Electronic Supplementary Material (ESI) for Lab on a Chip.

Resolving protein mixtures using microfluidic diffusional sizing combined with synchrotron radiation circular dichroism

C. Bortolini[1,2], T. Kartanas[1], D. Copic[3], I. Condado Morales[1], Y. Zhang[1], P. K. Challa[1], Q. A. E. Peter[1], T. Jávorfi[4], R. Hussain[4], G. Siligardi[4], M. Dong[2], Tuomas P. J. Knowles[1,5], and Jérôme Charmet[6]

[1] *Chemistry Department, University of Cambridge, Lensfield Road, Cambridge, CB3 0FF, UK*

[2] *Interdisciplinary Nanoscience Center, Aarhus University, Aarhus, 8000, DK*

[3] *Institute for Manufacturing, Engineering Department, University of Cambridge, Charles Babbage Road, Cambridge, CB3 0FS, UK*

[4] *Diamond Light Source, Harwell Science and Innovation Campus, Didcot, OX11 0DE, UK*

[5] *Cavendish Laboratory, University of Cambridge, J. J. Thomson Avenue, Cambridge, CB3 0HE, UK and*

[6] *Institute of Digital Healthcare, WMG, University of Warwick, Coventry, CV4 7AL, UK*

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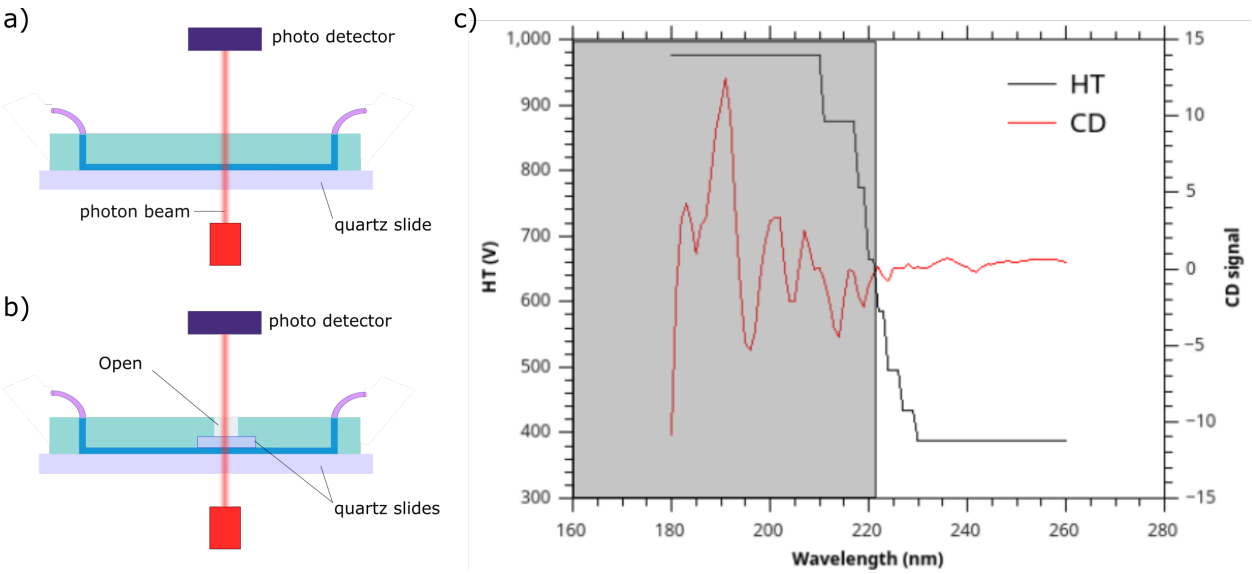

FIG. 1: Microfluidic devices made of PDMS are not compatible with SRCD. a) Sketch of a conventional PDMS based microfluidic device. In this case the photon beam goes through the PDMS and results in the spectra shown in (c). b) Sketch of a PDMS based microfluidics device with a quartz measurement window as proposed in the manuscript (second architecture). In this case the photon beam does not pass through the PDMS and enables measurements in the far-UV region. c) Plot of Wavelength (nm) *vs* CD signal (mdeg) and High Voltage (V) for the device presented in (a). The wavelength cut-off in our experiment is above 220 nm, however spectrum distorsion occurs as early as 230 nm.

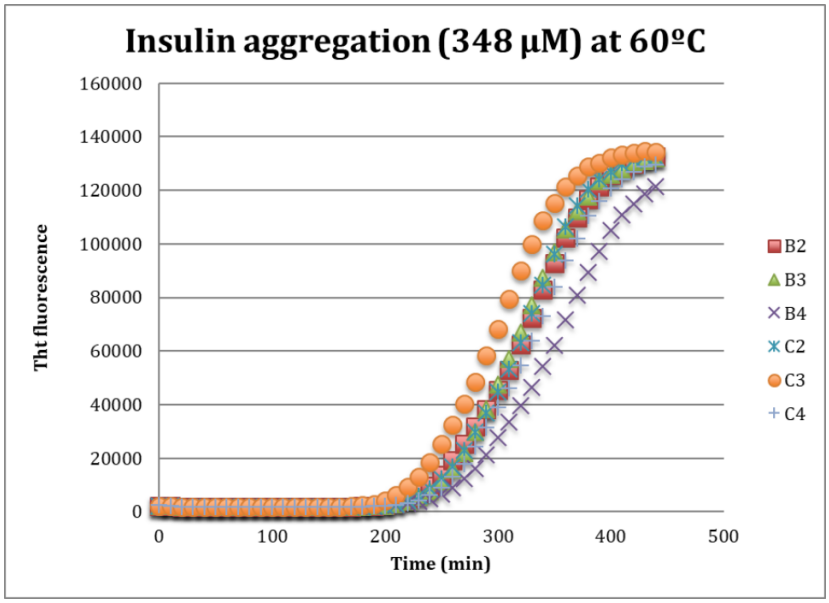

FIG. 2: Insulin fibrils evaluation: The fibrils were assembled in vitro by incubating bovine insulin (Sigma Aldrich) at a concentration of 348 μM (2 mg/ml) in HCl (pH 1.3) at 60C for 8 hours on non-binding plates (half-volume, CORNING 3881) with Tht (40 μM)

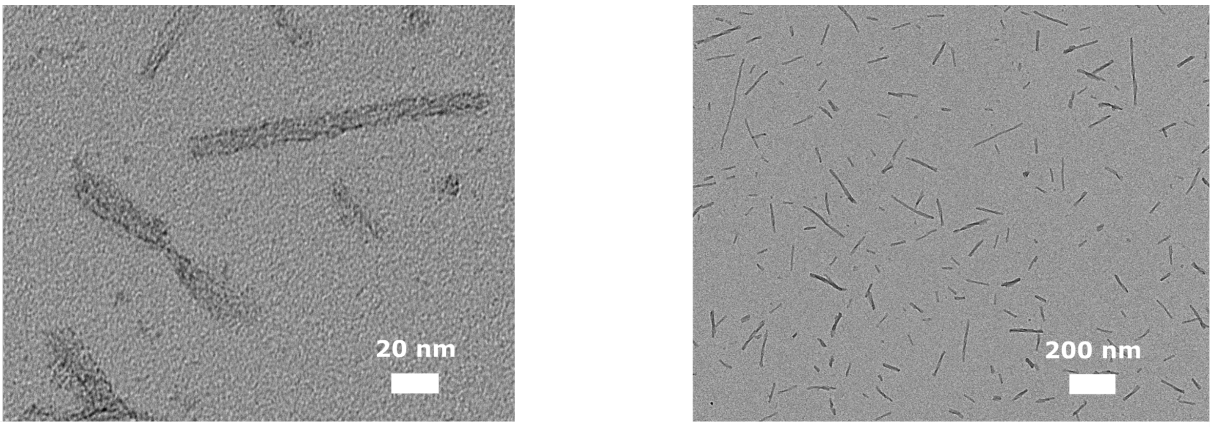

FIG. 3: Transmission Electron Microscope (TEM) micrographs of sonicated insulin fibers.

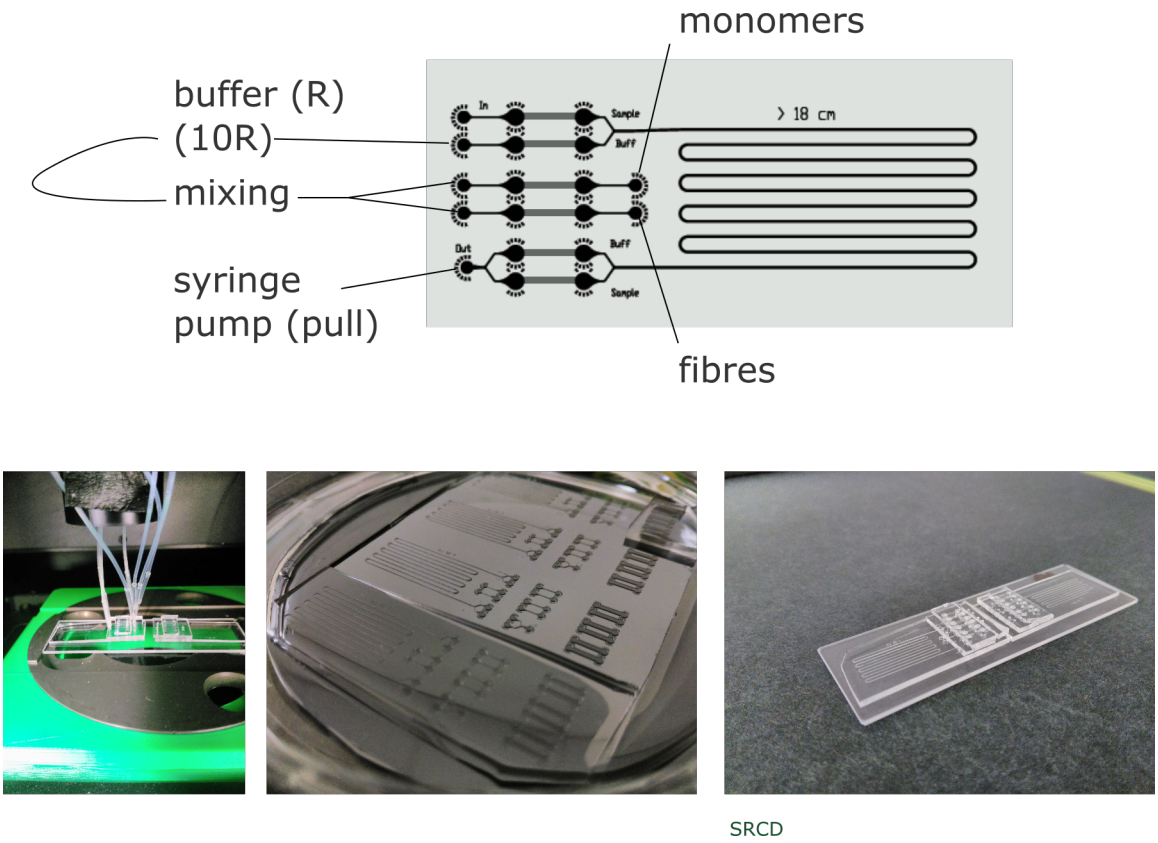

FIG. 4: Details of a 2 layer devices with integrated mixing of monomers and fibres. Top, sketch showing the connection. Bottom, optical images of the devices.

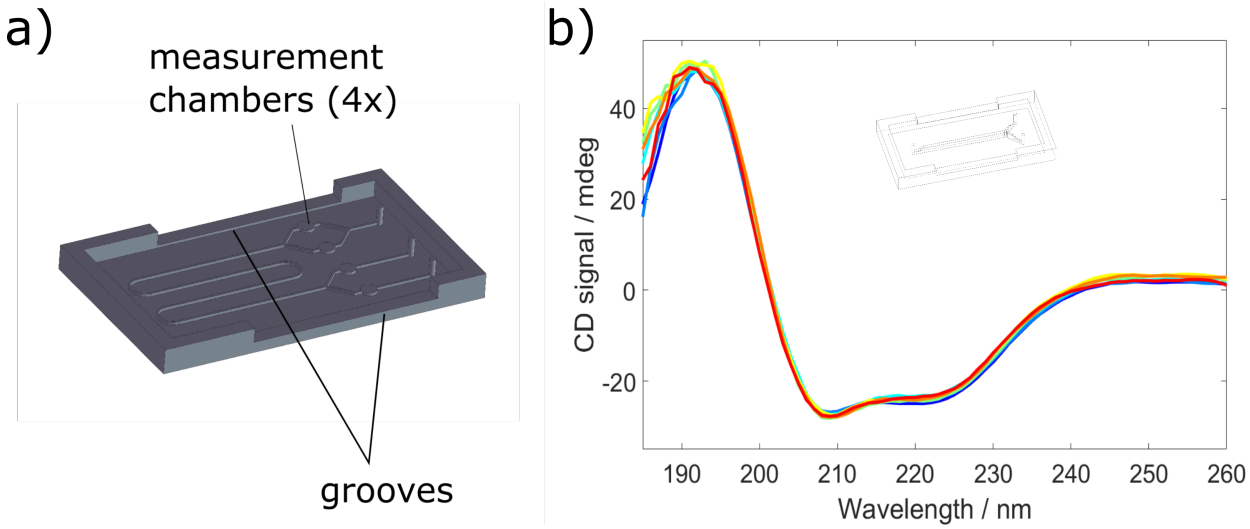

FIG. 5: Dilution of BSA (initial concentration 0.2mg/ml, pH 7) in a microfluidic device fabricated using a 3D printed master mould. a) Detail of a 3D printed master mould sketch based on the second device architecture. b) Normalised spectra of Fig. 4 (from manuscript) Zto show that the dilution did not induce any distortions and sketch of the 3D printed master (inset).

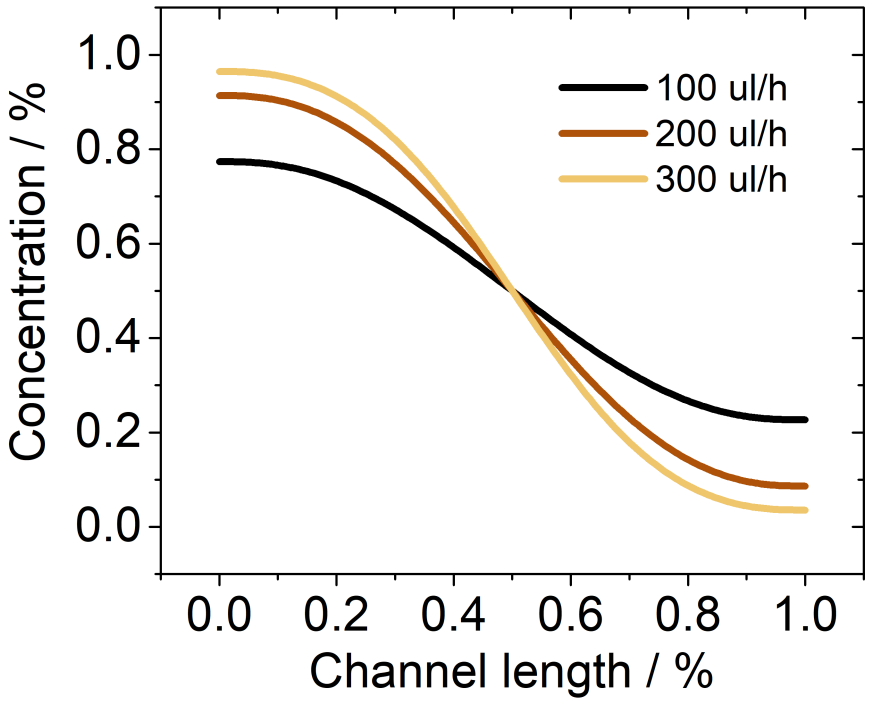

FIG. 6: Simulation of the concentration profile of insulin monomer across 100 μm wide channel after a diffusion length of 150 mm. The channel height = 50 μm corresponds to the pathlength.

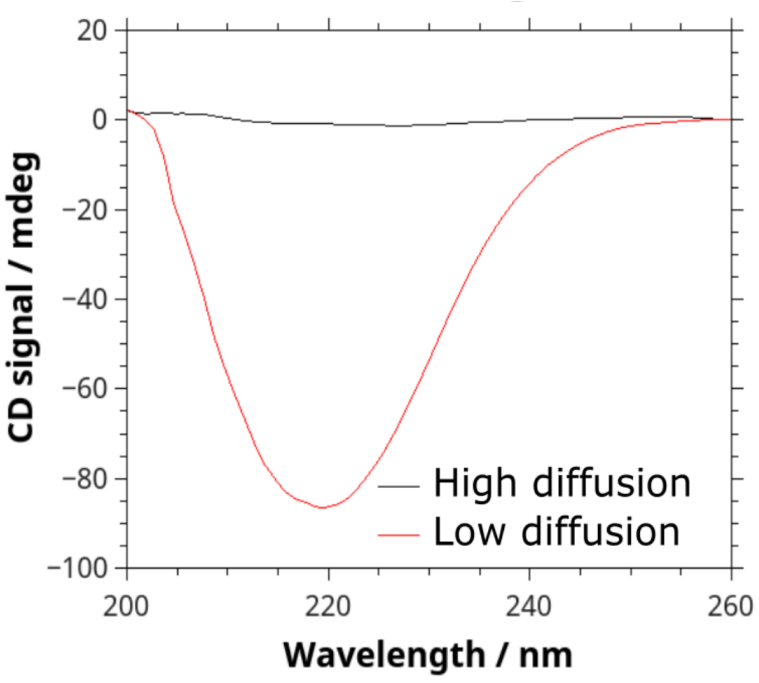

FIG. 7: Diffusion of insulin fibres (0.4 mg/mL) in the High- and Low-diffusion chambers. The negative band around 222 nm indicates the presence of a strong beta-sheet rich compound. The measured fraction in the high diffusion chamber corresponds to 2.3 % of the total fibrils concentration. This value compares well with the calculated values of 1.1 %.

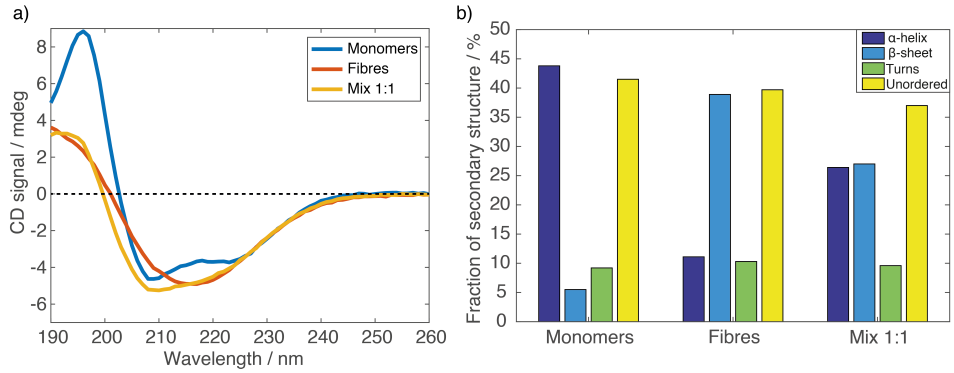

FIG. 8: Reference insulin samples (0.2 mg/mL) spectra and secondary structure content estimation (a) Reference CD spectra of insulin samples (monomers, fibrils and 1:1 mixture) obtained with a benchtop spectrometre (Chirascan); (b) Secondary structure fractions estimation obtained with web server BeStSel [9].

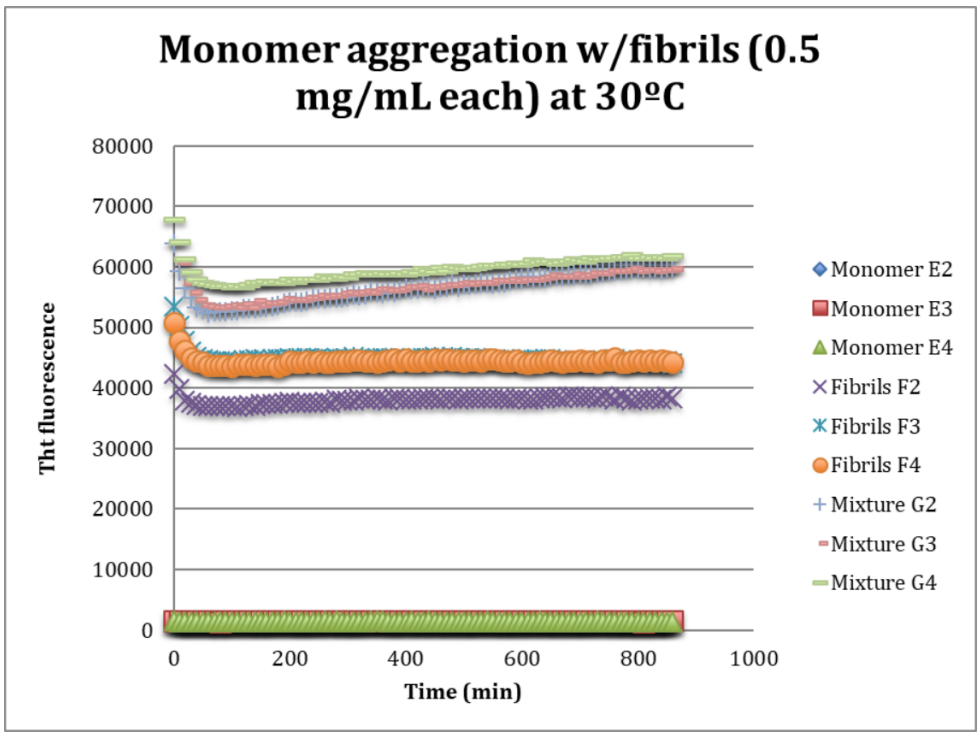

FIG. 9: Stability of monomers in presence of fibres at RT. Insulin monomer (0.5 mg/ml, 87 M), fibrils (0.5 mg/ml, 87 M) and a mixture of both (to a final concentration of (0.5 mg/ml, 87 M) each) were incubated in the presence of 40 M Tht at 30C overnight. The curves of the monomer and fibrils alone do not exhibit change in fluorescence intensity overtime, whereas the mixture of monomer and fibrils indicate the formation of aggregates for the contact of monomers with the fibrils, but the aggregation is slow.