

**Developing Biotherapeutics for Neurological Disorders,  
10 December 2018  
Royal Society of Chemistry, London, UK.  
Organised by the RSC Biotechnology Interest Group**

This was the fourth in a series of symposia (2009, 2013 and 2016) concerned with this important area of research. The symposium featured 8 speakers (one from Sanofi, France) and 8 poster presentations. The emphasis was on gaining a better understanding of the molecular processes that govern normal brain function and dysfunction. The presenters explained how their findings could translate into new therapeutic agents for brain disorders such as Alzheimer's and Parkinson's disease.

There were good interactions between the speakers and delegates and the posters initiated lively discussions during lunch and during the refreshment breaks.

A prize of £50 was awarded for the best poster. The recipient, Ms Sabine Ulamec from Leeds University received the prize from Professor Sir Christopher Dobson.

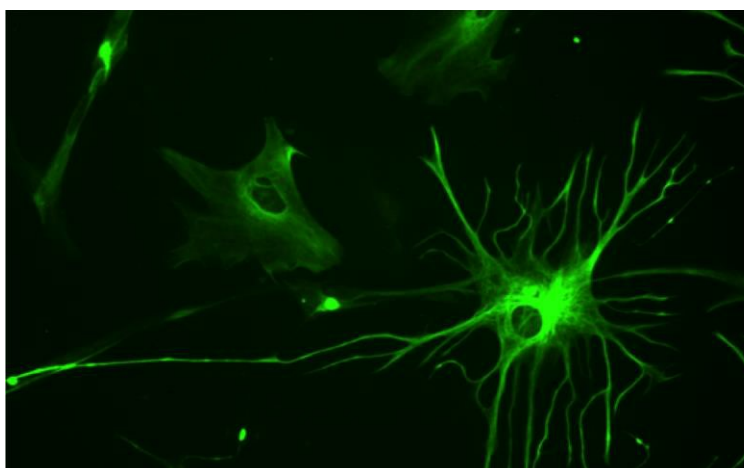
A number of delegates and presenters requested that we continue with this series of symposia holding one every 2 years. The full Programme, Speakers' Abstracts and Poster Abstracts are appended hereto.

We would like to thank: the catering and RSC Events staff of the *RSC Chemistry Centre* for Their hospitality and professional support contributed to the success of the event.

January 2019



## ***Developing Biotherapeutics for Neurological Disorders***



A stimulating one-day programme of presentations by key scientists and clinicians working at the forefront of Neurology Research & Development, organised by the:

**RSC Biotechnology Group**

**December 10, 2018**

## ***PROGRAMME and POSTER ABSTRACTS***

### ***Introduction***

Brain disorders such as; Alzheimer's disease, Parkinson's disease, epilepsy, cerebral vascular disease, including stroke, multiple sclerosis, brain trauma and migraine and their sequelae affect ~1bn people worldwide. The economic burden on health providers and governments is tremendous and worryingly this situation will escalate as the expected human life span increases. Throughout the world there are many interdisciplinary teams developing new innovations. Key research initiatives include: genomics, proteomics, cell imaging technologies, stem cell bioengineering, medicinal chemistry, molecular diagnostics, nanobiotechnology for drug delivery and biotherapeutics. Many significant advances have been made but still there remains a huge unmet clinical need for disease-modifying agents that will ameliorate these distressing disorders.

This conference will bring together inspiring speakers who will discuss progress in the understanding of the molecular processes that govern normal brain function and dysfunction and how their findings will translate into new clinical candidates. Furthermore, it will provide unique opportunities for specialists to interact and create new collaborations. An important component of the conference is that it will provide good networking opportunities for students, to meet and question the experts.

## Programme

**09.30 Coffee and registration**

**10.00 SESSION 1**

Chair: Doctor Irene François

**10.05 Professor Sir Christopher Dobson**, Dept. of Chemistry, University of Cambridge, UK

*The molecular origins of neurodegenerative disorders and the rational development of therapeutic strategies*

**10.40 Professor Sheena Radford**, Astbury Centre for Structural Molecular Biology, University of Leeds, UK

*The structural molecular mechanism of amyloid formation*

**11.15 Professor Michele Vendruscolo**, Dept. of Chemistry University of Cambridge, UK

*SAR by kinetics for drug discovery in protein misfolding diseases*

**11.50 Lunch and Poster Session**

**13.30 SESSION 2**

Chair: Doctor Paul Race

**13.35 Professor John Collinge**, UCL Institute of Prion Diseases & MRC Prion Unit, London, UK

*Therapeutic strategies for prion disease*

**14.10 Professor Louise Serpell**, School of Life Sciences, Biochemistry, University of Sussex, UK

*Tau self-assembly: a key target*

**14.45 Doctor Rosemary Staniforth**, Dept. of Molecular Biology & Biotechnology, University of Sheffield, UK

*The nature of a "GAIM" or general amyloid motif*

**15.20 TEA**

**16.05 SESSION 3**

Chair: Doctor Francis Lister

**16.10 Doctor Peter Astles**, Dept. of Discovery Chemistry Research, Eli Lilly, UK

*Protein misfolding and aggregation in Alheimers disease*

16.45 **Doctor Dominique Lesuisse**, CNS Barriers, Sanofi, Chilly Mazarin, Fr.

*Brain enhancement of biotherapeutics*

**17.20 CLOSING REMARKS:** Doctor Steven Burston

## Presentation Abstracts

# The Molecular Origins of Neurodegenerative Disorders and the Rational Development of Therapeutic Strategies

Christopher M. Dobson

Centre for Misfolding Diseases, University of Cambridge, Department of Chemistry,

Lensfield Road, Cambridge CB2 1EW, United Kingdom

Interest in protein misfolding, aggregation and amyloid formation has developed with extraordinary rapidity in recent years, such that this area of science is now a major topic of research across a wide range of disciplines. The reason for this surge of interest is primarily a result of the links between amyloid formation and a range of rapidly proliferating neurodegenerative disorders including Alzheimer's and Parkinson's diseases. This talk will discuss recent progress from our laboratory towards understanding the structural and physical properties of protein aggregates, the kinetics and mechanism of the process of their formation, and the nature and origins of their links with disease. In addition, the talk will discuss the ways in which protein aggregation and amyloid formation may be inhibited or suppressed, both to understand the nature of protein homeostasis in naturally functioning organisms and also to use this information to promote the development of effective strategies through which to combat the onset and progression of neurodegenerative disorders.

T.P.J. Knowles, M. Vendruscolo and C.M. Dobson. "The Amyloid State and its Association with Protein Misfolding Diseases", *Nature Rev Mol Cell Biol* 15, 384-396 (2014).

S.I. Cohen et al., "A Molecular Chaperone Breaks the Catalytic Cycle that Generates Toxic A $\beta$  Oligomers", *Nat Struct Mol Biol*, 22, 207-213 (2015).

F. Chiti and C.M Dobson, "Protein Misfolding, Amyloid Formation, and Human Disease: A Summary of Progress Over the Last Decade", *Annu Rev Biochem*, 86, 27-68 (2017).

FA. Aprile et al., "Selective Targeting of Primary and Secondary Nucleation Pathways in A $\beta$ 42 Aggregation Using a Rational Antibody Scanning Method", *Sci Adv*, 3, e1700488 (2017).

***The structural molecular mechanism of amyloid formation***

Sheena E Radford

Astbury Centre for Structural Molecular Biology, University of Leeds

Understanding how misfolded proteins aggregate, and how aggregated species cause cellular dysfunction and cell death, remain significant challenges. Whilst it is generally accepted that protein misfolding is required for the initiation of formation of amyloid, the structures of oligomers that are on- and off-pathway to amyloid fibrils, why and how oligomers cause cytotoxicity and cell death, and how these species convert into the cross-beta structure of amyloid, remain obscure. The structure of amyloid fibrils themselves, generated *in vitro* or formed *in vivo*, also remained elusive, until the advent of cryo-electron microscopy (cryo-EM) and advanced methods in solid state NMR methods, which now offer the opportunity to see amyloid structures in all-atom detail.

In this talk I will describe how we are using different structural and biophysical methods to delineate the mechanism by which the normally soluble protein  $\beta_2$ -microglobulin converts into amyloid fibrils that deposit in the joints, causing dialysis-related amyloidosis. By combining detailed kinetic analysis of the progress of amyloid formation with experiments using solution NMR and other methods, I will show how we have been able to determine the structure of  $\beta_2$ m oligomers that are on-pathway to amyloid fibrils in all-atom detail. In addition, I will show the structure of the amyloid fibrils themselves, obtained by combining cryo-EM and solid state NMR data. Together, we are beginning to piece together the entire pathway of amyloid formation in all-atom detail. Such knowledge is not only transforming our understanding of these amazing protein structures and how they form at a fundamental level, but it may also open the door to new strategies to combat dialysis-related amyloidosis and other amyloid diseases.

## References

A new era for understanding amyloid structures and disease. Iadanza, M.G., Jackson, M.P., Hewitt, E.W., Ranson, N.A. & Radford, S.E. (2018) *Nature Rev. Mol. Cell. Biol.*, **19**, 755–773

The structure of a  $\beta_2$ -microglobulin fibril suggests a molecular basis for its amyloid polymorphism. Iadanza, M.G., Silvers, R., Boardman, J., Smith, H.I., Karamanos, T.K., Debelouchina, G.T., Su, Y., Griffin, R.G., Ranson, N.A., & Radford, S.E. (2018) *Nature Comms.*, **9**, 4517 -4527

Comparison of the aggregation of homologous  $\beta_2$ -microglobulin variants reveals protein solubility as a key determinant of amyloid formation. Pashley, C.L., Hewitt, E.W. & Radford, S.E. (2016) *J. Mol. Biol.* **428**, 631–643

A population shift between sparsely populated folding intermediate determines amyloidogenicity. Karamanos, T.K., Pashley, C.L., Kalverda, A.P., Thompson, G.S., Mayzel, M., Orekhov, V.Y. & Radford, S.E. (2016) *J. Am. Chem. Soc.* **138**, 6271-6280

## SAR by kinetics for drug discovery in protein misfolding diseases

**Michele Vendruscolo**

Centre for Misfolding Diseases  
Department of Chemistry, University of Cambridge  
Lensfield Road, Cambridge CB2 1EW, UK

*To* develop effective therapeutic strategies for protein misfolding diseases, a promising route is to identify compounds that inhibit the formation of protein oligomers. In order to facilitate these efforts, I will describe a structure–activity relationship (SAR) approach based on chemical kinetics to estimate quantitatively how small molecules modify the reactive flux toward oligomers. This estimate can be used to derive chemical rules in the case of the amyloid beta peptide ( $A\beta$ ), which can then be exploited to optimize starting compounds to curtail  $A\beta$  oligomer formation. I will illustrate this approach by presenting an example in which by an inactive rhodanine compound is converted into an effective inhibitor of  $A\beta$  oligomer formation by generating chemical derivatives in a systematic manner. These results provide an initial demonstration of the potential of drug discovery strategies based on targeting directly the production of protein oligomers.

### **Tau self-assembly: a key target.**

Youssra Al-Hilaly<sup>1,2</sup>, Saskia Pollack<sup>1</sup>, Mahmoud Bukar Maina<sup>1</sup>, Karen Marshall<sup>1</sup>, Janet Rickard<sup>3</sup>, Michael Simpson<sup>5</sup>, John Storey<sup>4,5</sup>, Charlie Harrington<sup>3,5</sup>, Claude Wischik<sup>3,5</sup>, Louise Serpell<sup>1</sup>

<sup>1</sup> Dementia Research group, School of Life Sciences, University of Sussex, Falmer, E Sussex, BN1 9QG

<sup>2</sup> College of Sciences, Chemistry Department, Al-Mustansiriyah University, Baghdad, Iraq

<sup>3</sup> Institute of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, AB25 ZP <sup>4</sup>

TauRx Therapeutics Ltd., Aberdeen, AB25 2Z

<sup>5</sup> Department of Chemistry, University of Aberdeen, Aberdeen, AB24 3UE

Tau is a natively unfolded protein that self-assembles to form neurofibrillary tangles (NFTs) in Alzheimer's disease and other tauopathies. Tau performed many functions in the cell within the cytoplasm and nucleus. However, during the progress of disease, tau accumulates intracellularly as NFTs composed of paired helical filaments (PHF) and straight filaments (SF) composed of a cross-beta structure. Major advances have been made recently with the description of the cryo-EM structure of these filaments isolated from brain tissue. Tau assembly and disassembly has become a major target for the development of therapeutics to treat AD, including the development of a methylene blue derivative (methylthioninium) as well as immunotherapies targeting tau.

The trigger and the mechanism of tau self-assembly in vivo remains unclear. Using a truncated form of tau, we have defined specific conditions required to form PHF and SF that mimic those found in AD patients. Furthermore, we have utilised these model structures to examine the details of the mechanism by which a methylene blue derivative is able to prevent self-assembly in vitro, providing key information regarding the further development of drugs against tau assembly.

### **The nature of a "GAIM" or general amyloid interaction motif**

Peter Davis, Edward Curry, Alexander Lanz, Alex Taylor, Andrea Hounslow, Jon Waltho and Rosie Staniforth.

Department of Molecular Biology and Biotechnology, University of Sheffield, Firth Court, Western Bank, Sheffield S10 2TN. E-mail: R.A.Staniforth@sheffield.ac.uk

Sometimes the best discoveries are made by chance. The application of phage display technology to identify amyloid-binding peptides resulted in the discovery that control phage bind amyloid with nanomolar affinity. A bacteriophage protein is identified that binds to a range of amyloid aggregates from different precursors including amyloid  $\beta$ , Tau and  $\alpha$ -synuclein<sup>1</sup>. This binding leads to the re-modelling of fibrils to smaller, SDS-soluble assemblies. In both A $\beta$  and Tau transgenic mouse models of neurodegeneration (Tg2576 and rTg4510), administration of an engineered antibody containing two copies of the "GAIM" (generalised amyloid interaction motif) leads to a reduction in both A $\beta$  and Tau deposits as well as overall improvements in cognition tests<sup>2</sup>.



## Conference report

Here I present novel data on the nature of the interaction of this GAIM with amyloids mapped using hydrogen exchange / TROSY-NMR as well as a kinetic analysis of its impact on fibrillogenesis. The dynamic nature of the GAIM allows successful binding to amyloid and leads to ablation of disease.

<sup>1</sup> Krishnan R, Tsubery H, Proschitsky MY, Asp E, Lulu M, Gilead, Gartner M, Waltho JP, Davis PJ, Hounslow AM, Kirschner DA, Inouye H, Myszka DG, Wright J, Solomon B, Fisher RA. A Bacteriophage Capsid Protein Provides a General Amyloid Interaction Motif (GAIM) That Binds and Remodels Misfolded Protein Assemblies, *J Mol Biol* (2014) 426:2500-19

<sup>2</sup> Levenson, J. M., Schroeter, S., Carroll, J. C., Cullen, V., Asp, E., Proschitsky, M., Chung, C. H.-Y., Gilead, S., Nadeem, M., Dodiya, H. B., Shoaga, S., Mufson, E. J., Tsubery, H., Krishnan, R., Wright, J., Solomon, B., Fisher, R., Gannon, K. S. *Alzheimers Dement (N Y)*. (2016) 2:141-155.

### Protein Misfolding and Aggregation in Alzheimers Disease

**Dr Peter Astles  
Lilly Research Centre  
Eli Lilly and Co  
Erl Wood Manor  
Windlesham UK**

The leading cause of dementia in adults is Alzheimer's disease (AD). This progressive neurodegenerative disorder is defined by the accumulation of toxic amyloid plaques and neurofibrillary tangles in the brain, accompanied by synapse and neuron loss. These deposits are composed of misfolded protein aggregates the formation of which can be seeded in a prion-like manner. AD is therefore commonly characterized as a protein-misfolding disease.

This talk will present an overview of the current understanding of protein misfolding and aggregation processes in AD and how this may lead to potential therapeutic approaches.

### **Brain enhancement of biotherapeutics**

Dominique Lesuisse, PhD

Head of the CNS Barriers Research Group

Neuroscience Therapeutic Area

Sanofi Inc.,

Chilly Mazarin, France

## Conference report

The blood brain barrier with its network of highly tight and non-fenestrated endothelial cells, along with efflux transporters<sup>i</sup> remains a huge obstacle preventing access of biomolecules to the brain. This explains why some huge medical needs remain to be addressed in particular for difficult targets for which biologics are the main modality in therapeutic area such as neurosciences or oncology (such as CNS lymphoma or glioblastoma). This is actually also explaining why so few biologics are in development in the area of CNS. The very few biologics that are on the market are acting mostly peripherally (or else are given intrathecal). Several groups have shown that receptor-mediated transcytosis can be a route of choice to cargo antibodies the brain. Several mechanisms have been explored and are at various stage of development. The objective of the presentation is to review our work and others in this area.

### Reference

For general reviews see for instance Banks, W. A. From blood–brain barrier to blood–brain interface: new opportunities for CNS drug delivery. *Nat. Rev. Drug Discov* 15, 275 (2016); B. Obermeier, R. Daneman and R. M Ransohoff, Development, maintenance and disruption of the blood-brain barrier, *Nature Medicine* 19, 1584–1596 (2013).

## Poster Abstracts

### Exploring the links between the oral microbiome, ageing and sporadic Alzheimer's disease.

Debbie Shoemark<sup>1</sup> David Emery<sup>1</sup>, Shelley Allen<sup>1</sup> and Nicola West<sup>2</sup>

<sup>1</sup>University of Bristol, UK. <sup>2</sup>Bristol Dental Hospital, UK.

[deb.shoemark@bristol.ac.uk](mailto:deb.shoemark@bristol.ac.uk) (+44 11312146)

#### Abstract

Oral bacteria have been implicated in Alzheimer's disease (AD) from epidemiological, experimental, genetic and environmental studies. Gum disease results from the proliferation of mainly oral anaerobes which stimulate a robust, innate response from the oral mucosa dominated by the release of pro-inflammatory cytokines such as TNF $\alpha$ . Bacteria escaping the mouth or gut are said to be "immune-tolerated" by targeted cell-mediated and humoral responses, but can contribute to innate-mediated chronic inflammation; some are now considered causal in instances of atherosclerosis and back pain. The immune system's ability to mount targeted immune responses tends to wane with advancing age and as a result bacterial load increases and the more inflammatory innate responses gradually dominate. Prolonged exposure to TNF $\alpha$  can compromise the integrity of the blood brain barrier and A $\beta$  is now thought to be part of a host innate immune response. More recently, sensitive techniques such as bacterial 16S rRNA gene PCR detect the presence of low levels of bacteria in areas previously thought to be sterile. The question of how much a microbiome-induced general inflammation or low-level infiltration contributes to disease pathology has become the focus of study, especially as inflammatory processes have long been associated with AD. We (and others) find evidence of more bacteria in temporal cortex samples taken from patients with AD compared to subjects cognitively normal at death (SouthWest Brain Bank). Exploring the AD brain microbiome/host interplay, seeking to identify common components, their distribution, their route and mechanism of entry, may provide insights into identifying those most at risk to target preventive measures and develop new therapeutic strategies.

Sabine Ulamec

Asbury Centre for Structural Molecular Biology,

University of Leeds

Alpha-synuclein ( $\alpha$ Syn) is known to be involved in the neurodegenerative disorder Parkinson's disease (PD) which affects 10 million people worldwide. Patients show multiple motor (e.g. tremor, lack of coordination) and non-motor (e.g. depression, anxiety) symptoms, as well as pathological symptoms which are characterised by the loss of dopaminergic neurons and the formation of aggregated  $\alpha$ Syn-containing Lewy bodies in the brain. Understanding the process of amyloid formation from the intrinsically disordered monomer  $\alpha$ Syn to the complex  $\beta$ -sheet rich fibrils is therefore crucial.

We have engineered  $\alpha$ Syn mutants which lack several amino acids in the N-terminal region that are shown in various aggregation/ hydrophobicity calculators to be part of highly aggregation-prone regions. ThT fluorescence aggregation assays have shown that this N-terminal motif plays a critical role in aggregation. The importance of these regions were further analysed in molecular detail via paramagnetic relaxation enhancement (PRE) NMR experiments. Both inter- and intrachain PRE experiments were carried out on wildtype  $\alpha$ Syn and variants with deleted N-terminal stretches. Interestingly, the deleted regions are shown to be involved in both intra- and intermolecular interactions and are therefore likely to play a key role in driving aggregation.

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### **i A new approach to targeting the Ras superfamily *via* their regulatory GEF complexes**

Janine Gray

DPhil Candidate in Clinical Medicine,  
Nuffield Department of Medicine  
University of Oxford

Small GTPases are fundamental enzymatic switches, with roles in intracellular transport, receptor signalling, cell proliferation and development.<sup>1</sup> Due to their vital role in the cell, dysregulation or mutations that affect their activity can result in disease, including neurodegenerative disorders.<sup>2</sup> Ever since Ras, a small GTPase, was discovered as an oncogene, attempts have been made to produce small molecule inhibitors for these proteins, but efforts have been unsuccessful due to the underlying nature of these enzymes; the high picomolar affinity of GTPases for their nucleotide substrates has rendered competitive inhibitor strategies unproductive.<sup>3</sup> During this PhD project, a novel approach to targeting the small GTPases will be made through the development of a competitive nucleotide-binding site inhibitor of a GTPase complexed to its effector, a guanine nucleotide exchange factor (GEF). It is hypothesised this will be more successful than previous attempts as binding of the GEF to the GTPase reduces the affinity of the GTPase for GDP/GTP from picomolar to micromolar.<sup>3,4</sup> The propensity of the GTPase/GEF complex to form crystals, along with the necessity for structural information to ensure fragments are binding within the GDP/GTP binding site, makes it an ideal candidate for the XChem fragment screening technique developed by Frank von Delft's group at the Diamond Synchrotron, UK.

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This poster will cover the early stages of this project, including high-throughput production of over 30 proteins to form GEF/GTPase complexes, the successful development of three crystal systems and fragment screening of >1000 compounds on the Kalirin/Rac1 complex. Both components of this complex are associated with neurological disorders.<sup>2,5</sup> Medicinal chemistry strategies using structure-based design for hits and expansion of the project to other targets will be discussed.

1. Cherfils, J. & Zeghouf, M. Regulation of Small GTPases by GEFs, GAPs, and GDIs. *Physiol. Rev.* **93**, 269–309 (2013).
2. Stankiewicz, T. R. & Linseman, D. A. Rho family GTPases: key players in neuronal development, neuronal survival, and neurodegeneration. *Front. Cell. Neurosci.* **8**, 314 (2014).
3. Stephen, A. G., Esposito, D., Bagni, R. K. & McCormick, F. Dragging Ras Back in the Ring. *Cancer Cell* **25**, 272–281 (2014).
4. Goody, R. S. How not to do kinetics: examples involving GTPases and guanine nucleotide exchange factors. *FEBS J.* **281**, 593–600 (2014).
5. Remmers, C., Sweet, R. A. & Penzes, P. Abnormal kalirin signaling in neuropsychiatric disorders. *Brain Res. Bull.* **103**, 29–38 (2014).

## Structural evaluation of the effect of glycation on Abeta aggregation

Giulia Milordini<sup>1</sup>, Alessandro Emendato<sup>2</sup>, Elsa Zacco<sup>1</sup>, Annalisa Pastore<sup>1</sup>, Delia Picone<sup>2</sup>

<sup>1</sup>Maurice Wohl Clinical Neuroscience Institute, King's College London, London, UK;

<sup>2</sup>Department of Chemistry, University of Naples, via Cinthia, Napoli, Italy.

**Background:** Glycation, non-enzymatic addition of sugars to proteins, can be a pathological process leading to misfolding of the Abeta peptide into amyloids, hallmark of Alzheimer's disease (AD). [1,2] We aim to define the effects of glycation on the aggregation propensity of the peptides Abeta40 and Abeta42. These results could help understanding the high incidence of AD in Type 2 Diabetes (T2D) patients.

**Materials and Methods:** Methylglyoxal (MGO) was used as glycating agent. Circular dichroism illustrated the secondary structure variation upon glycation. Fluorescence-based assays and mass spectroscopy elucidated glycation and aggregation kinetics. High-resolution structural studies via atomic force microscopy (AFM) evaluated the morphological effects of glycation on amyloid fibrils.

**Results:** Abeta40 glycation allows the protein to retain its random coil conformation, while the non-glycated peptide forms amyloids after 24 hours. No significant variation of the secondary structure was observed upon glycation of Abeta42. However, the aggregation-associated fluorescence of both glycated Abeta40 and Abeta42 compared to the non-glycated versions shows a decrease in the final amount of total aggregates and a slower kinetics of aggregation with in MGO- concentration dependent fashion. AFM illustrates that the fibril formation is decelerated, and the

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peptides are found for a longer time in an oligomeric state.

**Discussion:** The glycation of Abeta 40 and Abeta42 has a strong impact on their structural stability and behavior [3]. The slower aggregation kinetics upon glycation translates into the stabilization of oligomeric forms, considered more toxic than the amyloids. These results could explain the higher incidence of AD in T2D patients.

**Conclusions:** Post-translational modifications can affect the structural behavior of aggregation-prone proteins and might be used to interfere with the development of AD and T2D.

#### References

Watson GS, Craft S: Insulin resistance, inflammation, and cognition in Alzheimer's Disease: lessons for multiple sclerosis. *J Neurol Sci.* 2006;245(1-2):21-33.

Sasaki N, Fukatsu R, Tsuzuki K, Hayashi Y, Yoshida T, Fujii N, et al. Advanced glycation end products in Alzheimer's disease and other neurodegenerative diseases. *Am J Pathol.* 1998;153(4):1149-55

Iannuzzi, C., et al: Differential effects of glycation on protein aggregation and amyloid formation *Front Mol Biosci.* 2014 1: 9.

## 1. Pulling apart the inter- and intramolecular interactions of $\alpha$ Synuclein reveals an important interaction motif in the N-terminus

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Ciaran P.A. Doherty, Sabine Ulamec, Sheena E. Radford and David J. Brockwell

### Abstract

Protein aggregation is linked with the onset of various neurodegenerative disorders including Parkinson's disease (PD) that is hallmarked by the aggregation of alpha synuclein ( $\alpha$ Syn). The pathways by which the protein aggregates however, are poorly understood. The toxic species in the aggregation cascade are still unknown, and so looking at the initial interaction conformations of  $\alpha$ Syn from both inter- and intramolecular perspectives is of high importance, as these interactions offer valid targets for clinical intervention. In this study, the initial dimerisation step in the aggregation pathway was probed at the single molecule level using force spectroscopy. Alongside these experiments, the conformations of monomeric  $\alpha$ Syn were studied via paramagnetic relaxation enhancement (PRE) NMR experiments in which long range, often transient interactions can be probed. From these experiments and aggregation assays, the importance of these interactions in  $\alpha$ Syn amyloid formation or inhibition have been hypothesised.

In this work, we have interrogated the self-association of  $\alpha$ Syn at a single molecule level by analysing the strength and conformation of self-association in different environmental conditions primarily by single molecule force spectroscopy (SMFS). The SMFS experiments show that force-resistant structure forms in the dimeric species of  $\alpha$ Syn and that this structure is dependent on the environmental conditions. SMFS utilising different immobilisation regimes of  $\alpha$ Syn have also allowed the location of a novel interaction interface involving the N-terminal region of the protein. Further SMFS experiments investigating the effects salt and hydrophobicity have on dimerisation, alongside bioinformatics analyses of the protein sequence led to the hypotheses that the dimeric interaction is driven by hydrophobic stretches in the N-terminal region, but modulated by local electrostatics. In vitro aggregation assays and SMFS on non-aggregation-prone synuclein homologues ( $\beta$ - and  $\gamma$ Syn)

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indicated that that this interaction is protective against aggregation, considering these finding with existing literature prompted speculation that the interactions observed in SMFS may indeed be physiologically relevant.

By utilising PRE NMR experiments, we have also shown that in conditions which promote aggregation, the key N-terminal region identified in SMFS studies makes more distil intramolecular contacts, further indicating the importance of this region in the modulation of aggregation. These results may therefore present an important finding in regards to targeting the aggregation process with disease modifying agents.

### The inhibition of alpha synuclein aggregation using a novel peptide-based inhibitor

J Torpey<sup>1</sup>, R Meade<sup>2</sup>, J Mason<sup>2</sup>, J Madine<sup>1</sup>

<sup>1</sup>University of Liverpool, <sup>2</sup>University of Bath

Alpha synuclein (aSyn) is a 140-residue protein localized at the presynaptic terminals. ASyn's physiological function remains poorly understood, yet it is a key player in Parkinson's disease (PD) and other neurodegenerative diseases. Disease progression is caused by the progressive loss neurons in the brain. Cell death appears to be associated with the aberrant aggregation of aSyn, and thus the inhibition of aggregation represents an enticing therapeutic strategy. Here we employ NMR to probe interactions between aSyn and a 10-residue peptide that has been previously shown to reduce aSyn fibril formation and associated cell toxicity (Cheruvvara et al. 2015). Using a series of NMR timecourse experiments we show that a structural rearrangement of the peptide occurs and that this may be of importance for binding to higher-order aSyn species. Electron microscopy shows the presence of more numerous yet much shorter fibrils when aSyn is incubated in the presence of the peptide, implying that the peptide prevents fibril extension. We hypothesize that binding of the peptide impedes the formation of the aSyn species responsible for cell toxicity. We now aim to elucidate the cellular pathways involved through the use of NMR metabolomics, using our previously published SH-SY5Y neuroblastoma metabolic profiles (Phelan et al. 2017).

### Bibliography:

Cheruvvara, H. et al., 2015. Intracellular screening of a peptide library to derive a potent

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peptide inhibitor of  $\alpha$ -synuclein aggregation. *Journal of Biological Chemistry*, 290(12), p.jbc.M114.620484. Available at: <http://www.jbc.org/content/290/12/7426.short> [Accessed October 6, 2015].

Phelan, M.M. et al., 2017. Using an NMR metabolomics approach to investigate the pathogenicity of amyloid-beta and alpha-synuclein. *Metabolomics*, 13(12), p.151.

Available at: <http://www.ncbi.nlm.nih.gov/pubmed/29142509> [Accessed November 17, 2017].

### **Tau self-assembly: a key target.**

Youssra Al-Hilaly<sup>2</sup>, Saskia Pollack<sup>1</sup>, Mahmoud Bukar Maina<sup>1</sup>, Karen Marshall<sup>1</sup>, Janet Rickard<sup>3</sup>, Michael Simpson<sup>5</sup>, John Storey<sup>4,5</sup>, Charlie Harrington<sup>3,5</sup>, Claude Wischik<sup>3,5</sup>, Louise Serpell<sup>1</sup> Dementia Research group, School of Life Sciences, University of Sussex, Falmer, E Sussex, BN1 9QG

<sup>2</sup> College of Sciences, Chemistry Department, Al-Mustansiriyah University, Baghdad, Iraq

<sup>3</sup> Institute of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, AB25 ZP  
<sup>4</sup> TauRx Therapeutics Ltd., Aberdeen, AB25 2Z

<sup>5</sup> Department of Chemistry, University of Aberdeen, Aberdeen, AB24 3UE

Tau is a natively unfolded protein that self-assembles to form neurofibrillary tangles (NFTs) in Alzheimer's disease and other tauopathies. Tau performed many functions in the cell within the cytoplasm and nucleus. However, during the progress of disease, tau accumulates intracellularly as NFTs composed of paired helical filaments (PHF) and straight filaments (SF) composed of a cross-beta structure. Major advances have been made recently with the description of the cryo-EM structure of these filaments isolated from brain tissue. Tau assembly and disassembly has become a major target for the development of therapeutics to treat AD, including the development of a methylene blue derivative (methylthioninium) as well as immunotherapies targeting tau.

The trigger and the mechanism of tau self-assembly in vivo remains unclear. Using a truncated form of tau, we have defined specific conditions required to form PHF and SF that mimic those found in AD patients. Furthermore, we have utilised these model structures to examine the details of the mechanism by which a methylene blue derivative is able to prevent self-assembly in vitro, providing key information regarding the further development of drugs against tau assembly.

### **Extracellular Cl<sup>-</sup> ion reduction mimics the effect of bumetanide on optic nerve fibres, supporting a resting electroneutral Na<sup>+</sup> influx coupled to Cl<sup>-</sup>**

Ella Richards\*, Lavinia Przyborowski\* and Mark D Baker  
equally

\*authors

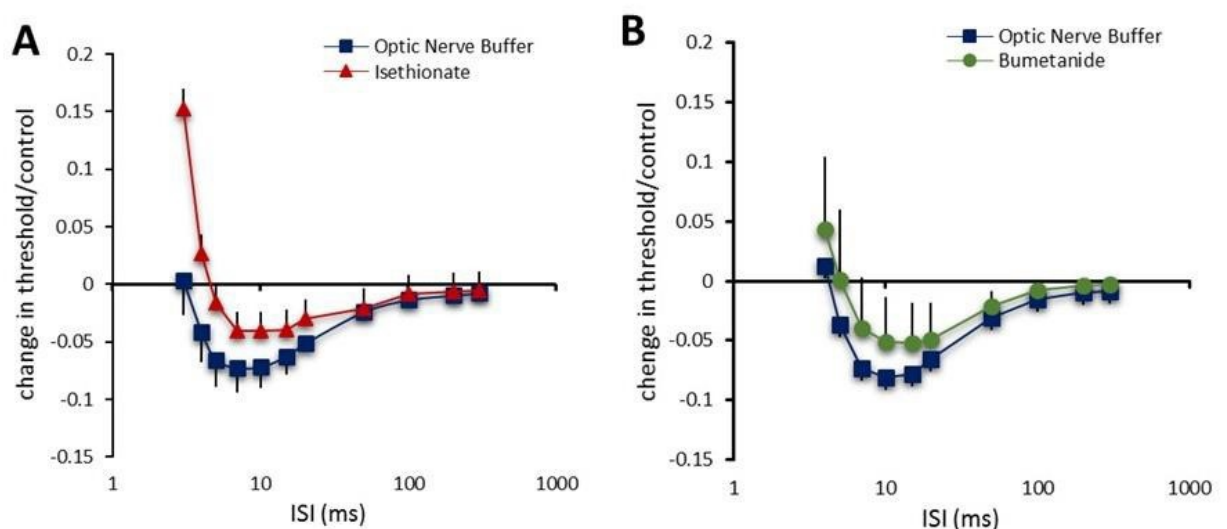
Contributed



**Introduction**  $\text{Na}^+$  movements into nervous tissue occur during impulse signalling (e.g. Hodgkin & Katz, 1949) but also happen at rest. The nature of this resting influx has not been fully elucidated although some is accepted as being through open  $\text{Na}^+$  channels. Understanding the movement  $\text{Na}^+$  in axons is important because  $\text{Na}^+$  influx requires energy expenditure to reverse, intracellular  $\text{Na}^+$  build-up occurs in diseased human brain (Biller et al., 2016), and because blocking  $\text{Na}^+$  entry is expected to be neuroprotective. However, most of the resting influx in optic nerves may not be through channels, but rather via electroneutral transport mechanisms. Key evidence for this idea is the result of raising temperature, a manoeuvre expected to *enhance*  $\text{Na}^+\text{-Cl}^-$  cotransport, giving rise to a membrane potential hyperpolarization by secondarily recruiting the electrogenic  $\text{Na}^+$ -pump (Coates et al., 2015; Kanagaratnam et al., 2017). It is not possible directly to record membrane potential in optic nerve axons, but it is possible to measure biophysical parameters using threshold-tracking (known as the recovery cycle- shown in Fig 1A,B) following an action potential that are known to be sensitive to changes in membrane potential.

**Method** We have previously concluded that the loop diuretic bumetanide is able to reduce resting  $\text{Na}^+$  entry into optic nerve axons, and in doing so causes a depolarization by reduction of a continuous and restorative  $\text{Na}^+$ -pump current (Coates et al., 2015). In these experiments we replaced extracellular  $\text{NaCl}$  with  $\text{Na-Isethionate}$ , and compared this to the application of Bumetanide, by following the effects on the recovery cycle in F-fibres in rat optic nerve.

**Results** Reducing  $\text{Cl}^-$  and exposing the optic nerves to bumetanide affects recovery cycles in a similar way, consistently increasing refractoriness and reducing superexcitability following an action potential (Fig. 1). The effect of reducing  $\text{Cl}^-$  was found to be reversible.



**Figure 1. Recovery cycles recorded following an action potential in optic nerve F-fibres, by tracking threshold at incrementing inter-stimulus intervals (ISI)** (A) Isethionate increased refractoriness and reduced superexcitability consistent with depolarization ( $n = 5$ ),  $p = 0.002$ , paired-t-test. (B) Exposure to bumetanide ( $5 \mu\text{M}$ ) produced an indistinguishable effect ( $n = 4$ ),  $p = 0.026$  paired t-test. Data plotted as means  $\pm$  SEM.

**Conclusion** Reducing extracellular  $\text{Cl}^-$  and exposing the optic nerves to bumetanide have indistinguishable effects on recovery cycles, consistent with the transporter NKCC1 being responsible for a part of the resting  $\text{Na}^+$  influx into the axons. We suggest NKCC1 is a potential novel target for reducing resting  $\text{Na}^+$  influx into axons and hence for neuroprotective compounds.

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