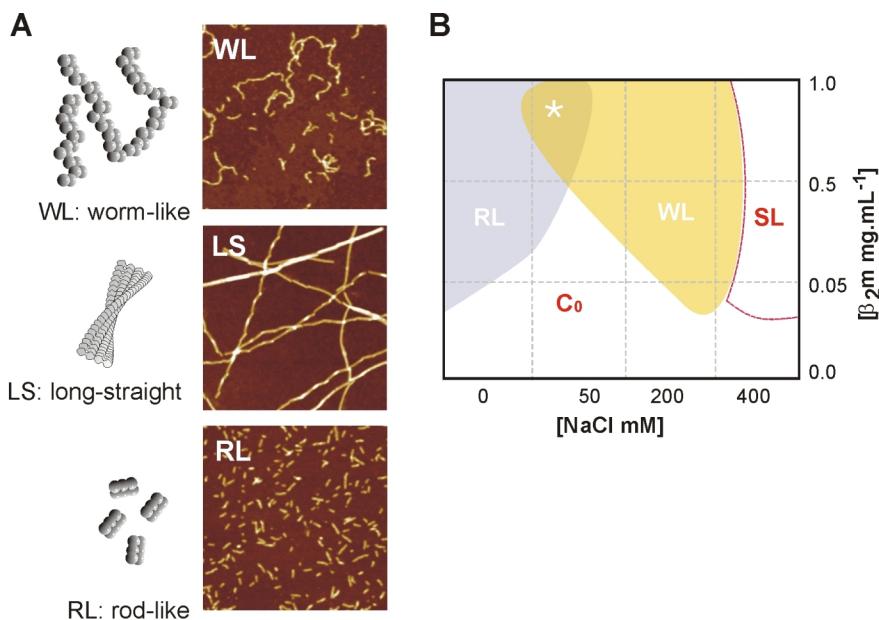


# Amyloid under the atomic force microscope

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## Introduction

Amyloid fibrils are the underlying physical feature in a subset of protein misfolding diseases known collectively as the amyloidoses, and amyloid-like fibrils are also found in other areas of biology. The precise mechanism by which proteins assemble into amyloid is unknown, although it is beginning to emerge that the assembly of proteins into amyloid is heterogeneous, a property which has been used to explain the existence of prion-strains in prion-related biology. Furthermore, the self-assembly pathway, both *in vitro* and *in vivo*, is often associated with the existence of small ‘prefibrillar’ assemblies (*i.e.* either pore-like, small globular oligomers, and/or worm-like (flexible) fibrils), which form prior to the long, straight fibrils classically observed in amyloid disorders. In cell-viability assays, ‘prefibrillar’ assemblies have been shown to be toxic, and thus determining their role in the mechanism of amyloid formation is imperative. Utilising atomic force microscopy (AFM), we have studied the self-association mechanism of  $\beta_2$ -microglobulin ( $\beta_2$ m) into amyloid-like fibrils *in vitro*.



**Fig. 1.** **A.** AFM images ( $1\mu\text{m}^2$ ) and schematic diagrams of the various types of fibrillar morphologies formed when  $\beta_2$ m assembles into amyloid-like fibrils under different conditions *in vitro*. **B.** Example state-diagram describing fibril morphology *versus* solution conditions, determined using AFM. In this case the effect of NaCl and protein concentration, in pH 3.5 buffer, on the end-point morphology after 2–4 weeks of incubation at 37°C is shown. Here (\*) denotes heterogeneous regions, while SL is the solubility limit of monomeric and/or polymeric  $\beta_2$ m, and  $C_0$  the apparent critical concentration for assembly.

## The heterogeneous nature of $\beta_2$ m assembly

The deposition of  $\beta_2$ m, a 99 residue all- $\beta$ -sheeted protein, into amyloid fibrils is associated with the condition ‘haemodialysis-related amyloidosis’, which affects all patients with renal failure on long-term dialysis. *In vitro* at low pH,  $\beta_2$ m forms distinct classes of amyloid fibrils, as defined by differences in their morphology and persistence-length. The appearance of these fibril types (worm-like (WL); rod-like (RL) and long, straight (LS)) is highly dependent on the solution conditions (Fig. 1A). In order to determine the factors governing these differences, we used AFM to map systematically the final end-point fibril morphology after long periods of incubation (~4 weeks), varying the conditions such as pH, salt and protein concentration. These data, encompassing over 500 AFM images, were then used to

construct state diagrams (Fig. 1B), and used to define regions where different fibril morphologies are favoured over others. To determine the relationships between various fibril types a series of experiments are currently being performed in which fibrils of one morphological type are formed and the solution conditions are then rapidly changed such that they cross a ‘state-boundary’. Current data suggest that fibrils of different morphology form on distinct and competitive pathways of assembly, defining an energy landscape that rationalises the sensitivity of fibril morphology on the solution conditions.

### **Publications**

Gosal, W.S., Myers, S.L., Radford, S.E. and Thomson, N.H. (2005) Amyloid under the atomic force microscope. *Protein & Peptide Letters*. **In press**.

Gosal, W.S., Morten, I.J., Hewitt, E.W., Smith, D.A., Thomson, N.H. and Radford, S.E. (2005) Competing pathways determine fibril morphology in the self-assembly of  $\beta_2$ -microglobulin into amyloid. **Submitted**.

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