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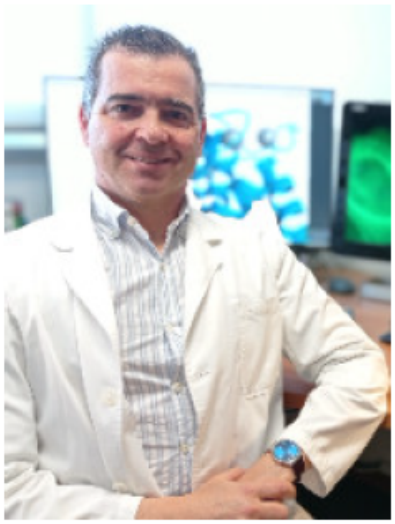

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Dr. Gomes is a biochemist specialized in protein folding, structural biochemistry, and biophysics working at the University of Lisbon. In his lab, Dr. Gomes and his team explore molecular mechanisms underlying protein misfolding and aggregation, from the molecular scale to cells and organisms. They are driven to gain in-depth insights into proteostasis regulation in the human brain, with a particular focus on its impact on Alzheimer's Disease and other neurodegenerative conditions. Their research has uncovered novel chaperones and mechanisms that delay pathological interactions. Dr. Gomes's mission is to bridge the gap between the intricate biochemistry of neuronal dysfunction and practical applications for improved treatments in neurodegeneration.
https://folding.campus.ciencias.ulisboa.pt/

Abstract

Aims

Proteostasis and protein misfolding and aggregation are central across neurodegenerative diseases and tauopathies. In AD, aggregation of Aβ and tau proceeds during prodromal stages, implying a heightened proteostasis burden along the disease continuum. While Aβ self-assembles primarily outside cells, intracellular tau aggregates are promptly secreted, spreading pathology to nearby cells also as toxic oligomers. Therefore, chaperones with intra and extracellular activity are key proteostasis regulators controlling molecular mechanisms in the diseased brain.

Methods

Molecular, cellular, biochemical, and structural approaches show that S100B is associated with novel neuroprotective functions as holdase-type chaperone, modulating tau aggregation. The S100-family calcium-binding proteins have intra and extracellular activities, playing roles during neuronal dysfunction. While S100 proteins co-localize with inclusions, their response to proteotoxic insults is only now emerging [1].

Results

S100B functions as a Ca^{2+} -switched chaperone inhibiting Tau aggregation under sub-stoichiometric conditions [2], as well as Aβ42 [3-4]. S100B interacts dynamically with PHF-segments in tau-MBTR and with tau in living cells, even in microtubule-destabilizing conditions. In agreement with a role as an extracellular chaperone and its accumulation near tau positive inclusions, we show that S100B blocks proteopathic tau seeding. Recently, we established that S100B modulates Tau LLPS, incorporating into Tau droplets as inferred from FLIM-FRET studies, efficiently reversing tau demixing [5].

Conclusions

Overalll, S100 proteins are a group of important proteostasis regulators acting broadly on tau and protein aggregation and condensation phenomena across the neurodegeneration continuum. Acknowledgments: Collaborators, research team and funders are gratefully acknowledged. Funded by EU-TWIN2PIPSA/GA101079147 and FCT-Portugal (MCS)/UID/MULTI/04046/2020-BioISI).

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[3] Cristóvão et al (2018) Sci. Adv. https://doi.org/10.1126/sciadv.aaq1702
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[5] Moreira & Gomes (2023) J. Neurochem. https://doi.org/10.1111/jnc.15756

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Shift 1 - 0146

CHAPERONE MULTIMERS SUPPRESS THE GENERATION OF AB42 NEUROTOXIC OLIGOMERS IMPLICATED IN ALZHEIMER´S DISEASE

POSTER SHIFT 01 (MARCH 6-7): THEME A: B-AMYLOID DISEASES / A02.J. THERAPEUTIC TARGETS, MECHANISMS FOR TREATMENT: PROTEIN AGGREGATION, MISFOLDING, CHAPERONES

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Aims: Alzheimer's disease (AD) involves extracellular aggregation of Aβ42 into toxic oligomers and fibrils, whose emergence is regulated by molecular chaperones. These include S100B alarmin, a homodimeric EF-hand protein with intra and extracellular functions which acts as a Ca^{2+}-switched Aβ42 anti-aggregation chaperone. However, S100B occurs also as a homotetramer, with uncharacterized neuroprotective roles. Here, we compared the chaperone activities of both S100B multimers and explored their impact on the formation of Aβ42 oligomers (AβO).
Methods: S100B anti-aggregation activity was evaluated by thioflavin-T (ThT) Aβ42 aggregation assays. Aβ42 conformers targeted by S100B were accessed by computational and structural-biophysical spectroscopies. Aβ42 oligomer distributions were determined through mechanistic analysis of fibril formation and via early detection of Aβ42 species using the X-34 fluorophore.
Results: Aβ42 aggregation kinetics revealed that, unlike the dimer, tetrameric S100B delays Aβ42 aggregation and reduces the amounts of fibrils formed 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 upon tetramerization of S100B, with which monomeric and fibrillar Aβ42 interact (Figueira et al JMB 2022). Kinetic and mechanistic analysis revealed that dimeric and tetrameric S100Bpreferentially inhibit Aβ42 fibril surface-catalyzed nucleation, decreasing the reactive influx towards oligomers down to <10%. Such results comply with an independent screening of AβO using a combination of the thioflavin-T and X-34 fluorophores (Figueira et al Front. Neurosci. 2023).
Conclusions: Our study sheds new insights on the functional landscape of S100B chaperone multimers, suggesting its critical role in the regulation of proteopathic Aβ42 aggregation and oligomerization in AD.
Funded by EU-TWIN2PIPSA/GA101079147 and FCT-Portugal BD/06393/2021 (AJF)/UID/MULTI/04046/2020 (BioISI).

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Shift 2 - 0445

HETEROTYPIC INTERACTIONS DRIVE ANTI-AGGREGATION ACTIVITY OF NANOBODIES AGAINST S100B ON TAU AGGREGATION

POSTER SHIFT 02 (MARCH 8-9): THEME B: TAUPATHIES / B02.F. THERAPEUTIC TARGETS, MECHANISMS FOR TREATMENT: PROTEIN AGGREGATION, NFT, MISFOLDING, CHAPERONES

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Aims: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the World Health Organization as a public health priority. The pathology is characterized by the aggregation of amyloid-β (Aβ) and Tau protein into amyloid-β plaques and neurofibrillary tangles, respectively. Neuroinflammation is also implicated in AD and is responsible for the secretion of alarmins, which include the S100B protein. S100B is highly studied in the context of AD, and it's known for its dual function as a detrimental pro-inflammatory mediator and a beneficial anti-aggregation chaperone over Aβ and tau, making it an amenable drug target. Since there is still no cure for AD and is highly attractive to target chaperones with an already reported anti-aggregation activity, a library of single-domain antibodies (or nanobodies) targeting S100B was developed to potentiate S100B chaperone activity and modulate tau aggregation.
Methods: Here, we employed ThT-monitored kinetics of heparin-induced K18 aggregation to study the effect of nanobodies alone and in combination with S100B on K18 aggregation. Moreover, we performed mechanistic analysis to determine which step of the aggregation reaction are targeted by the nanobodies.
Results: Several nanobodies potentiated S100B inhibitory effect over K18 (Tau244-372), possibly by harnessing S100B in a more competent conformation to bind K18. Surprisingly, control experiments revealed that some nanobodies alone significantly inhibit K18 aggregation even at sub-stoichiometric ratios. This striking observation is discussed in the context of possible heterotypic interactions between the nanobody CDR3 region and Tau/K18. Further, mechanistic analysis demonstrates that different nanobodies target multiple steps of K18 fibrillation.
Conclusions: These findings uncover the therapeutic potential of anti-S100B nanobodies, which can be used as modulators of K18 aggregation or as activators of S100B chaperone activity. Funded by EU-TWIN2PIPSA/GA101079147 and FCT-Portugal BD/11023/2022 (MCS)/UID/MULTI/04046/2020 (BioISI).

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Shift 2 - 0444

TAU LIQUID-LIQUID PHASE SEPARATION IS REGULATED BY THE CALCIUM-BINDING S100B CHAPERONE

POSTER SHIFT 02 (MARCH 8-9): THEME B: TAUPATHIES / B02.F. THERAPEUTIC TARGETS, MECHANISMS FOR TREATMENT: PROTEIN AGGREGATION, NFT, MISFOLDING, CHAPERONES

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Aims: 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-β (Cristóvão et al. 2018 Sci Adv) and tau aggregation/seeding (Moreira et al. 2021 Nat Commun). 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.
Methods: PEG-induced tau LLPS was followed by spectroscopy measurements of light absorbance (400nm) and tau fluorescently labelled. Co-localization of S100B within tau droplets was achieved using fluorescence-labelled proteins and FLIM-FRET. Evaluation of droplet fluidic characteristics encompassed fluorescence recovery after photobleaching (FRAP) and observation of fusion events.
Results: Phase diagrams indicate significant suppression of tau droplet formation by Ca^{2+}-S100B, preserving droplet liquid properties. The 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. Likewise, S100B effectively halts PEG-free Zn^{2+}-induced tau LLPS due to its combined Zn^{2+}-buffering and tau-interaction capabilities.
Conclusions: Our results establish S100B as a calcium-dependent suppressor of tau LLPS. Collectively, these findings suggest that S100B, functioning as a chaperone, regulates the formation of various pathological conformers and phase-separated systems, strengthening its pivotal role as a proteostasis regulator in early neurodegeneration. Acknowledgments: EU for funding Twinning Grant EU-TWIN2PIPSA/GA101079147, FCT/MCTES (Portugal) for funding UIDB/04046/2020 and UID/MULTI/04046/2020 (BioISI) and PhD grant 2020.06443.BD (GGM) and Agilebio for funding LabCollector Scientific Award 2021 (CMG).

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Shift 1 - 0315

EXPLORING EARLY EXTRACELLULAR DISEASE MECHANISMS: CROSS-INTERACTIONS BETWEEN TAU AND AMYLOID BETA IN ALZHEIMER'S DISEASE

POSTER SHIFT 01 (MARCH 6-7): THEME B: TAUPATHIES / B01.A. DISEASE MECHANISMS, PATHOPHYSIOLOGY: TAU AGGREGATION, PHOPHORYLATION, ACETYLATION & MODIFICATIONS

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Aims: Since the underlying mechanisms of early Alzheimer's Disease are not well understood, we aimed to explore plausible extracellular cross-interactions between Tau and Amyloid beta (Abeta) that could condition disease development, as well as the effect of the early-stage chaperone S100B in this context.
Methods: The effect of Abeta on Tau was assessed following the aggregation of a fixed concentration of the Tau AD core (TADC, $Tau_{306-378}$) by monitoring X-34 fluorescence intensity in the presence of Abeta42 monomers under cofactor-free conditions. To study the effect of Tau over Abeta, the *in vitro* aggregation of a fixed concentration of Abeta42 monomers in the presence of increasing proportions of TADC and Tau-K18 ($Tau_{244-372}$) was monitored by ThT fluorescence. Finally, the influence of S100B on TADC aggregation, with and without heparin, was similarly evaluated with the fragment alone and in the presence of Abeta42 monomers. The toxicity of end-point species was assessed by liposome leakage assays.
Results: TADC aggregation was accelerated in the presence of Abeta42 in a concentration-dependent manner, and the end-point species present were fully toxic for liposomes. On the contrary, Abeta42 aggregation was inhibited by both TADC and K18. S100B had a dual-behavior on TADC aggregation, accelerating it at substoichiometric proportions and fully inhibiting it at equimolar proportions, though this inhibition was not complete when heparin was present. Finally, under mixed conditions with Abeta42, the inhibitory effect of S100B over TADC aggregation was lost.
Conclusions: Tau and Abeta have opposite effects on each other's *in vitro* aggregation. S100B, while able to inhibit TADC aggregation and toxicity, showed a concentration-dependent dual-behavior, accelerating its aggregation at lower proportions. These results highlight the complex interplay of events in early-disease scenarios in Alzheimer's Disease.

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Shift 1 - 0302

DEVELOPMENT OF CELL MODELS OF SPORADIC ALZHEIMER'S DISEASE BY CAPTURING PATIENT-DERIVED TRANSCRIPTOMIC FINGERPRINTS

POSTER SHIFT 01 (MARCH 6-7): THEME A: B-AMYLOID DISEASES / A06.G. CELL, MOLECULAR AND SYSTEMS BIOLOGY: OTHER

Alicia González Díaz[1], Andrea Possenti[1], Gustavo Antonio Urrutia[2], Benedetta Mannini[3], Michele Vendruscolo[1]
[1]University of Cambridge, Yusuf Hamied Department Of Chemistry, Cambridge, United Kingdom, [2]WaveBreak Therapeutics, Cell Biology Platform, Cambridge, United Kingdom, [3]University of Florence, Department Of Experimental And Clinical Biomedical Sciences Mario Serio, Florence, Italy

Aims: While the aetiology of familiar Alzheimer´s Disease (fAD) is determined by genetic mutations in well-characterised genes, sporadic AD (sAD) arises from a complex interplay between genes and environment that remains poorly understood. This project rises from the hypothesis that early causative disease states, triggered by genetic and external factors, highly difficult to model, can be defined by transcriptomic fingerprints that functionally drive the cells into the disease phenotype. In this context, we aimed to integrate such fingerprints in human cells for the generation of sAD models.
Methods: We have developed a pipeline for the validation of early transcriptomic alterations identified from post-mortem patient samples which hold the potential to be causative of sAD. Bioinformatically-predicted candidates were analysed in human neuroblastoma SH-SY5Y cells to assess the effect of their knockdown (KD) on Aβ aggregation. Shortlisted candidate genes were then validated in iPSC-derived cortical neurons to study whether AD relevant phenotypes were triggered upon early expression perturbation.
Results: Our SH-SY5Y pre-screening assay identified 12 candidates which increased Aβ aggregation upon KD. FBXO2, a subunit of the ubiquitin protein ligase complex SCF, was chosen for further validation. The early downregulation of *FBXO2* in wild-type human cortical neurons triggered amyloidopathy, tauopathy and both functional and structural network impairment, hence enabling the generation of a comprehensive model of sporadic AD.
Conclusions: We report a neuronal model of sAD that recapitulates a set of key molecular AD hallmarks. We envision to expand our strategy towards the generation of panels of preclinical models that faithfully capture the molecular complexity of the full spectrum of AD patients, steering the drug discovery field towards personalised medicine.

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Shift 2 - 0473

EFFECTS OF SPLICE VARIANTS ON THE PATHOGENIC AGGREGATION OF ALPHA-SYNUCLEIN

POSTER SHIFT 02 (MARCH 8-9): THEME C: A-SYNUCLEINOPATHIES / C01.A. DISEASE MECHANISMS, PATHOPHYSIOLOGY: A-SYNUCLEIN AGGREGATION

Alexander Röntgen, Zenon Toprakcioglu, Samuel Dada, Owen Morris, Michele Vendruscolo
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Aims: The aberrant aggregation of alpha-synuclein has long been associated with Parkinson's disease (PD) and related synucleinopathies. The predominant isoform of alpha-synuclein is expressed as a 140-amino acid protein (aSyn-140). However, alpha-synuclein does not exist as a single molecular species in cellular environments but as a variety of proteoforms, generated by variations in splicing, with increasing evidence suggesting that such variants are involved in the overall aggregation process and in the progression of the disease. We therefore investigate the role of splice variants in aggregation and cytotoxicity, as well as their aggregation in mixtures.
Methods: We employed computational solubility predictors, fluorescence-based aggregation assays, kinetic analysis and modelling, as well as electron microscopy to elucidate the mechanism of alpha-synuclein aggregation. In parallel, we used cell viability assays to determine the cytotoxicity of the formed aggregates on SH-SY5Y neuroblastoma cells.
Results: Variations in splicing led to marked differences in the aggregation kinetics and mechanisms, as well as morphological and cytotoxic properties of the proteoform aggregates. Moreover, we determined that the aggregation of aSyn-140 was influenced and modulated by the presence of splice variants.
Conclusions: Our findings imply that the mechanisms leading to the production of diverse proteoforms of alpha-synuclein may be involved in the pathogenesis of synucleinopathies. These results may open the possibility to further understand the role of alpha-synuclein splice variants in the pathological aggregation of this protein and therefore the development of synucleinopathies.