Electronic Supplementary Material (ESI) for Nanoscale.
This journal is © The Royal Society of Chemistry 2014

Supporting Information

Stability and Cytotoxicity of Crystallin Amyloid Nanofibrils

Manmeet Kaur[a], Jackie Healy[a], Madhusudan Vasudevamurthy[a], Moritz Lasse[a], Ljiljana Puskar[b], Mark Tobin[b], Celine Valery[c], Juliet A. Gerrard*[,a,d,e] and Luigi Sasso*[,a,d]

[a] Biomolecular Interaction Centre and School of Biological Sciences, University of Canterbury, Christchurch 8140, New Zealand

[b] Australian Synchrotron, Clayton VIC 3168, Australia

[c] School of Medical Sciences and Health Innovations Research Institute, RMIT University, Bundoora VIC 3083, Australia

[d]MacDiarmid Institute for Advanced Materials and Nanotechnology, Wellington 6140, New Zealand

[e] Callaghan Innovation Research Ltd, Lower Hutt 5040, New Zealand

Table of Contents:

ThT fluorescence of buffers used for normalisation

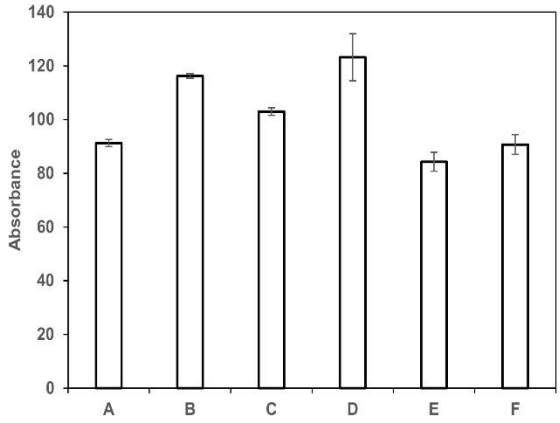

Figure S1. ThT fluorescence of buffers used for normalising ThT fluorescence of PNFs in appropriate buffer. Sample buffers from left to right: (A) 0.1 M sodium acetate, pH-2.0, (B) 0.1 M sodium acetate, pH-4.0, (C) 0.1 M sodium phosphate, pH-6.0, (D) 0.1 M sodium phosphate , pH-7.2, (E) 0.1 M HEPES, pH-8.0, (F) 0.1 M HEPES, pH- 9.0, and (G) sodium borate, pH-11.0. Error bars represent the SD of three replicates.

ThT fluorescence of solvents used for normalisation

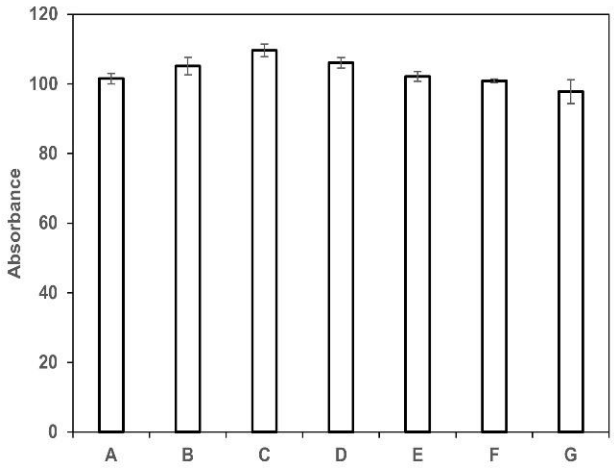

Figure S2. ThT fluorescence of solvents used for normalising ThT fluorescence of PNFs in appropriate solvent. Samples from left to right: (A) MeOH, (B) EtOH, (C) DMSO, (D) iPrOH, (E) ACN, and (F) water. Error bars represent the SD of three replicates.

Representative TEM images of fibrils over a wide range of pHs

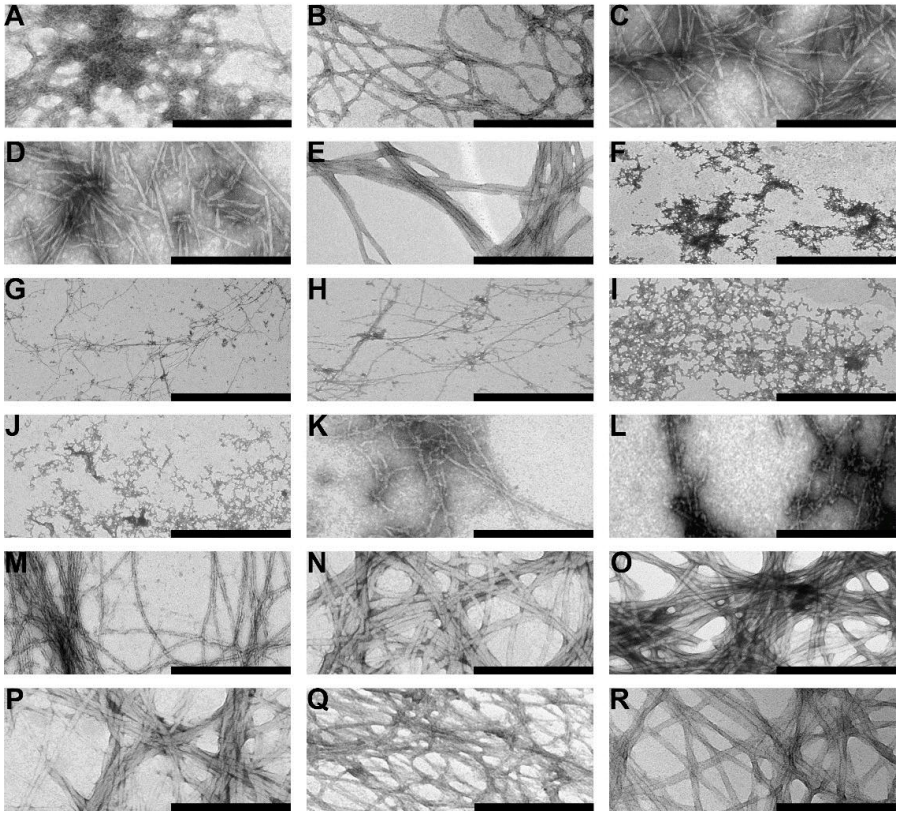

Figure S3. Representative TEM images of fibrils over a wide range of pH: insulin PNFs - (A) pH-2.0, (B) pH-4.0, (C) pH-6.0, (D) pH-8.0, (E) pH-9.0 and (F) pH-11.0; whey PNFs - (G) pH-2.0, (H) pH-4.0, (I) pH-6.0, (J) pH-8.0, (K) pH-9.0 and (L) pH-11.0; crystallin PNFs - (I) pH-2.0, (M) pH-4.0, (N) pH-6.0, (O) pH-8.0, (P) pH-9.0 and (Q) pH-11.0. Scale bar is 100 nm.

The dark aggregates observed in the samples resuspended in buffer could be due to the formation of the salt crystals upon drying of the TEM grids, and are unlikely to be dissolved or fragmented fibrils. The buffer is interacting with the stain used for TEM, as this was present in the sample with a buffer and not in samples with water (Fig. S3 compared to Fig. 2B-g,n,u). Uranyl acetate used as a staining agent can form complexes with various buffers leading to the formation of different complexes with different charge (Hayat, M.A. Principles and techniques of electron microscopy: biological applications, 4th ed, Cambridge University Press, Cambridge, U.K., 2000).

Representative TEM images of PNFs at variety of temperatures

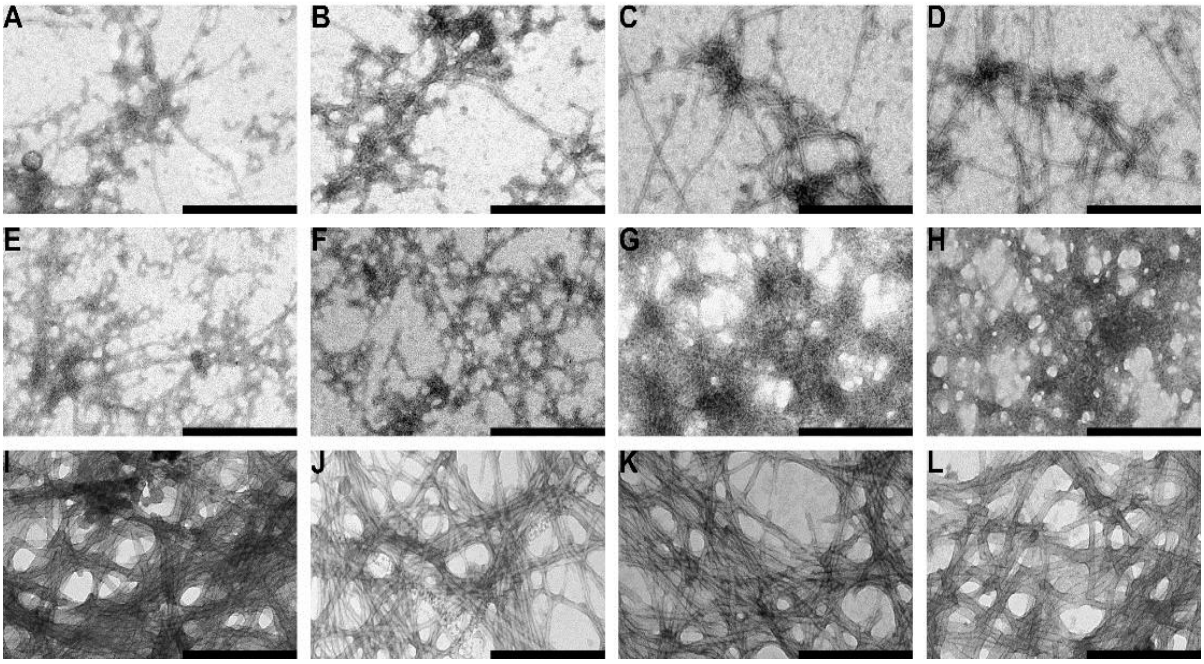

Figure S4. Representative TEM images of PNFs at variety of temperatures: Row 1-insulin PNFs at (A) -20 °C, (B) 22 °C, (C) 37 °C, and (D) 80 °C; Row 2-whey PNFs at (E) -20 °C, (F) 22 °C, (G) 37 °C and (H) 80 °C; Row 3-crystallin PNFs at (I) -20 °C, (J) 22 °C, (K) 37 °C, and (L) 80 °C. Scale bar is 100 nm.

IR spectrum of full amide fingerprint region

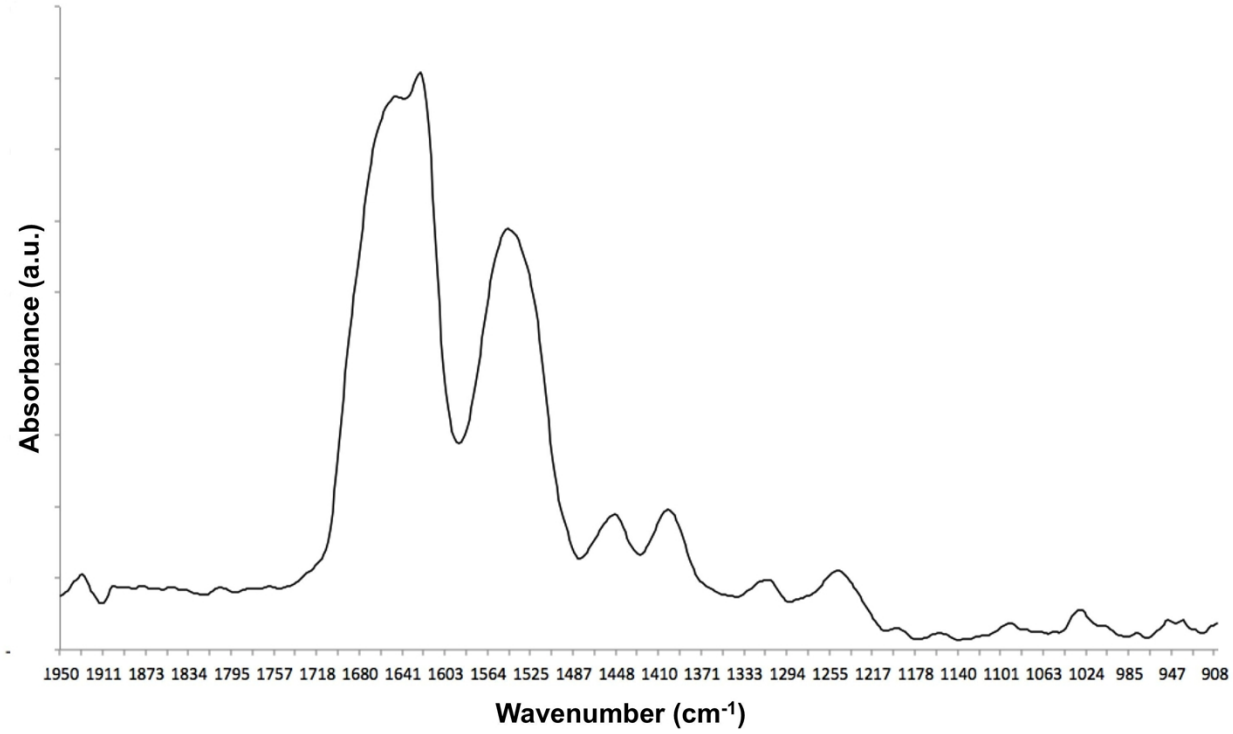

Figure S5. Typical IR absorbance spectrum of amyloid crystallin nanofibrils, across the whole amide fingerprint region, showing the baseline.