytosis. We also show that oligomers are preferentially internalised over amyloid beta monomers, fibres, or fibres sonicated to a similar size to oligomers. Using newly established correlative cryo-florescence and cryo-soft X-ray tomography imaging techniques to explore cellular architecture in 3D in the native state, we also show that lysosomes in oligomer treated neurons are less X-ray dense suggesting they contain less carbon-rich material than untreated cells. Furthermore, we observe an increase in carbon dense lipid vesicles in oligomer treated cells. We suggest that the preferential uptake of oligomeric forms of amyloid beta by neurons leads to lysosomal dysregulation, and the consequential stalling of the endosomal-lysosomal pathway is an early event in the process that leads to cell death.

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