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Claire Louisa Tinker-Mill

Nanoscale Imaging and Characterisation of Amyloid-β

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Claire Louisa Tinker-Mill

Nanoscale Imaging and Characterisation of Amyloid-β

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Mayes, J., Tinker-Mill, C., Kolosov, O., Zhang, H., Tabner, B. & Allsop, D.
Journal of Biological Chemistry. 289, p. 12052–12062 11 p. 25/04/2014

Ultrasonic force microscopy for nanomechanical characterization of early and late-stage amyloid-β peptide aggregation
Tinker-Mill, C., Mayes, J., Allsop, D. & Kolosov, O.
Scientific Reports. 4, 7 p. 4004, 2014

A novel retro-inverso peptide inhibitor reduces amyloid deposition, oxidation and inflammation and stimulates neurogenesis in the APPswe/PS1ΔE9 mouse model of Alzheimer's Disease
Parthsarathy, V., McClean, P. L., Hölscher, C., Taylor, M., Tinker, C., Jones, G., Kolosov, O., Salvati, E., Gregori, M., Masserini, M. & Allsop, D.
PloS ONE. 8, 1, 11 p. e54769, 2013

Also in preparation

Retro-inverso peptide inhibitory nanoparticles (PINPs) as potent inhibitors of aggregation of the Alzheimer's Aβ peptide
Gregori, M., Taylor, M., Tinker-Mill, C., Michael, M., Kolosov, O., Salvati, E, Re, F., Minniti, S., Zambelli, V., Masserini, M. & Allsop, D.

Comparison of photothermal and opto-acoustic response of nanoscale probes for Mid-IR photothermal microspectroscopy (PTMS) of nanostructured biological materials
Tovee, P.D., Tinker-Mill, C., Kjoller, K., Allsop, D., Weightman, P., Surman,' M., Siggel-King, M., Wolski, A. & Kolosov, O.V.

Scanning thermal microscopy imaging of Amyloid-β
Tovee, P.D., Tinker-Mill, C., Allsop, D., & Kolosov, O.V.

Presentations at Conferences

Nanoscale SPM Characterisation of Nacre Aragonite Plates and Synthetic *Human Amyloid Fibres.*
Grishin, I., Tinker, C., Allsop, D., Robson, A & O.V. Kolosov.
Nanotech 2012 Santa Clara, California.
Proceedings of Nanotech—2012, TechConnect World 2012, pages 2, 940 ISBN: 978-1-4665-6278-3, NSTI, CRC press, Santa Clara, USA **(2012).**

Nanoscale morphology and nanomechanical characterisation of recombinant human Amyloid-β 1-42 via tapping mode and ultrasonic force microscopies.
Tinker, C., Allsop D. & Kolosov, O.
Seeing at the Nanoscale, July 2012, Bristol University.

Nanoscale morphology and nanomechanical characterisation of recombinant human Amyloid-β 1-42 via tapping mode and ultrasonic force microscopies.
Tinker, C., Allsop D. & Kolosov, O.
European Microscopy Conference, September 2012, Manchester.

Nanoscale dynamics of Amyloid-β fibres on poly-L-Lysine substrate in air and liquid environments via atomic force and ultrasonic force microscopy
Tinker, C., Allsop, D., Kolosova, K., Dinelli, F & Kolosov, O
Multifrequency Conference, October 2012, Madrid.

Nanoscale imaging of Alzheimer's Disease: Getting to the core of it
Tinker, C., Allsop, D., Robson, A. & Kolosov, O
Lancaster University Sci-Tech Christmas Conference, 17th December 2012, Lancaster.

Ultrasonic force microscopy studies of the nanoscale structure of Amyloid-β fibres in a liquid environment
Tinker C., Allsop, D., Kolosova, K., Dinelli, F. & Kolosov O.
Bristol University Nanomaterial Futures Conference, 17th February 2013, Bristol.

Nanostructure of Amyloid Fibres using Ultrasonic Force Microscopy
Tinker, C., Allsop D. & Kolosov, O.
Waterloo University talk as a visiting student, September 2013, Waterloo, Canada.

Nanomechanical and nanothermal mapping of initial stages of amyloid fibres formation
Kolosov, O., Tovee, P., Tinker-Mill, C. & Allsop, D.
Bristol Nanoscience Symposium 15/09/2014, Bristol.

Press Articles

http://www.labnews.co.uk/news/sewing-machine-inspires-imaging-tool-for-alzheimers/
21/04/14: (Lab News)
Tinker-Mill, C., Mayes, J., Allsop, D. & Kolosov, O.

Imaging tool gives insight into origins of Alzheimer's
2/04/14: (Medical Express)
Tinker-Mill, C., Mayes, J., Allsop, D. & Kolosov, O.

New imaging tool provides fresh insight into origins of Alzheimer's, Parkinson's disease
2/04/14: (News medical)
Tinker-Mill, C., Mayes, J., Allsop, D. & Kolosov, O.

Sewing machine' idea gives insight into origins of Alzheimer's
1/04/14: (USA Daily news) (Science daily) (Deccan chronicle) (Business Standard)
Tinker-Mill, C., Mayes, J., Allsop, D. & Kolosov, O.

Tools of the Trade
26/02/14: (BioTechniques - The International Journal of Life Science Methods)
Tinker-Mill, C., Mayes, J., Allsop, D. & Kolosov, O.

Awards

Juno Award for Research Excellence 2014
Juno Award for Research Excellence 2012

Supervisor's Foreword

It is my great pleasure and true honour to write this foreword to Claire Tinker-Mill's thesis. I was always amazed with Claire, a mainstream biologist by training, who has been able to successfully develop highly sophisticated physical methods for exploring nanoscale properties of materials that only a handful of research groups worldwide was privy to. Her work is an excellent example how a truly interdisciplinary exploration of nature should be conducted.

The subject of Claire's thesis came from my ongoing collaboration with Prof. David Allsop at Lancaster University Health and Medicine, who was working on uncovering molecular mechanisms of Alzheimer's disease and developing paradigms for the novel drugs that can stop or, better, reverse this debilitating disease. While there were multiple optical and chemical methods for detecting an aggregation of misfolded amyloid peptides, the known cause for the disease, the exact mechanisms of their aggregation and the source of pathological changes was not at all clear.

While researchers' ultimate goal would be to directly observe such folding, this was not an easy task, given that most relevant biomolecular interactions are happening in the liquid, and the interacting structures are of the order of few nanometres in size. When Claire joined my group, we were perfecting a rather powerful method for nanoscale characterisation and imaging of inorganic materials—e.g. semiconductor quantum dots or metallic interfaces—ultrasonic force microscopy or UFM. This approach combined a near-atomic resolution of scanning probe microscopy with the sensitivity of ultrasonic imaging to local mechanical properties of surface and near-surface areas.

Claire has successfully applied UFM for the detection of the early-stage toxic amyloid aggregates by combining UFM with methods for capturing all range of peptide assemblies from the monomers to the fully formed amyloid plaques. She explored a small universe of diverse substrates, coatings and buffers, to home in on the winning combination that allowed to capture practically all peptide components on the perfectly flat surface. The nanomechanical contrast provided by UFM allowed her to pinpoint toxic amyloid peptides and to reliably differentiate them

from the substrate. Claire also established that the nanoscale ultrasonic probe had another huge advantage—the minute but very-high-frequency ultrasonic vibrations used in UFM allowed a nanoscale tip of the probe to gently poke into the biological molecules, leaving them intact, while still identifying a wrongly folded ones. The approach successfully uncovered that a significant portion of the initial amyloid peptide even at later stages of assembly was in the small molecular weight stage that is regarded as triggers of cellular death in Alzheimer's disease. This work allowed to develop the novel retro-inverso peptide inhibitor based on nanoscale liposomes as carriers for the anti-Alzheimer's drugs. These have shown potential for 1000 times higher efficiency in scrubbing harmful fibres from biological fluids and reducing the peptide deposition in this disease, hence paving the way to the new paradigm for the future drugs.

While expanding this avenue of research would be more than sufficient for a solid Ph.D. thesis, Claire did not pause at all and explored other physical methods to study amyloid proteins. She used a relative of UFM—heterodyne force microscopy, or HFM, to measured time response of peptides. Claire found that pathological misfolded peptides have a characteristic time signature, linked to their mechanical relaxation that was different from the normal proteins, adding new method that may help to detect early stages of Alzheimer's disease in the future.

Finally during her Ph.D. work, Claire explored yet another powerful method allowing to directly identify biomolecules with nanoscale resolution—via combining chemical identification by infrared optical excitation with the scanning probe microscopy. As high-power tunable infrared sources are not easy to come by, she was studying amyloid aggregates on the Daresbury ALICE accelerator that powered a free electron laser, where she became a dedicated commissioner responsible for safety of this complex physical instrument. This work, instigated by Claire, can create an ultimate instrument for early detection of Alzheimer's disease.

Overall, Claire's thesis not only provides a deep insight into a novel scanning probe methodology for nanoscale exploration of biological molecules and their aggregates, but also shows an inspiring example of how to solve a complex interdisciplinary problem at the interface of biology, physics and chemistry. It will be a useful reference both for established researchers and graduate students working in these highly active areas of modern research.

Lancaster
May 2016

Prof. Oleg Kolosov

Abstract

In this work, several novel scanning probe microscopy (SPM) methods have been applied to the study of the amyloid peptide implicated in the pathogenesis of Alzheimer's disease (AD). Amyloid-β (Aβ) undergoes a hierarchy of aggregation following a structural transition, making it an ideal subject of studying with SPM.

The application of SPM-based techniques to biological samples has become increasingly common place. However, these techniques are not always immediately suitable for imaging delicate samples of proteins and adaptions must be made before imaging can be considered successful. AD is the most common form of dementia worldwide, and a growing concern for health authorities. As a result, it has attracted the attention of a wide range of disciplines. There has been much work conducted which combines the main pathogenic peptide, Aβ, with atomic force microscopy (AFM) in order to elucidate more about its aggregation behaviour; however, these techniques offer little more than structural comments, with only the most advanced forms of cryo-electron microscopy (EM) providing more details on the nanoscale. Presented here is a method for reliably and robustly producing samples of Aβ by capturing them at various stages of aggregation, as well as the results of subsequent imaging by various methods of AFM. Each of the AFM techniques studied provides additional "added value" to the data which can typically be collected by AFM, either nanomechanical, elastic, thermal or spectroscopical.

By imaging samples of Aβ with ultrasonic force microscopy, a detailed substructure to the morphology could be seen, which correlates well with the most advanced cryo-EM work. In addition, this technique was ideal for detecting the most toxic form of Aβ, early aggregates, in a sensitive and nondestructive fashion robustly differentiating them from the underlying layer of another peptide (poly-L-Lysine) that was designed to reliably capture the Aβ aggregates. Early work investigating the potential for combining an established method of thermal AFM with a mid-IR laser system also shows promise for detecting the response of the protein.

It was also the focus of this work to study the aggregation of Aβ using dynamic light scattering (DLS), in order to confirm whether the technique could identify

differences between populations throughout the aggregation process. This was applied in conjunction with potential therapeutics which targets the early aggregates to prevent their accumulation, as well as block formation of fibrils.

Ultimately, this work aims to show with care to the initial protocols used, physical techniques such as AFM and DLS can be added to the existing methods of monitoring aggregation. Synergistic use of these techniques can generate a clearer overall picture of the effect of metal ions/developing therapeutics on Aβ aggregation and provide more detail than classical biological techniques alone.

Acknowledgments and Dedications

Acknowledgments

I would first like to thank Prof. David Allsop and Dr. Oleg Kolosov for their patience and guidance throughout the completion of this work. I would also like to extend my eternal gratitude to my colleagues in both the Department of Physics and Biomedical Life Sciences for their help and advice.

I am blessed to have made so many friends throughout this Ph.D., both within the university and elsewhere: Jenny Mayes and Alex Robson, thank you for brightening my morning with many a cup of coffee and chatter, in addition to all your support with my research; Riccardo Mazzocco, who will always be "my favourite Italian"; Kylie O'Shea and Ben Shreeve, without whom I would not have enjoyed nearly as much cake as I have currently; the wonderful Taylor Lura, Emily Smith and Christie Herd, who have made me laugh so hard I have cried; My "Bookend" Louise Walker, for fostering my madness and creating the candyfloss world with me. I would like to thank Dr. Christine Shirras for her guidance and support as my teaching mentor. Lastly, my friends in Canada: Ronnie and Liz Drolle, who I would never have had the joy of meeting if it were not for this Ph.D. All of these people, and more, have made this experience the happiest of my life. You have brought so much joy, sparkle and laughter as we have journeyed together. I'm so lucky to have you in my life.

My family's support and belief in me has been paramount throughout my studies, and I thank them from the bottom of my heart of believing in me, comforting me and giving me the strength I have.

Most importantly, I want to thank my long-suffering husband, Richard. Without you I would not be me, and this would not have happened. We share this achievement as we do everything. I love you now, forever and always and cannot thank you enough for your support and love.

Dedications

The work in this thesis is dedicated to those that sadly did not get the opportunity to see me finish this journey, but offered their unconditional support and love throughout my life: Vera Pennington, who sadly lost her own battle with Alzheimer's disease inspired with her tireless work as a daughter, mother and grandmother; Rex Pennington, who went long before this journey began, thank you for the memories; Marjorie and Ernest Tinker, without whom I would not have been able to begin this journey, I love and miss you both so much and am eternally grateful for all that you did for me, and It breaks my heart you will not see me graduate.

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