Designing a Single-molecule *In Vitro* Assay and Targeting C5

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INTRODUCTION

- Alzheimer's disease (AD) is the most common form of dementia, effecting over 850,000 people in the UK and over 50 million worldwide, with a global economic impact of over £1 trillion according to Alzheimer's Research UK
- AD is a neurodegenerative disease leading to progressive loss of neurons and brain atrophy. It is characterised by the formation of plaques in the brain by oligomerisation of the peptide amyloid-β.
- The complement system has become a potential therapeutic avenues for treating neurodegenerative diseases as complement system controls the body's inflammatory response
- Targeting C5 complement protein will allow the complement system to retain its immune roles while reducing the inflammation and cell death caused by the cleavage of C5 to C5a and C5b
- This project will allow to build the foundation for designing a new class of blood-brain barrier penetrant small molecular drugs for neurogenerative diseases.

METHODS

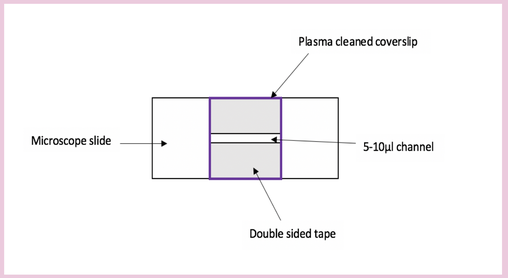

Figure1. The design for an in vitro assay

- Different concentrations of apolipoprotein E (apoE), amyloid-beta and factor H (FH) were used to obtain an ideal concentration for single molecule imaging
- The solutions were flowed into the channel and incubated in a hydrating chamber
- Single molecule florescence microscopy was used to detect the fluorescently labelled proteins with specific wavelengths
- 1000 frames were recorded and analyzed in MATLAB
- C5 was then imaged under different conditions such as positive control, negative control and using BSA to block non-specific bindings

RESULTS

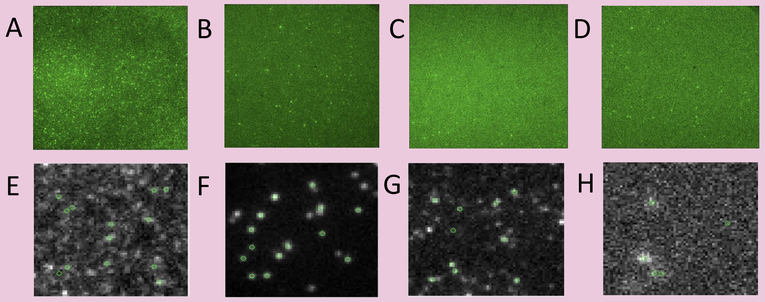

Fig A-D. Frame images of (A) C5 in 1:100 dilution, (B) without C5, (C) C5 in 1:1000 dilution and (D) C5 in 1:1000 dilution+BSA respectively (from left to right).
Fig E-H. Images of tracking spots in (E) C5 in 1:100 dilution, (F) without C5, (G) C5 in 1:1000 dilution and (H) C5 1:1000 dilution+BSA respectively (from left to right).

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Fig 1-2. Comparison of spots intensity between solutions

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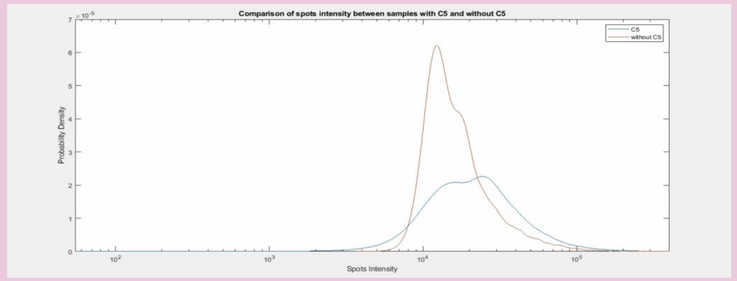

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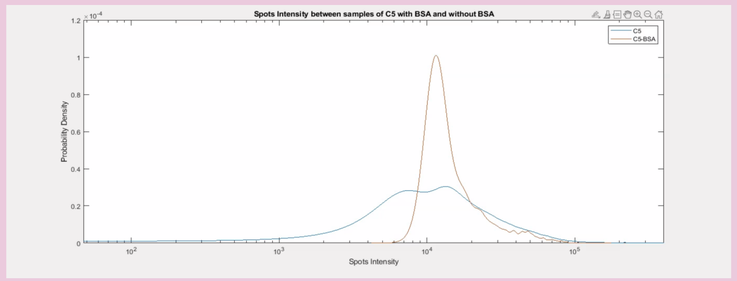

Fig 3-4. Plots of probability against spots intensity for two sets of samples (respectively)

AIMS

- Design an in vitro assay for single molecule fluorescence microscopy using apolipoprotein E(apoE), amyloid-beta and factor H(FH), which are proteins found in the pathogenesis of AD
- Observe C5 with different controls under the fluorescence microscope

DISCUSSION

- After performing the assay with different dilutions of apoE,FH and amyloid beta, Ideal concentration for single molecule imaging was determined to be between
- Solutions with C5 >>>> higher spot intensity
- However, when comparing the samples of C5 positive and other solutions, the spot intensities were within a similar range
- This could be due to the random crops of the video size from 562x562 to 64x64, certain frames or coordinates in the frame being brighter due to aggregates of C5, causing increased light
- Imaging of C5 allowed us to build foundation in understanding the neuroinflammation involving complement system in AD

CONCLUSION

- Method for in vitro assay to image under the single-molecule microscopy was optimized after experimenting with different concentrations
- This allowed the observation of complement C5 with labelled proteins

FUTURE WORK

- Results of imaging complement protein C5 will be useful in observing enzymatic cleavage of C5 into C5a and C5b by elastase in real time under the single-molecule microscopy
- Further work can be done by designing C5 cleavage inhibitor, which will stop the inflammation and complement activation, possibly creating a treatment for AD and other neurodegenerative diseases.

ACKNOWLEDGEMENTS

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