

# Towards an understanding of polymorphism in protein aggregation

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## Introduction

In an ageing society diseases such as Alzheimer's, Parkinson's and Type II Diabetes are on the increase. Amyloid protein aggregates are believed to play an important role in the onset of such conditions. Both amyloid fibrils and amyloid spherulites have been found in the plaques of Alzheimer's patients. Yet little is known about what role different environmental conditions play in determining the final morphologies.

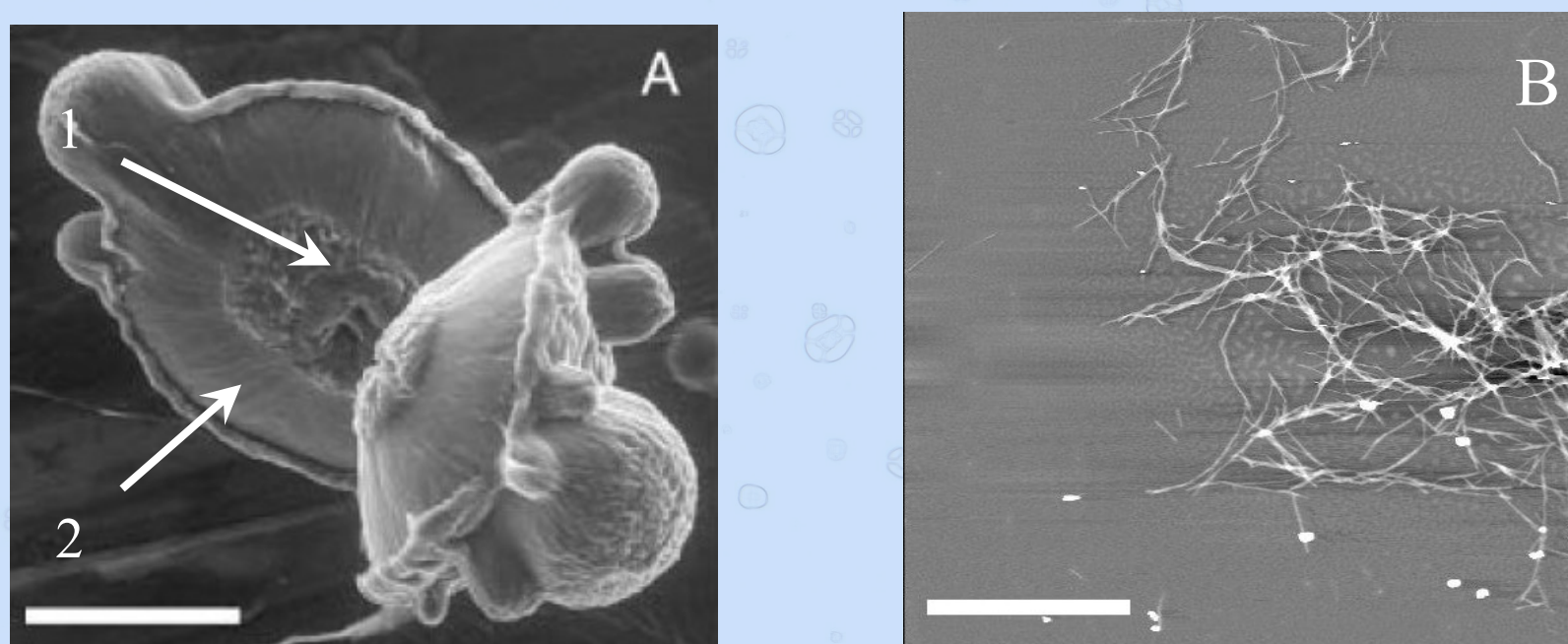


Fig 1. Aggregate morphologies: A) ESEM image of an Amyloid Spherulite which has split open (Scale bar 30 $\mu$ m), showing an amorphous core (1) and radially oriented fibrils (2). B) AFM image of Amyloid Fibrils. (Scale Bar 3 $\mu$ m)

## Experimental

Amyloid aggregates form from misfolded proteins under denaturing conditions. In this study we use Bovine Insulin which we heat in solution at low pH. Spherulites are visible using polarised microscopy (see insets Fig 2. & 6). From which measurements of the radius and number of spherulites can be obtained.

## Salt Concentration

Increasing NaCl concentration results in:

- a decrease in the radius of observed spherulites.
- decrease in the number of clearly detected spherulites
- Shorter fibrils

At low NaCl concentration:

Fibrils shows elongated structure micrometric range

Intertwined fibrils and bundles.

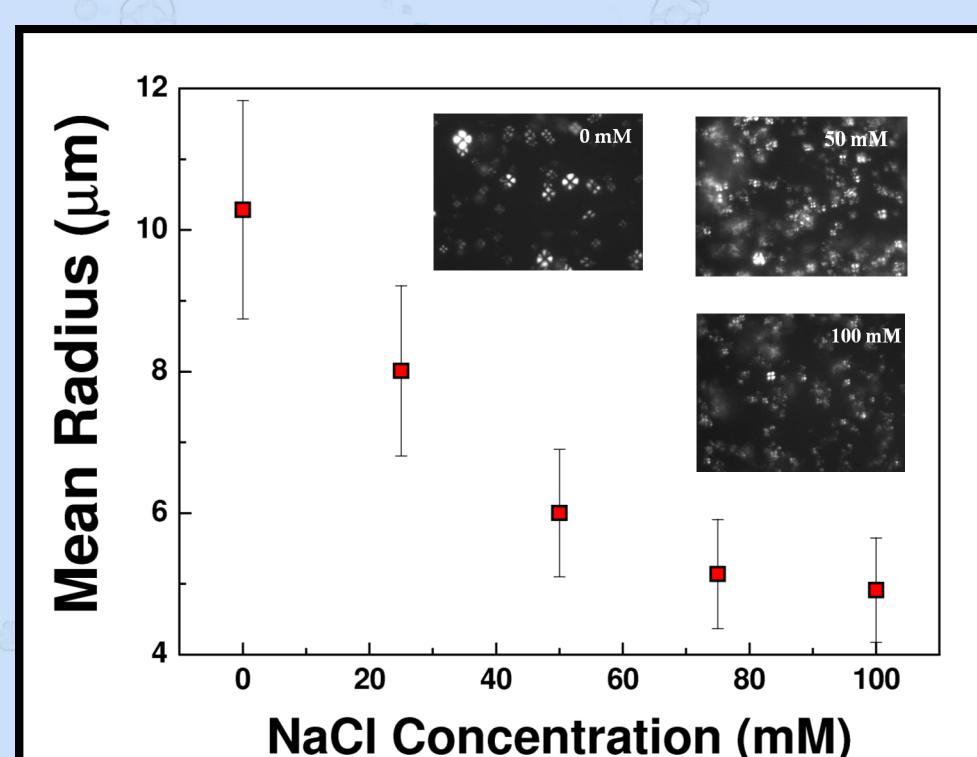
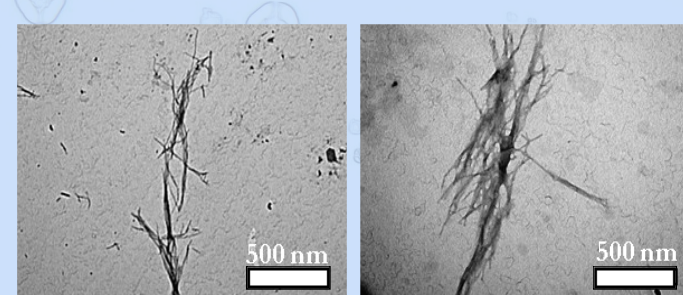


Fig 2. Main Panel) Dependence of spherulite radius with NaCl Concentration Inset) Amyloid Spherulites viewed through crossed polarisers after 24 hours incubation (80°C, pH 1).

**0mM NaCl**



**100mM NaCl**

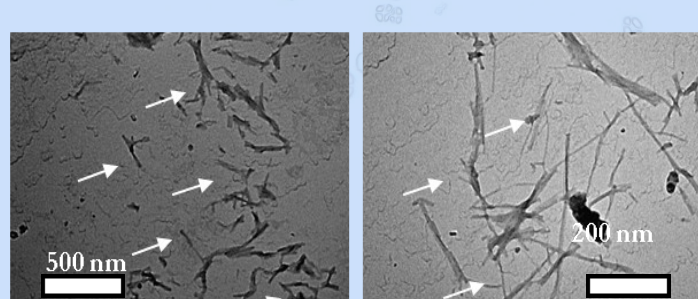


Fig 3. Typical TEM images of fibrils with and without 100mM NaCl. Fibrils show increased length at low salt concentration

## Temperature

Increasing temperature results in an increase in the observed number of spherulites and a decrease in their radii.

The balance of aggregates is unaffected by temperature (See Fig 4 inset: volume fraction of protein in spherulites does not change with temperature)

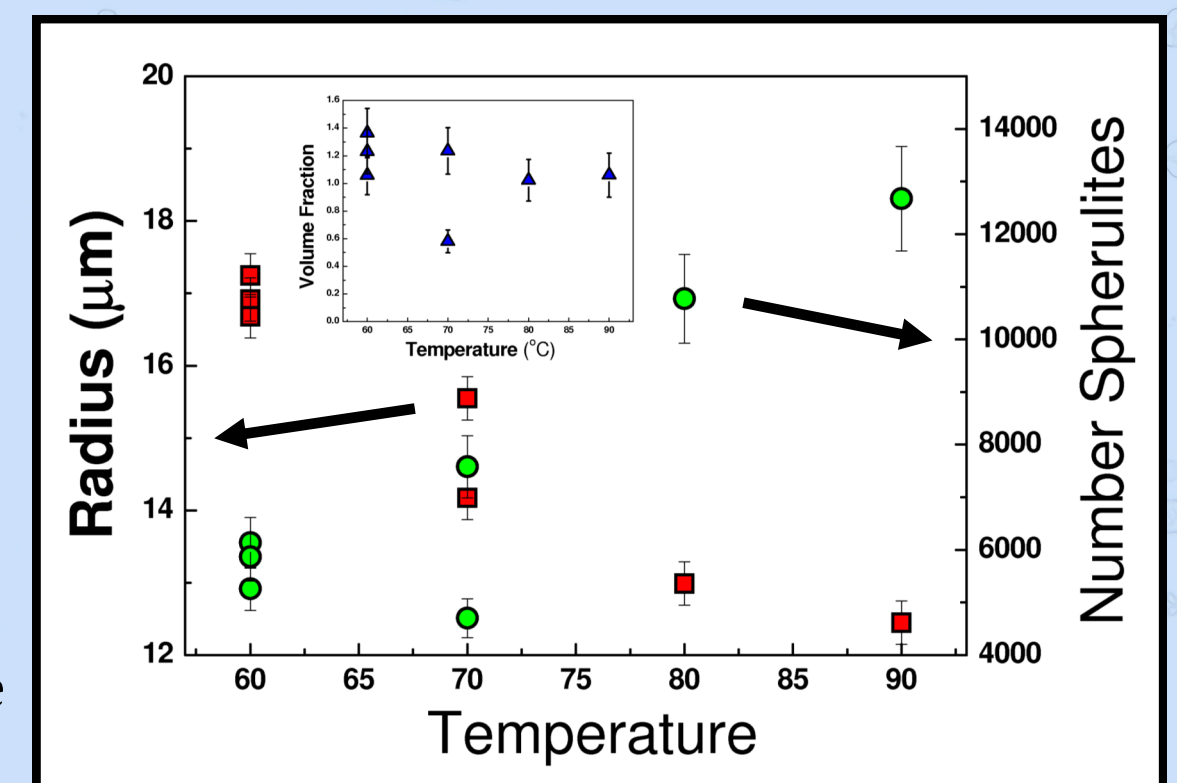


Fig 4. Effect of temperature on the final size and number of spherulites present in 25 $\mu$ l (4 mgml<sup>-1</sup>, pH 1.75, 25mM NaCl). Inset) the temp dependence of the volume fraction.

## Protein concentration

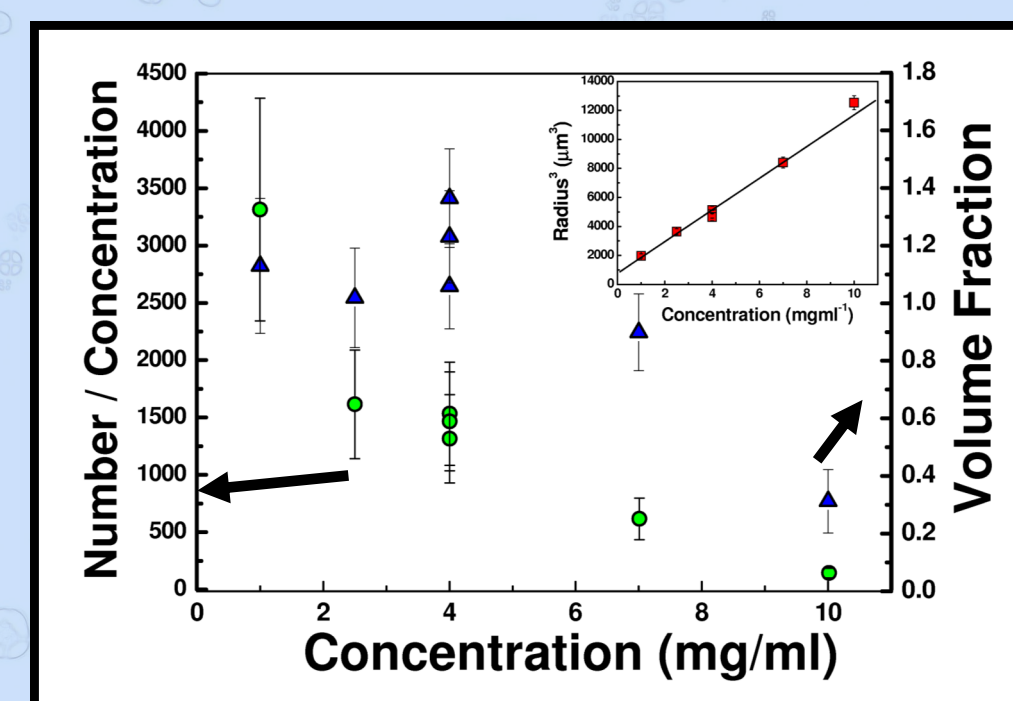


Fig 5. Effect of protein concentration on the relative abundance of spherulites (T=60°C, pH 1.75, 25mM NaCl). Inset the size dependence of spherulites on concentration

Final volume of the spherulite scales linearly with the concentration

Fewer spherulites nucleated at higher protein concentrations

Volume fraction of spherulites decreases with concentration

→ Shift in balance of aggregate morphologies

## pH dependence

Final radii of spherulites exhibit a complex dependence upon the pH

Dramatic decrease in size between pH 1.75 & 2

We observe large increases in the number of spherulites with pH at 10mgml<sup>-1</sup>

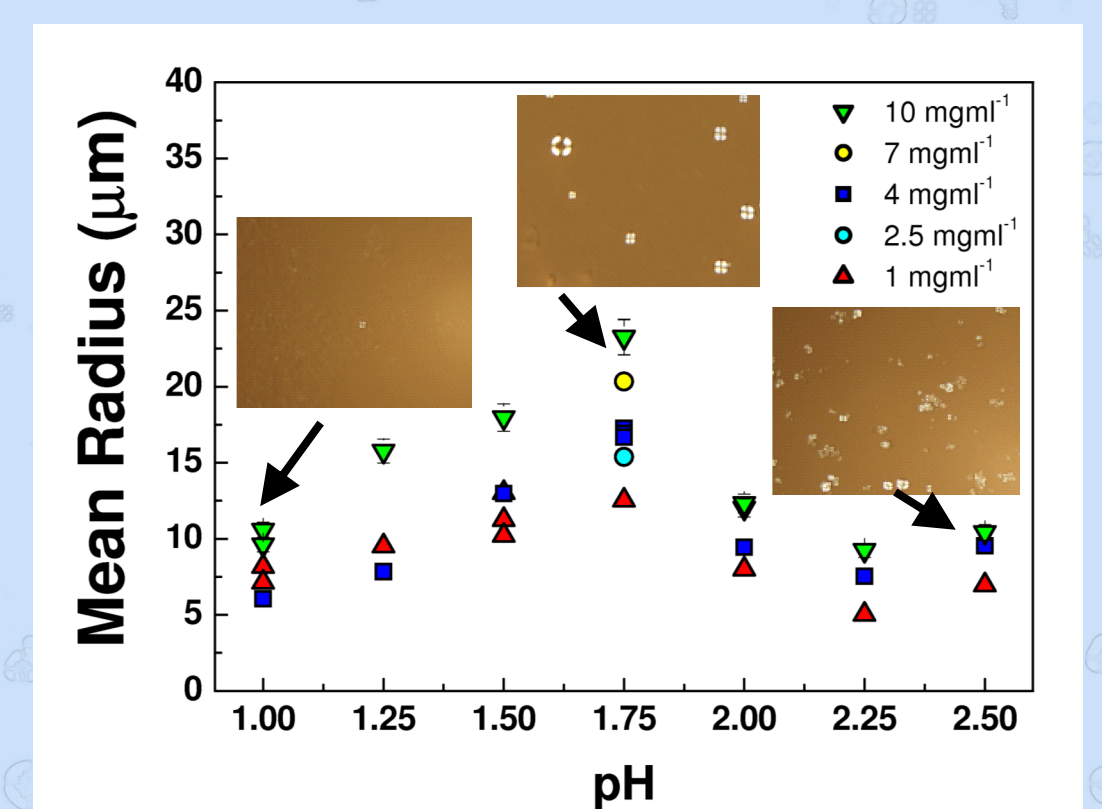


Fig 6. pH dependence of the spherulite radii showing a peak at pH 1.75 (T=60°C, 25mM NaCl). Inset) typical polarised microscopy images of spherulites

## Discussion

- Decreases in colloidal stability (increasing salt concentration, higher temp) result in the formation of larger numbers of smaller spherulites.
- Corresponding changes are also observed in isolated fibrils
- Higher protein concentrations shift the balance of morphologies from predominantly spherulites to fibrils.

## Conclusions

Environmental conditions influence spherulite and fibril morphologies, and their relative populations in solution.



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