Supporting Information

# Mechanical stress affects glucagon fibrillation kinetics and fibril structure

*Francesca Macchi[1], Søren V. Hoffmann[2], Martin Carlsen[3], Brian Vad[1], Alberto Imparato[3], Christian Rischel[4*], Daniel E. Otzen[1]**

[1] iNANO, Center for Insoluble Protein Structures, Department of Molecular Biology, Aarhus University, Gustav Wieds Vej 10C, DK – 8000 Aarhus C, Denmark

[2]Institute for Storage Ring Facilities, ISA, Aarhus University, Ny Munkegade 120, Bldg. 1520, DK-8000 Aarhus, Denmark

[3] Department of Physics and Astronomy, Aarhus University, Ny Munkegade 120, Bldg. 1520, DK – 8000 Aarhus C, Denmark

[4] Protein Structure and Biophysics, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Måløv, Denmark

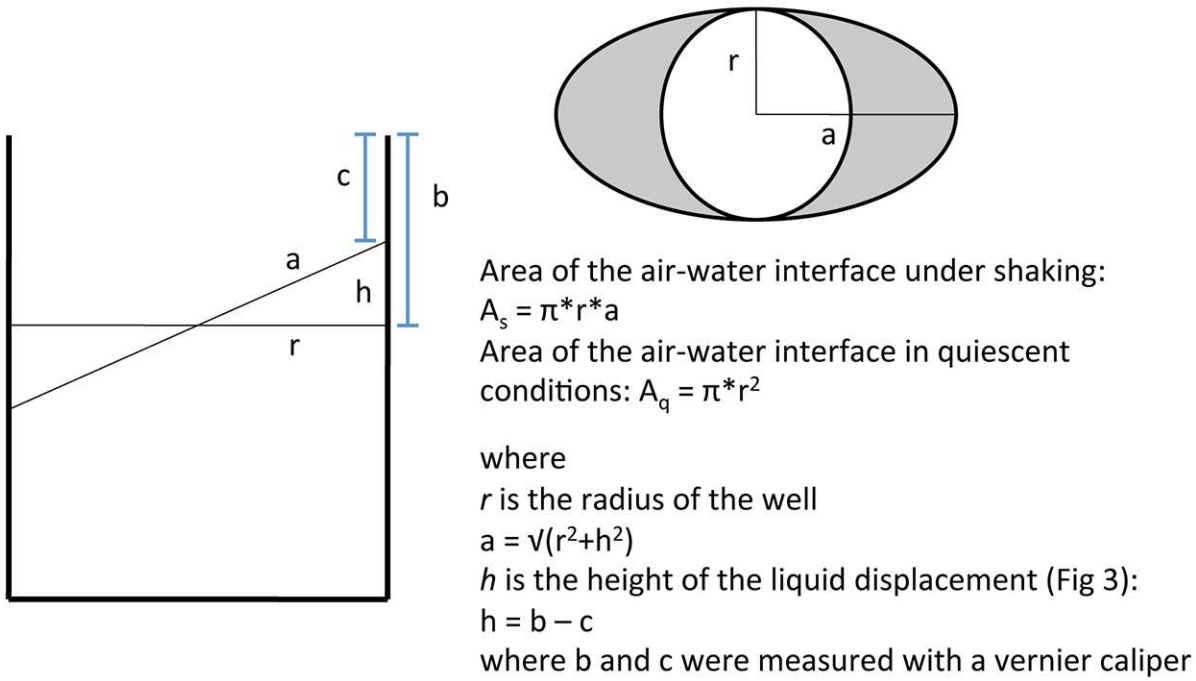



Figure S1: Geometrical argument used for the calculation of the increased surface area under shaking conditions.

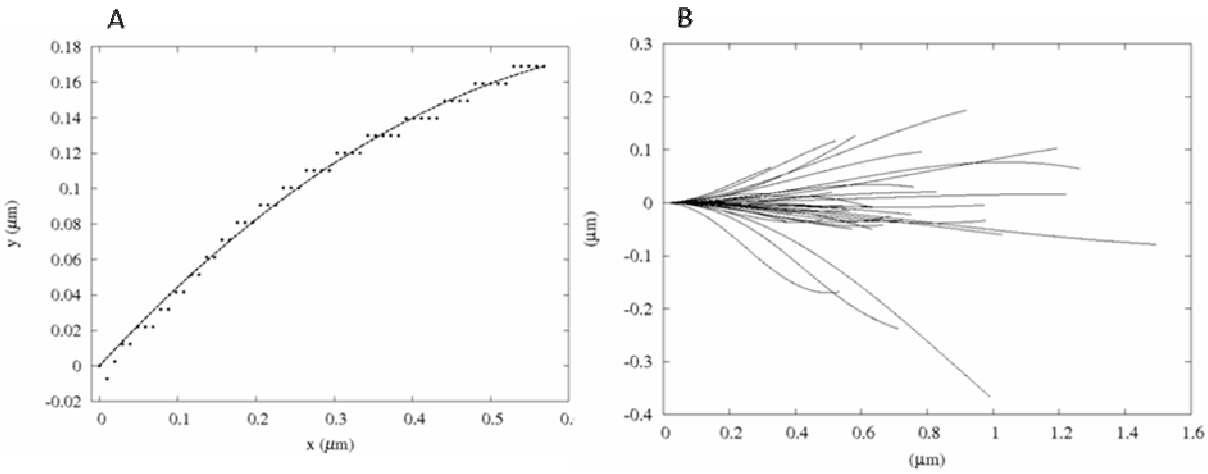



Figure S2: Least-squares cubic spline fit used to obtain the contour of a single fiber (panel A). Sample of fibril contours obtained at pH 3, 3mg/ml, 500 rpm (panel B). The fibrils have been rotated and translated such that all the fibrils have the same initial tangent.

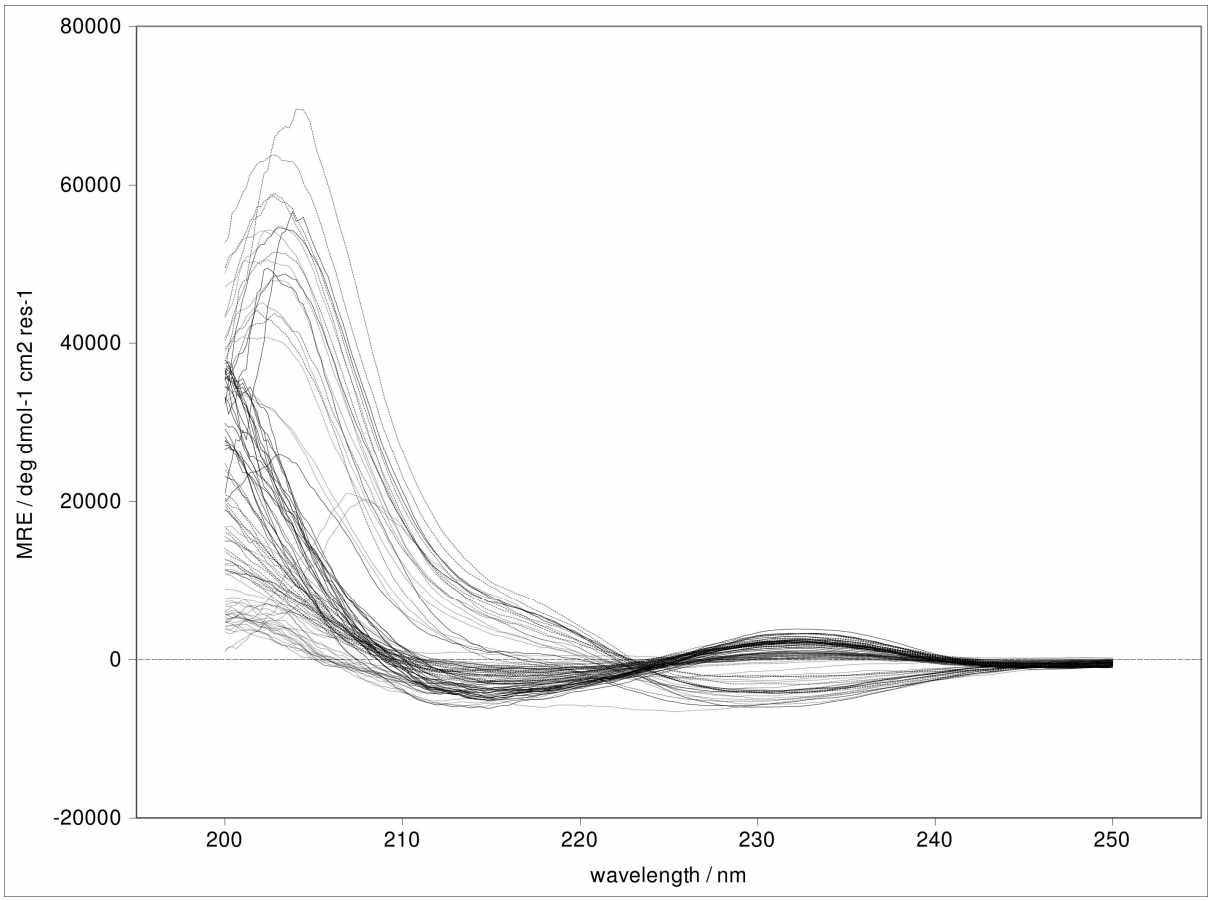



Figure S3: CD spectra of all fibrils samples retrieved from the microtiter plates: experiments at 720 rpm (solid line), at 300 rpm (dashed line) and at 180 rpm (dotted line).

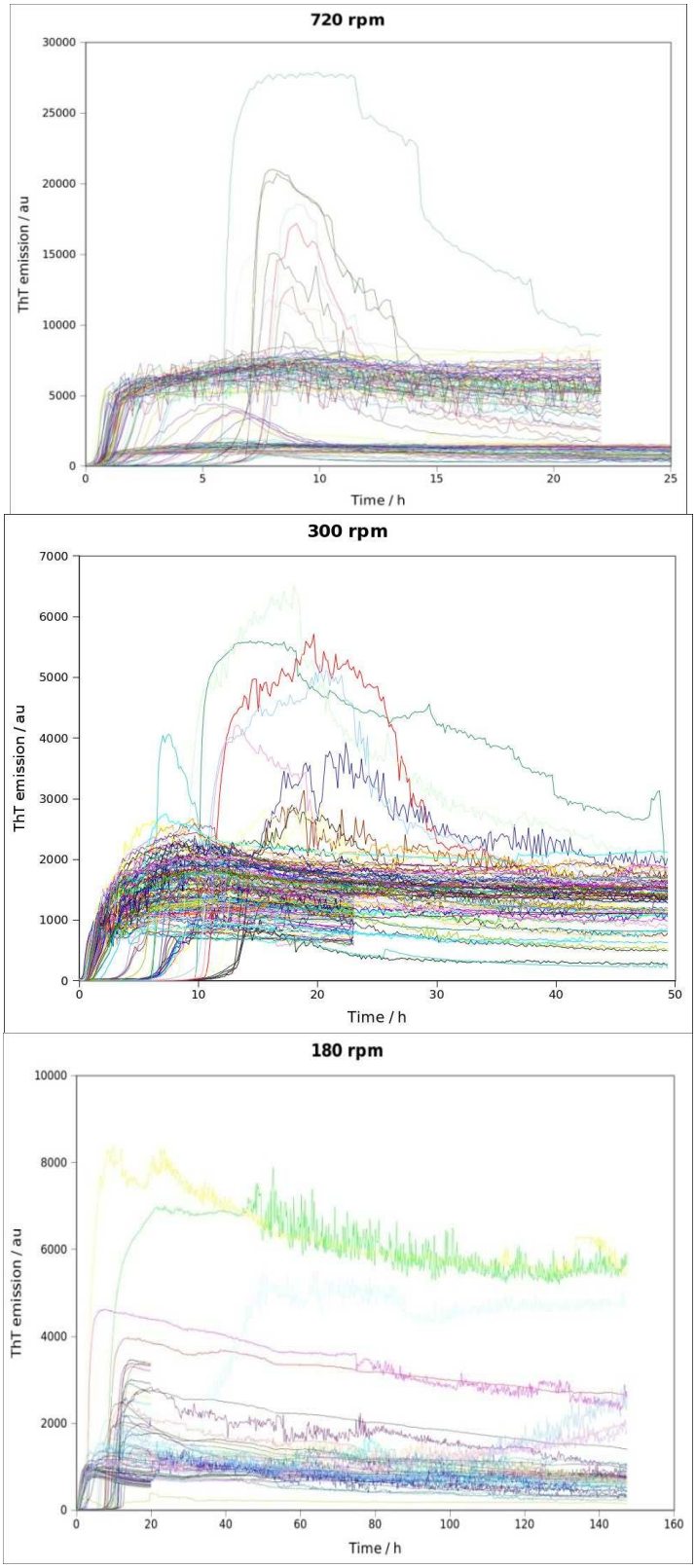



Figure S4: Kinetic profiles of all the plate reader experiments performed in this study

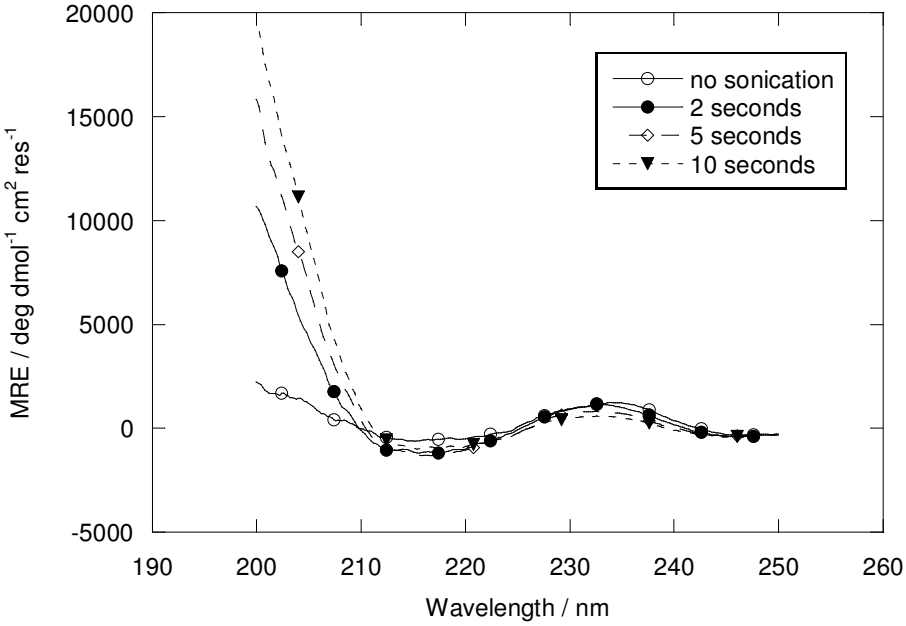



Figure S5: Effect of sonication on the CD spectra of class HS fibrils

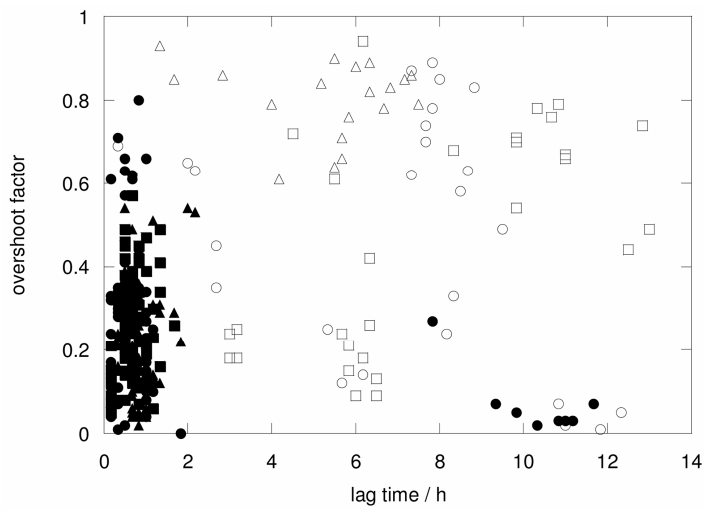



Figure S6: Correlation between lag time and overshoot factor. The colors were assigned according to the C factor: HS fibrils are characterized by positive C factor (filled symbols) and short lag time, whereas LS fibrils had negative C factor (open symbols) and longer lag time. The symbols were assigned according to the rpm value used during the fibrillation assays: 180 rpm (circles), 300 rpm (squares), 720 rpm (triangles). Each data point represents one individual kinetic profile.

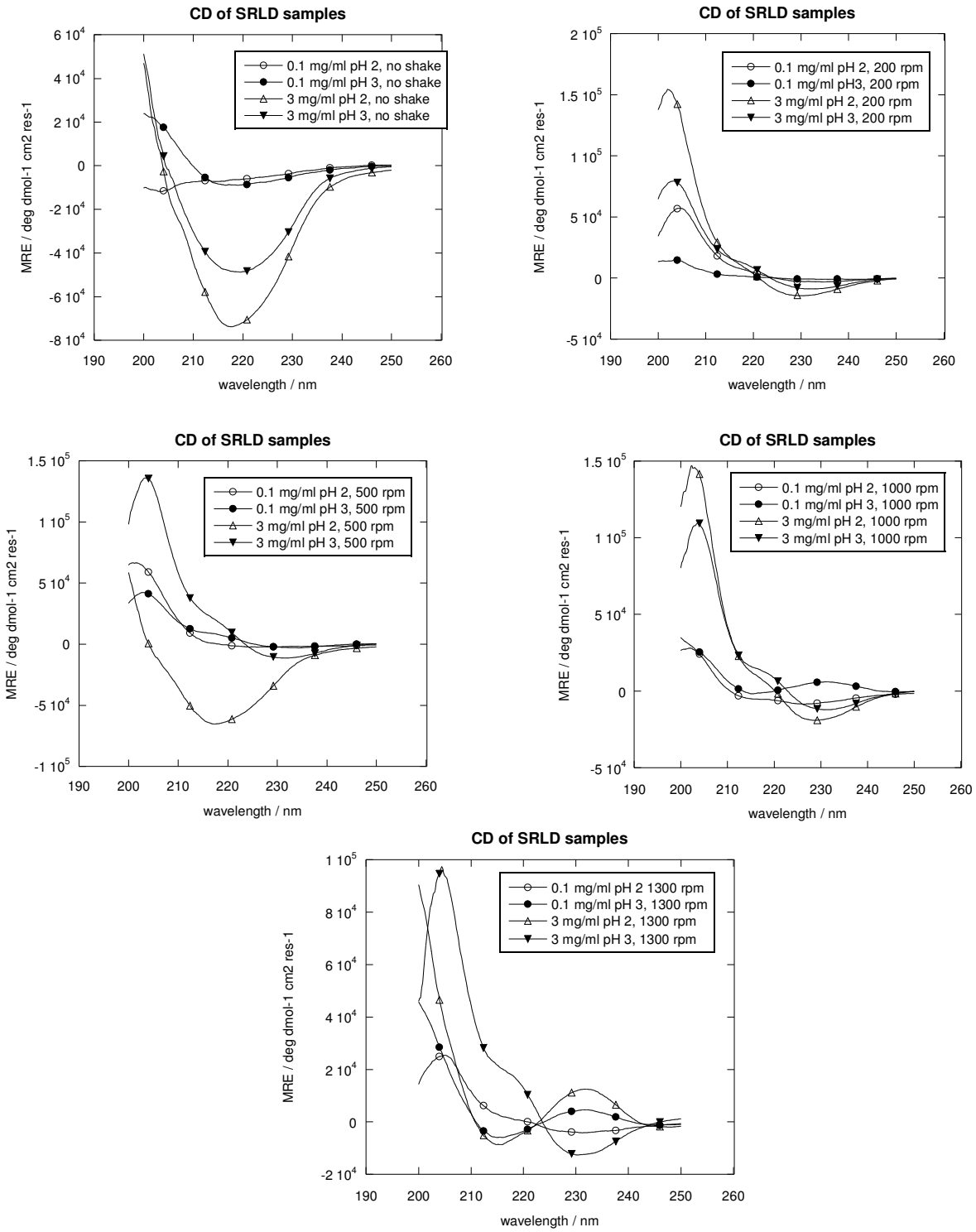



Figure S7: CD spectra of the samples reported in Table 1

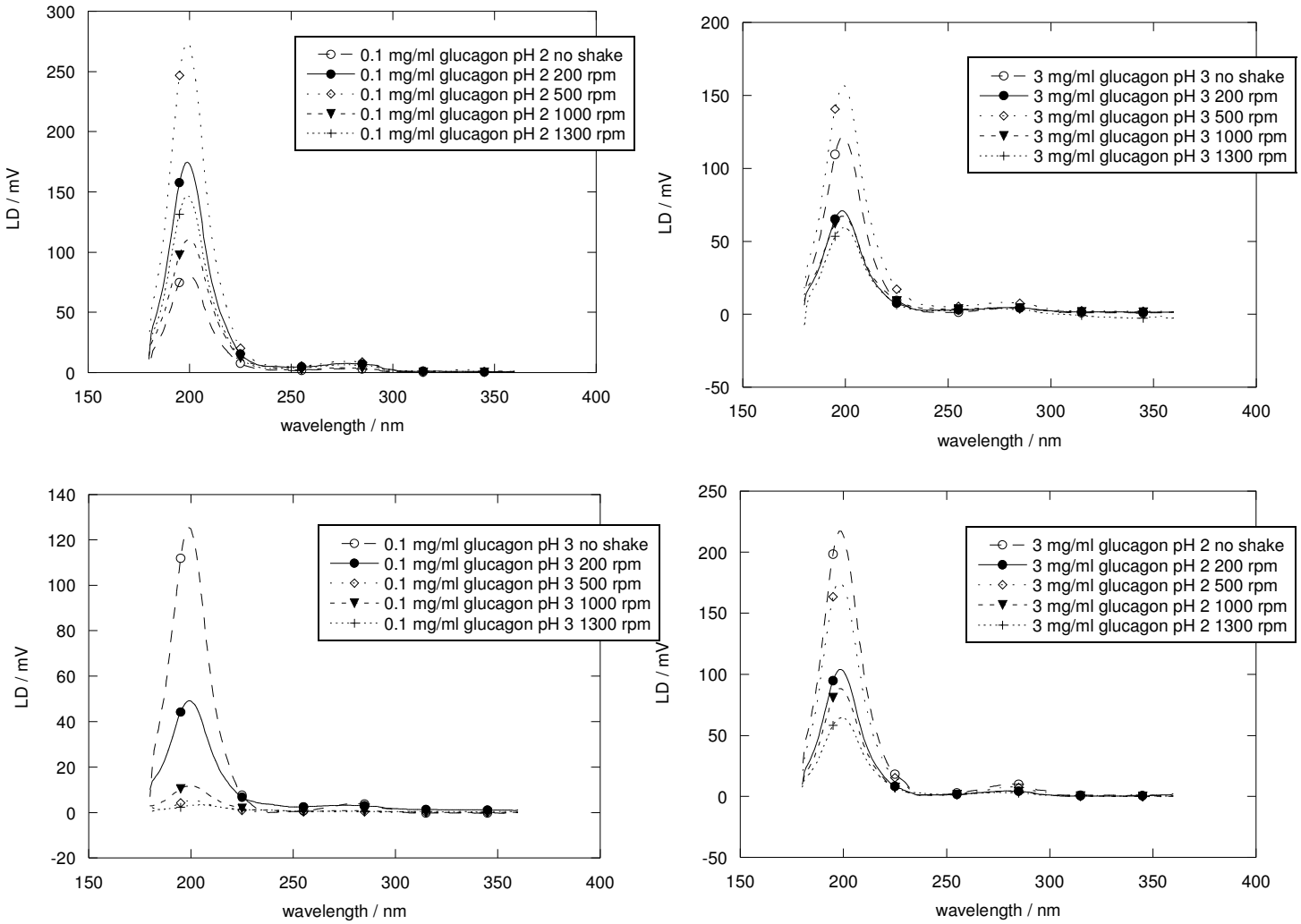



Figure S8: SRLD spectra of all samples grown in the eppendorf shaker

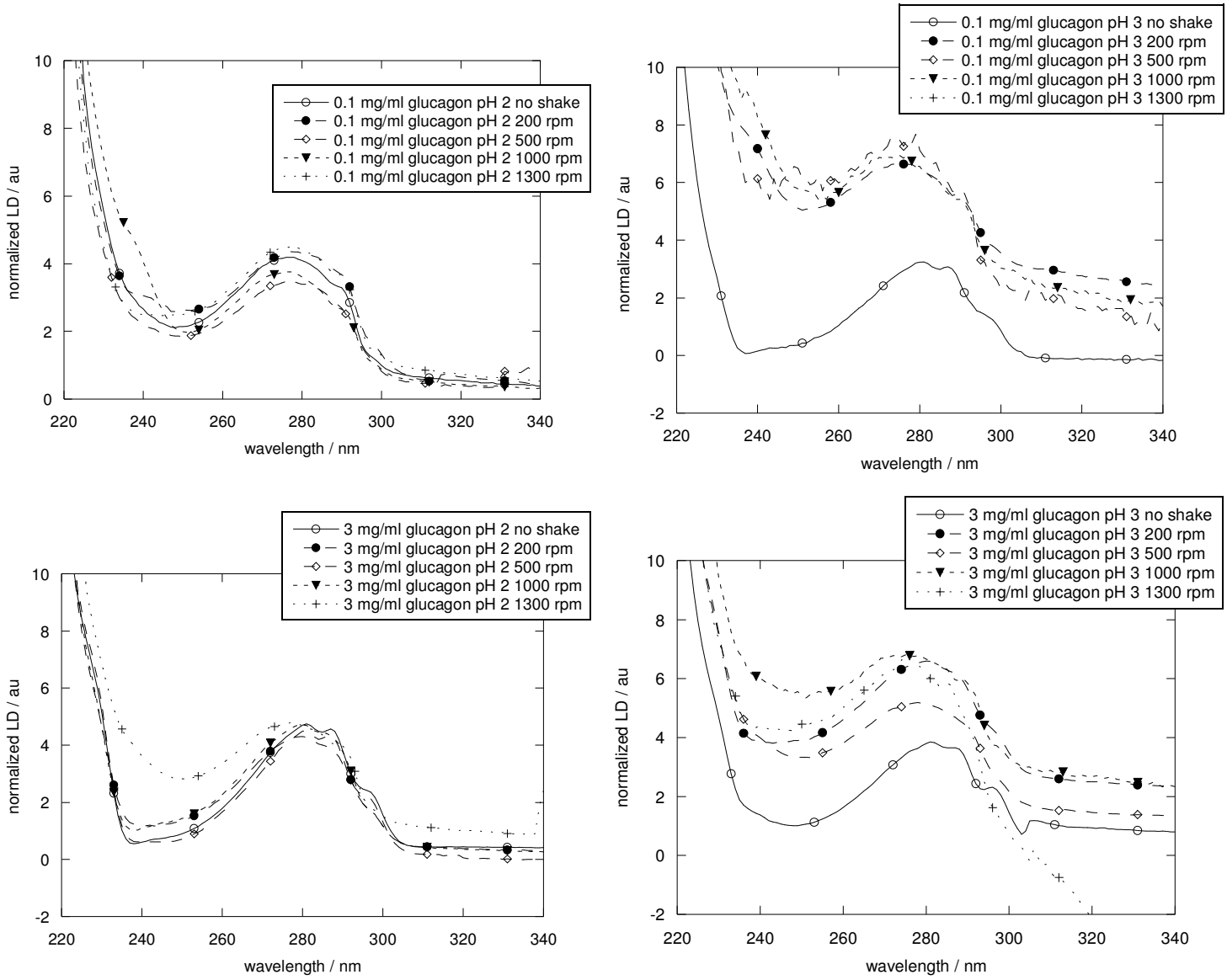



Figure S9: Near UV region of the SRLD spectra, shown after the normalization of the data according to the backbone peak

A

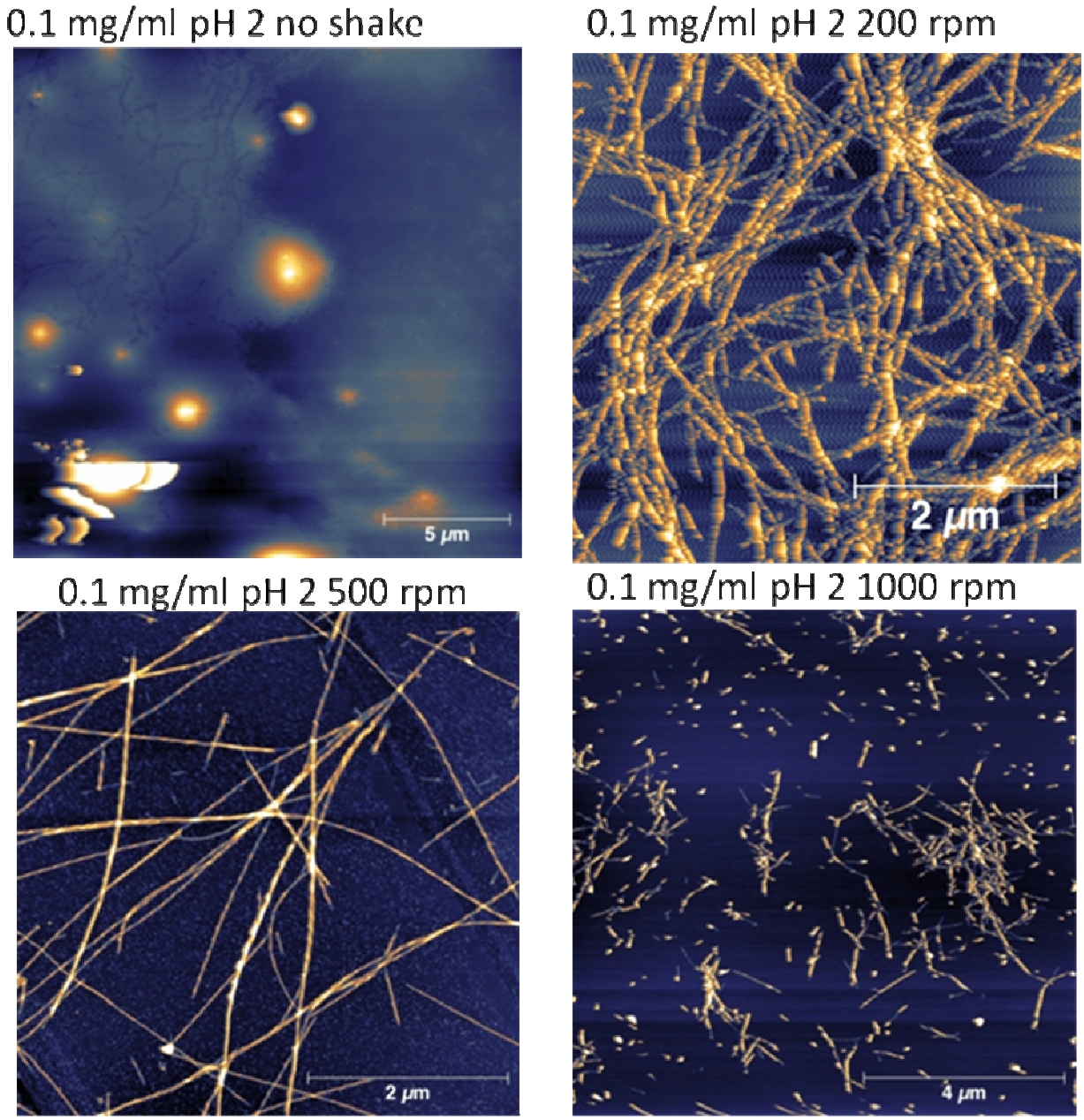
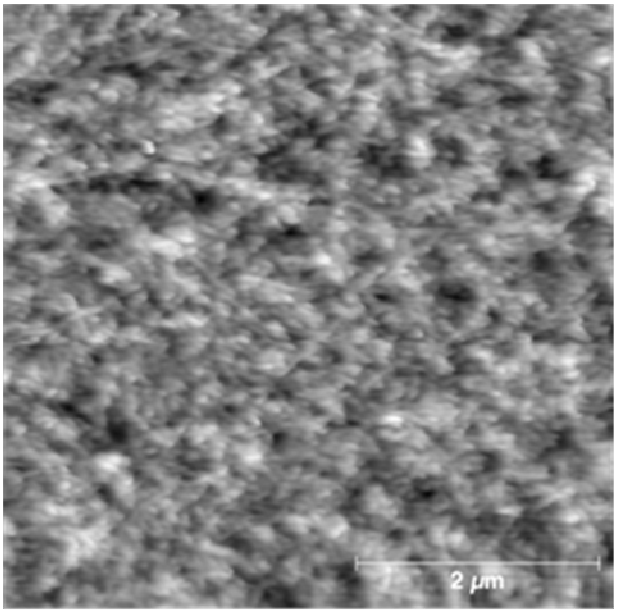


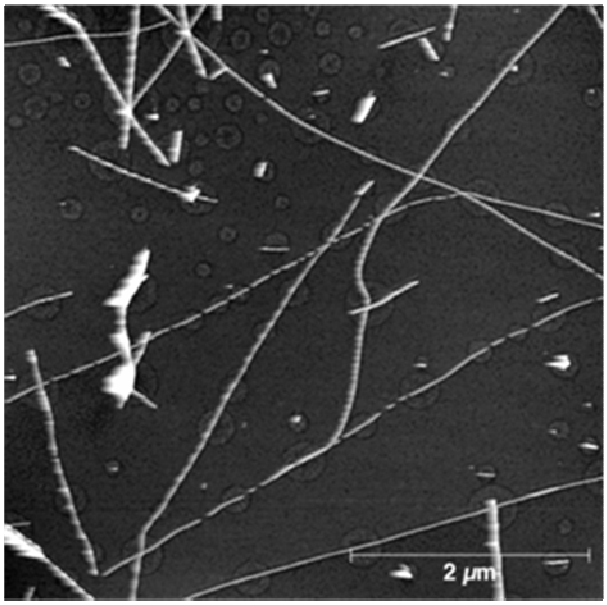

Figure S10 A-D: examples of AFM images used for the calculation of the bending rigidity as in Fig. 10.

B

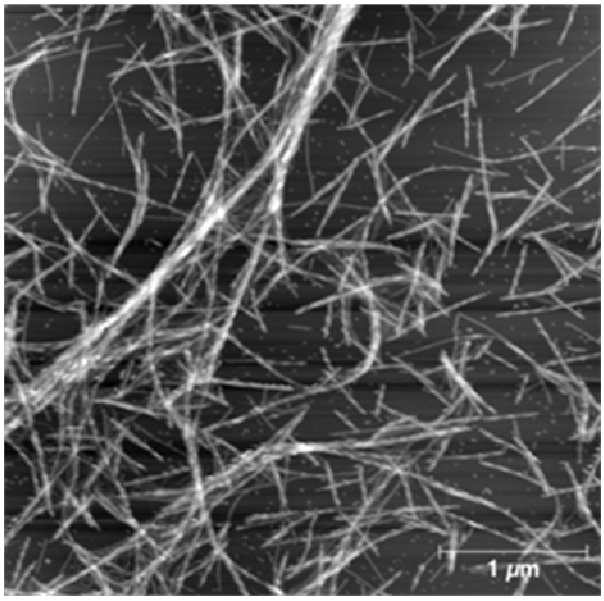
3 mg/ml pH 2 no shake




3 mg/ml pH 2 200 rpm

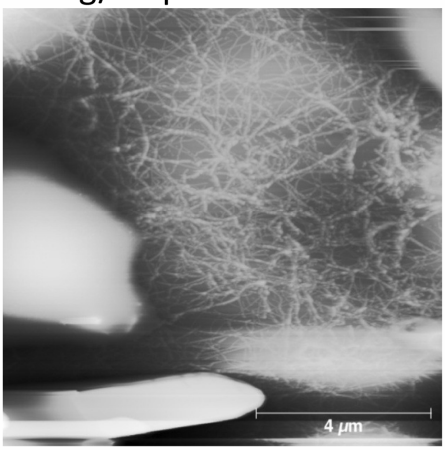



3 mg/ml pH 2 1000 rpm

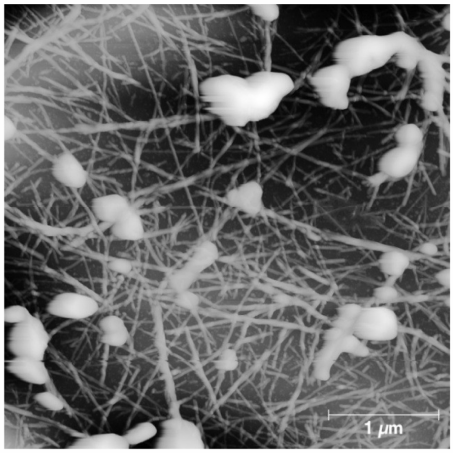


C

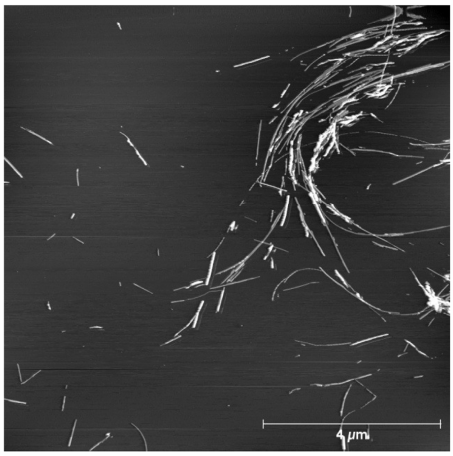
3 mg/ml pH 3 no shake



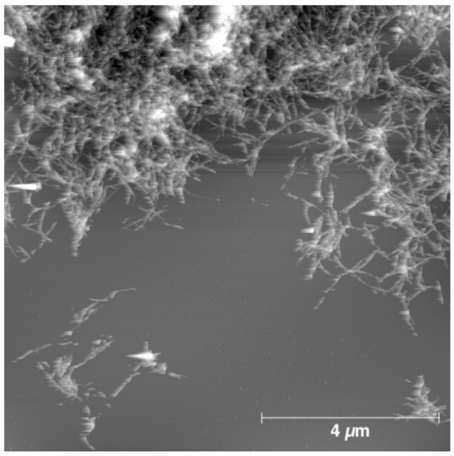

3 mg/ml pH 3 200 rpm



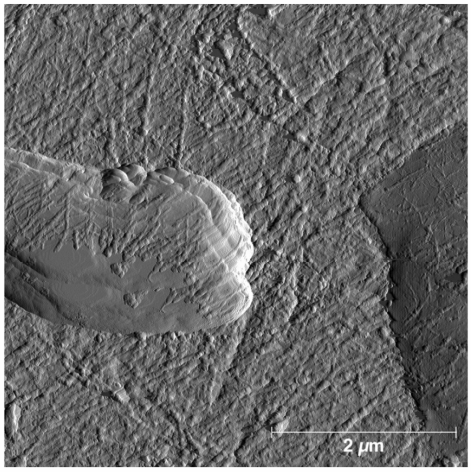

3 mg/ml pH 3 500 rpm



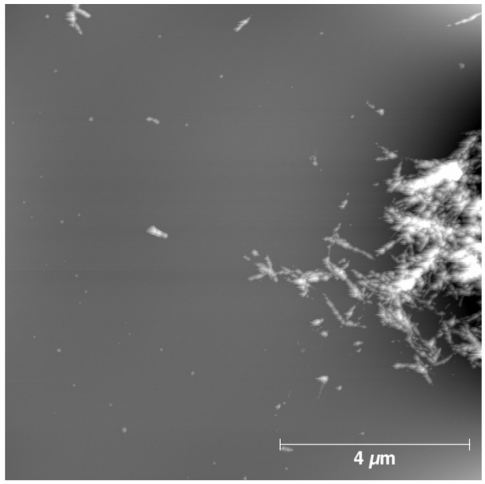

3 mg/ml pH 3 1000 rpm




D




0.1 mg/ml pH 3 no shake




0.1 mg/ml pH 3 1000 rpm