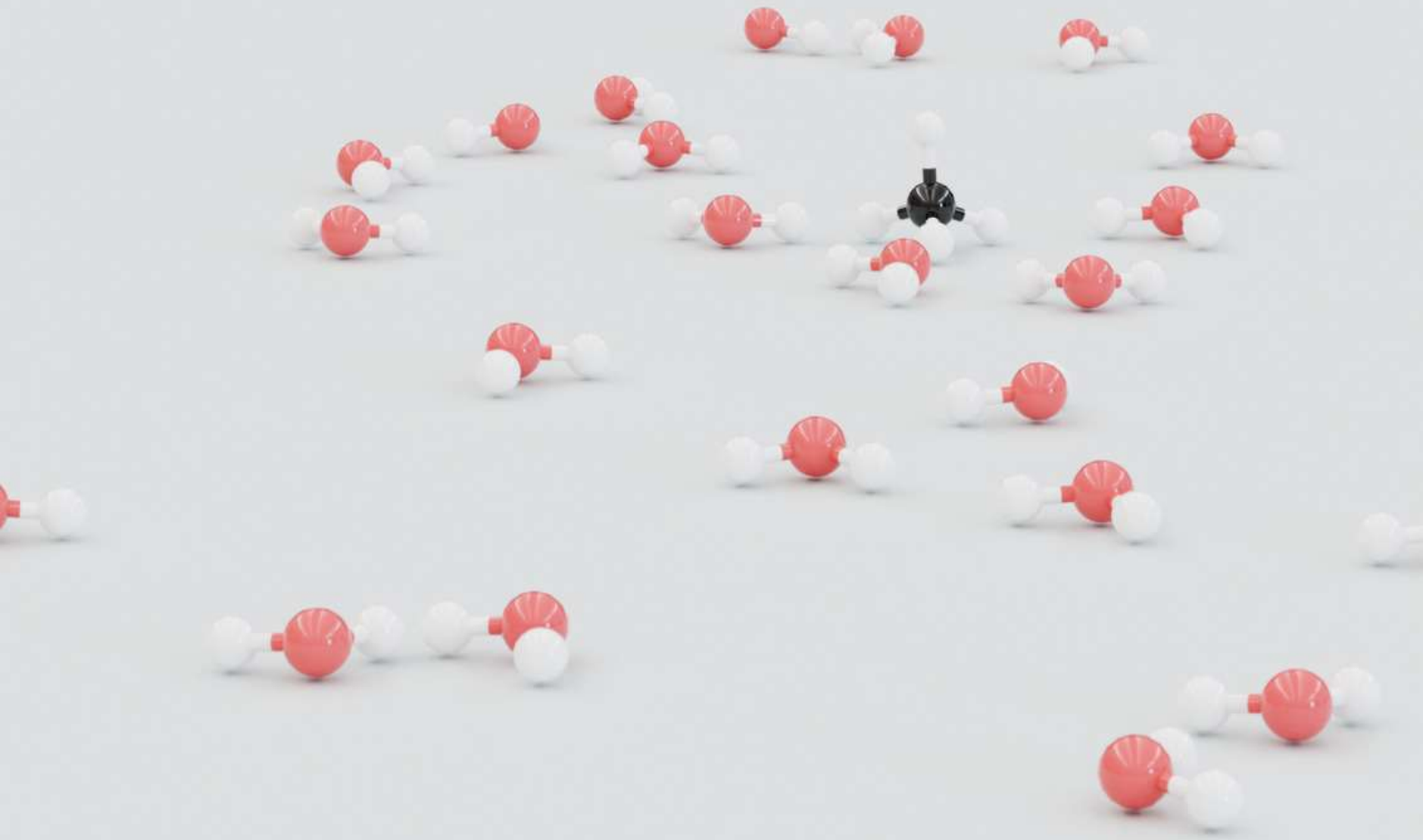
BOOK OF ABSTRACTS

4th Chem & Biochem Students Meeting



June 27th, 2024

4th Chem & Biochem Students Meeting

Oral Communications

4th Chem & Biochem
Students Meeting
June 27, 2024
FCUL - Lisboa

OC1 - Structural biology and biophysical analysis of the EARS2 protein and disease variants: unveiling their role in Leukoencephalopathy

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Aminoacyl-tRNA synthetases (aaRS) proteins are responsible for adding an amino acid to the corresponding tRNA and, consequently, they are indispensable for the translation process in the cell. Interestingly, mutations in genes encoding for mitochondrial aaRS (mt-aaRS) have been associated to particular mitochondrial disorders (MD), and an increasing number of protein variants are being identified, including in Portugal. Hence, it is essential to clarify the molecular mechanisms behind these MDs and, in particular, to provide information on mt-aaRS structure, conformation, and function to decipher the impact of the identified mutations. We aim to contribute to the field by studying the mitochondrial glutamyl-tRNA synthetase (EARS2), employing biochemical and biophysical methods to make for the first time, to our knowledge, a structural characterization of the human EARS2 wild-type and three disease variants associated with leukoencephalopathy with thalamus and brainstem involvement and high lactate (LTBL). We have heterologously expressed and purified the EARS2 wild-type in E. coli, achieving a purity yield higher than 90%. Analysis of secondary and tertiary structure revealed that the purified protein presents a folded conformation, and an apparent melting temperature of 45℃. Regarding disease variants, we established protocols using co-expression with molecular chaperones to increase the bacterial expression yield. We are also optimizing the purification of the EARS2-p.E96K variant. We believe that implementing the purification protocol for this mt-aaRS will open new avenues for characterizing variants and, in the future, aid in designing disease therapies.

References

Acknowledgements

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OC5 - Tau phase separation is regulated by the calcium-binding S100B chaperone

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The phenomenon of liquid-liquid phase separation (LLPS) involving tau is increasingly acknowledged as a contributory process in the onset of tau aggregation and the generation of pathogenic conformers within Alzheimer's disease (AD). Neuroinflammation accompanies tau pathology, with late-stage astrocyte-released alarmin exacerbating the condition, while early inflammatory responses encompass protective functions. This applies to the Ca^{2+}-binding protein S100B, which we recently implicated as a proteostasis-regulator that inhibits amyloid-beta [1] and tau aggregation/seeding [2]. These findings suggest a broad holdase-type chaperone function for S100B in counteracting the malformation of protein structures. Our study aims to elucidate S100B's role in tau LLPS. We employed PEG-induced tau LLPS followed by light absorbance. Co-localization of S100B within tau droplets was achieved using fluorescence-labelled proteins. Evaluation of droplet fluidic characteristics encompassed FRAP and fusion events. Phase diagrams indicate significant suppression of tau droplet formation by Ca^{2+}-S100B, preserving droplet liquid properties. Introduction of Ca^{2+} to PEG-induced LLPS with apo-S100B promptly reduces tau droplet levels, highlighting the dynamic, calcium-triggered nature of Ca^{2+}-S100B's action. Also, S100B effectively halts PEG-free Zn^{2+}-induced tau LLPS due to its combined Zn^{2+}-buffering and tau-interaction. Our results establish S100B as a Ca^{2+}-dependent suppressor of tau LLPS. Collectively, these findings suggests that S100B, functioning as a chaperone, also regulates phase-separated systems, strengthening its pivotal role as a proteostasis regulator in early neurodegeneration.

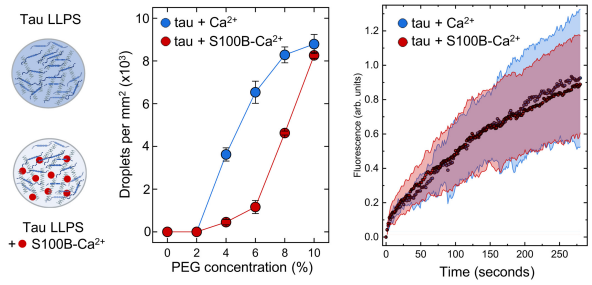

Figure 1: S100B-Ca^{2+} inhibits PEG-induced tau LLPS and colocalizes with tau droplets without compromising liquid-like droplet properties.

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OC7 - Untying the Knot: Unraveling the Functional Mysteries of Knotted Proteins via UCH-L1 Investigation

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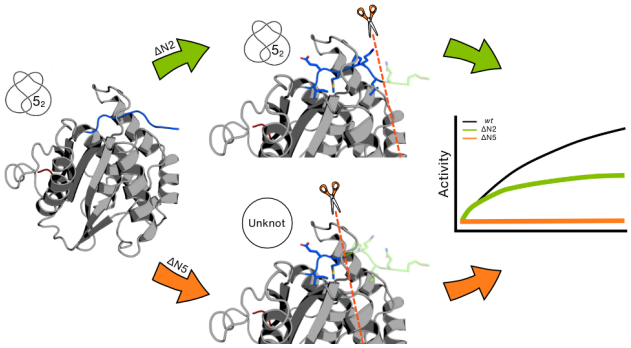
Knotted proteins, distinguished by the presence of an open knot within their native conformation, gained attention following the advent of a knot detection method in 2000 [1]. Despite their complex folding kinetics, these proteins are evolutionarily conserved across all life kingdoms, fulfilling roles such as enhancing stability and resisting degradation [2]. However, a consensus on knots functional implications remains unclear. Hence, we focus on UCH-L1, a monomeric protein with a 5_2 knot, pivotal in the ubiquitin-dependent proteolytic pathway, and linked to neurodegenerative diseases. UCH-L1 acts as a cysteine protease, featuring a catalytic triad. In its apo structure, the triad is misaligned for catalysis, but upon ubiquitin binding, a conformational rearrangement occurs bringing the residues into closer proximity, enhancing enzymatic activity. The unanswered question concerning the function of the knot stands as the primary driving force behind this research. In order to seek an answer we conducted classical MD simulations alongside in vitro experiments to investigate the impact of the knot on the catalytic activity of UCH-L1. By engineering unknotted variants via N-terminus truncation, we discovered that removing the first two N-terminal residues causes partial loss of enzyme activity, while maintaining the secondary structure and knotted topology. In contrast, removing five N-terminal residues, which disrupts the native structure and topological state, results in complete loss of enzymatic activity. These results demonstrate the dependence of UCH-L1's catalytic activity on N-terminus integrity and overall structure, intricately linked to its knotted topology.



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Flash Pitches

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F1 - Identification and characterization of a novel library of single-domain antibodies which compete for RAGE binding and modulate S100B neurotrophic activity

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S100B is a multifunctional protein associated with intra- and extracellular roles, primarily found in the brain, where it plays crucial roles in cell proliferation, differentiation, and cell survival [1]. Depending on S100B concentration, this protein can have neurotrophic and neurotoxic activity, both which are receptor for advanced glycation end products (RAGE)-mediated [2]. The neurotoxic activity of S100B triggers proinflammatory responses exacerbating conditions such as traumatic brain injury and neurodegeneration [2]. Here, we report the discovery and characterization of nanobodies (VHHs) targeting dimeric and tetrameric S100B, which are the two most abundant oligomeric functional forms of the protein with the goal of modulating S100B-mediated RAGE activation. The selected VHHs recognized structural epitopes present in both S100B conformers but not in other S100 proteins. Two of the selected VHHs bind tetrameric S100B with high affinity, as determine by biolayer interferometry analysis and stable complex formation. Structural and docking analysis revealed preferential interaction sites of the VHHs on S100B implicated in RAGE interaction. In accordance, VHHs modulated RAGE-mediated neurotrophic activity of S100B in SH-SY5Y cells by inhibiting this activity. To specify which RAGE-domains are affected by VHHs binding to S100B, we performed competition binding assays and identified VHHs that selectively inhibit S100B engagement with specific RAGE-domains. These finding uncover VHHs as powerful investigational tools to elucidate molecular and cellular mechanisms through the modulation of RAGE-mediated S100B functions and inspire potential therapeutic applications.

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F5 - Striving for new Bioadhesives inspired by Sea Urchin proteins

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Nowadays there is a great need for biological adhesives that are non-cytotoxic and efficient in wet/humid environments for biomedicine and biotechnology applications (for example, to be used in surgical adhesives). It is known that marine invertebrates produce secretions with remarkable adhesive properties in the presence of seawater (similar to physiological fluids in its high dielectric and ionic strength), which can inspire the development of new biomimetic adhesives. We have been studying sea urchins' adhesives, having identified Nectin from Paracentrotus lividus as an important adhesive protein present in its adhesive organs (tube feet) and adhesive secretions [1]. Nectin has six galactose-binding discoidin-like (DS) domains, which are thought to be important for its adhesive function [1-3]. Aiming to develop a new bioadhesive inspired in sea urchins, we are currently developing a project focused on the study of constructs whose design is based on the Nectin protein sequence. We have successfully produced one construct, that we are now in the process of characterising, in respect to structure, conformational stability, aggregation propensity, and adhesive strength, using techniques like circular dichroism (CD), fluorescence, TEM and surface coating assays. Interestingly, in the presence of certain salts at high concentration, like seawater conditions, the construct appears to aggregate as indicated by Thioflavin-T fluorescence assays and TEM imaging, evidencing possible fibre formation. Moreover, preliminary data of surface coating assays indicates that the construct adsorbs to glass surfaces forming an heterogenous coating.

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F11 - The role of post-translational modifications in the dimerization of alpha and beta splicing isoforms of STAT3

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STAT3 plays a key role in development, immune response, and cancer. STAT3 has 4 splicing isoforms, with STAT3α and STAT3β being the most abundant, which can form heterodimers. While the canonical functions of STAT3 are clearly mediated by Y705 phosphorylation, the impact of more than 80 possible post-translational modifications (PTMs) is unclear. The main goal of this work is to elucidate the putative role of PTMs on the heterodimerization, subcellular localization and transcriptional activity of STAT3α and STAT3β isoforms. To address this, we designed molecular tools based on BiFC to analyse the dynamics of STAT3α-STAT3α and STAT3α-STAT3β heterodimers, including plasmids with mutations in residues preventing key PTMs (Y705F and K685R). The response of STAT3 heterodimers to TNF-α and LIF cytokines was tested in HeLa STAT3-/- cells. STAT3α-STAT3β dimers present a nuclear-cytoplasmic distribution and accumulate in the nucleus in the presence of LIF, but not TNF-α. STAT3α homodimers with asymmetric PTM-resistant mutations at K685, K49 and S727 can accumulate in the nucleus in response to LIF. STAT3α-STAT3β heterodimers with PTM-resistant mutations at K685 are also able to accumulate in the nucleus in response to LIF. These results enhance our understanding of the role of STAT3 on fundamental cellular processes relevant to development, cancer, neurodegeneration, and inflammation.

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Acknowledgements

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Posters

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P1 - Targeting Amyloid Deposits with Radiation Therapy: A New Frontier in Neurodegenerative Disease Management

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Amyloid deposits, resulting from abnormal protein accumulation, play a crucial role in causing organ dysfunction and degeneration. Radiotherapy (RT), a mainstay in cancer treatment for over 50% of patients, has the potential to extend beyond oncology and address extra-cranial amyloidosis. Furthermore, RT shows promise in treating amyloid-associated neurodegenerative disorders such as Alzheimer's, and Huntington's diseases [1]. Proton Therapy (PT) offers significant clinical advantages over conventional RT by minimizing damage to healthy tissues [2]. Our research investigates the potential of various RT modalities to combat toxic amyloid proteins involved in neurodegenerative disorders. Initial gamma-irradiation experiments conducted at C2TN demonstrated a dose-dependent reduction in pathological protein levels. Similar outcomes were observed with photon and electron irradiation experiments performed at ULSSM. PT experiments will be conducted at CMAM supported by dosimetry measurements. Monte Carlo simulations using TOPAS will be employed to model radiation effects on protein deposits. Additionally, an in vitro model is under development to study the impact of radiation on protein aggregation, using Thioflavin T as a probe. Our presentation will showcase the results from gamma-irradiation and current photon and electron experiments. We aim to broaden the applications of PT, enhancing its scope and potentially altering the progression of neurodegenerative disorders.

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Acknowledgements

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P4 - Exploring Halogen Anisotropy: Impact on Membrane Permeability of Halogenated Drugs

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Halogenation is a common approach to enhance drug-like properties, including membrane permeability. However, halogen atoms can interact with biologically relevant targets, such as proteins and nucleic acids through halogen bonds (XBs) or hydrogen bonds (HBs). This ability arises from the anisotropic electrostatic potential of covalently bonded halogens (X), which creates a positive region (σ-hole) on X opposite to the R–X bond axis. Recent findings [1] suggest that XBs play a role in ligand-membrane interactions, hinting at their potential influence on drug permeation. However, the extent of this effect remains unclear. In this work, six commercially used halogenated drugs - diazepam, bromazepam, clonidine, metolazone, furosemide, and amiodarone - were studied through molecular dynamics simulations combined with umbrella sampling. The permeability coefficients (Pcalc) were derived using the inhomogeneous solubility-diffusion model (ISDM) and a ranking score based on the difference between the maximum and minimum free energies (δGranking). The study revealed very high correlations (R > 0.9) between the calculated and experimental permeability values when using the ISDM model. Incorporating an EP (to emulate σ-hole) facilitated the accurate sampling of XBs without disrupting the sampling of HBs and affecting the Pcalc. These findings highlight the importance of XBs in drug permeation and provide a robust methodology for future studies.

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P7 - Exploring The Role of Sacsin and S100B chaperones in Cytoskeleton Organization in ARSACS Disease

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Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a recessive neurodegenerative disorder caused by mutations in the SACS gene, resulting in truncated or defective forms of the 520 kDa multidomain protein sacsin. The biological role of sacsin is barely known, although it displays chaperone activity and is related to mitochondrial behavior and function. While ARSACS studies have focused on neuronal cells, we have recently observed that sacsin is highly expressed in astroglia and developed a glial cell model of ARSACS to study their role in the disorder [1]. Sacsin knockout leads to an accumulation of the intermediate filaments in the juxtanuclear area and an upregulation of the S100B chaperone. S100B can play a protective role in neurodegenerative disorders by interfering with the formation of toxic protein aggregates [2]. Sacs-/- cells exhibited S100B accumulation near the intermediate filament aggregates. Additionally, we are studying the effects of S100B knockdown on ARSACS cell phenotype. Withaferin A, an inhibitor of vimentin organization, induces juxtanuclear aggregation of glial intermediate filaments resembling Sacs-/- cell phenotype. However, S100B levels did not increase in response to Withaferin A. We are currently exploring the differences between the genetic and pharmacological models of ARSACS. Our results may provide relevant information for the future treatment of ARSACS but also advance our basic understanding of the function of sacsin and S100B proteins in cytoskeletal and mitochondrial organization.

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P11 - SPAX8-related mutations in NKX6-2 form stable aggregates

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NKX6-2, a transcriptional factor, influences neurons, oligodendrocytes, and pancreas cell fates, as well as myelin formation and maintenance. Loss-of-function mutations of NKX6-2 are the cause of Spastic Ataxia 8 (SPAX8), a childhood-onset neurodegenerative disease characterized by hypomyelinating leukodystrophy. Our previous work [1] explored SPAX8-related mutations in a NKX6-2-Venus fluorescent protein fusion system, where we noted the aggregation process in most cases. Currently, we are investigating the nature of these aggregates using timelapse microscopy and FRAP assays, observing that SPAX8-related mutations form solid and stable aggregates. In parallel, we are using bioinformatic databases and tools, such as AlphaFold 3.0, to uncover possible insights on NKX homeodomain's interaction with DNA and tinman domain's interaction with the Gro/TLE family. As it was previously observed [2] that the repression complexes form by Gro/TLE family are important for optimal function of multiple proteins of the Sonic the Hedgehog pathway during neuron cell fate, including some members of the NKX family. Our aim is to understand the molecular mechanisms behind SPAX8 and NKX6-2 as whole, as an important step to design a therapeutic strategy for this rare disorder.

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P21 - Exploring tau and amyloid-β cross-interactions in Alzheimer's Disease

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A hallmark of Alzheimer's Disease (AD) is the emergence of proteinaceous aggregates in the brain, mostly composed by amyloid β (Aβ) in extracellular plaques, and tau in intracellular tangles, with protein dysregulation starting from earlier stages. Despite their different localization, tau exits cells and spreads throughout the brain, highlighting the extracellular milieu as a space where potential interactions between tau and Aβ might occur, that could influence the progression of the pathology. Furthermore, S100B, a late-stage alarmin, can act as an early-stage extracellular chaperone against tau [1] and Aβ2 [2] aggregation. Thus, we explored plausible cross-interactions between tau and Aβ in vitro, and the effect of S100B in this context. As a tau model we used the tau fibril core (TADC, $tau_{306-378}$), which aggregates without heparin, making it closer to in vivo settings. It was evidenced that TADC aggregation was accelerated in the presence of Aβ42 in a concentration-dependent manner but Aβ42 aggregation was inhibited by tau. S100B showed a dual-behaviour on TADC aggregation, accelerating it at lower ratios and fully inhibiting it at equimolar ones, though this inhibition was not complete when heparin was present, suggesting that its presence conditions the type of species generated, which were also differentially detected by the amyloid-sensitive fluorophores ThT and X-34. Lastly, in mixed conditions with Aβ42, the inhibitory effect of S100B was lost, hinting to a competition for the S100B dimer. Overall, these results underline the complex interplay of events taking place in early AD scenarios, stressing the need to explore them further to better understand AD progression.

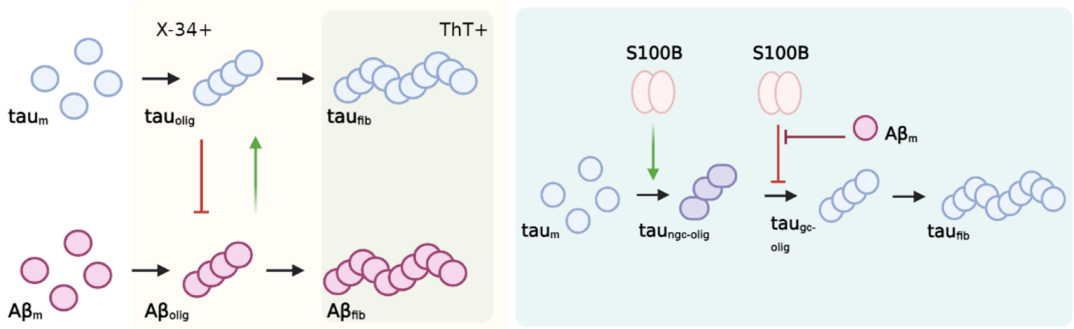

Figure 1: Aβ aggregation was inhibited by tau while tau aggregation was enhanced by Aβ. S100B has a dual-effect over TADC aggregation, with Aβ interfering if present.

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P22 - Acylations as modulators of mitochondrial beta oxidation proteins

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Metabolic regulation involves a complex interplay of genomic, proteomic and metabolic adjustments within cells. A particular group of non-enzymatic post-translational modification (PTMs) known as acylations, such as succinylation and glutarylation, have emerged as important regulators of mitochondrial enzymes [1]. The extent of acylations is closely associated with the accumulation of intermediate metabolites such as Succinyl-CoA and Glutaryl-CoA, that occur under certain conditions such as caloric restriction and in several metabolic disorders, creating a unique scenario for anomalous protein acylation [2]. Although several studies have identified enzymes that are acylated, and sirtuin substrates (enzymes responsible for modification reversion), the impact at the protein structural and functional level of these modifications remains to be fully addressed. Taking advantage of our know-how with mitochondrial beta oxidation enzymes, we have been studying acylation's impact in different proteins using biochemical and biophysical techniques. We have shown that glutaryl-CoA dehydrogenase (GCDH) is prone to high levels of glutarylation, due to increased glutaryl-CoA production stimulated by lysine catabolism, which diminishes enzyme activity and is regulated by sirtuin5 (Sirt5) [3]. In contrast Medium Chain Acyl-CoA Dehydrogenase (MCAD) and Electron Transfer Flavoprotein (ETF) are prone to succinylation. Interestingly, succinylation increases MCAD activity but decreases ETF activity, although no major differences were shown in neither proteins' structure nor stability. Further, Sirt5 incubation reverts succinylation and brings function of both proteins to unmodified levels.

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M4 - Phase Separation and Misfolding of TDP-43 in Neurodegenerative Diseases

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Transactive response DNA-binding protein 43 (TDP-43) is a nucleic acid-binding-protein with important physiological roles in RNA metabolism, regulating transcription and translation, mRNA splicing, transport and stabilization, non-coding RNA processing and stress granule (SG) assembly in neurons. TDP-43 has been implicated in a wide range of neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTLD), Alzheimer's and Lewy bodies disease. It has been suggested that the mechanisms underlying TDP-43 neuropathology consist of nuclear loss-of-function and cytoplasmic gain-of-function through depletion of TDP-43 in the nucleus and mislocalization and further aggregation in the cytoplasm. TDP-43 undergoes liquid-liquid phase separation (LLPS) to form membraneless organelles, likely contributing to RNA processing spatiotemporal control in the nucleus. Moreover, upon transient cellular stress, the intrinsically disordered low complexity domain (LCD) of TDP-43 promotes LLPS mediating reversible assembly of SG in the cytoplasm which can become irreversible. In neurodegenerative diseases, mislocalization of TDP-43 enhances the transition of TDP-43 liquid droplets into a solid state leading to spontaneous aggregation and neurodegeneration. Furthermore, it has been evidenced that misfolded TDP-43 might propagate toxicity in a prion-like manner. This mini review aims to provide an overview of the current understanding of TDP-43's role in health and disease. I will discuss the complex mechanisms, including toxic self-assembly and phase separation, that contribute to neurodegeneration, and the existing knowledge gaps in this area.

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