Supporting Information

Absolute Quantification of Amyloid Propagons by Digital Microfluidics

Manuela Pfammatter,[†] Maria Andreasen,[‡,§] Georg Meisl,[‡] Christopher G. Taylor,[‡] Jozef Adamcik,[⊥] Sreenath Bolisetty,[⊥] Antoni Sánchez-Ferrer,[⊥] David Klenerman,[‡] Christopher M. Dobson,[‡] Raffaele Mezzenga,[⊥] Tuomas P. J. Knowles,[‡,∥] Adriano Aguzzi,[†] and Simone Hornemann[†,]*

[†]Institute of Neuropathology, University of Zurich, CH-8091 Zurich, Switzerland

[‡]Department of Chemistry, University of Cambridge, Cambridge CB2 1EW, United Kingdom

[§]Interdisciplinary Nanoscience Center (iNANO), Aarhus University, DK-8000 Aarhus, Denmark

[⊥]Department of Health Sciences and Technology, ETH Zurich, CH-8092 Zurich, Switzerland

[∥]Cavendish Laboratory, Department of Physics, University of Cambridge, Cambridge CB3 1HE, United Kingdom

*Corresponding author: E-mail: simone.hornemann@usz.ch, phone: +41 44 255 21 07.

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SI Materials and Methods

Atomic force microscopy. A droplet of 20 µL of insulin fibril solution (1 µM in HCl, pH 2.0) was deposited on freshly cleaved mica substrate, incubated for 2 min at room temperature, rinsed with deionized water to remove unbound fibrils, and dried with compressed air flow. The samples were scanned on a MultiMode VIII Scanning Probe Microscope (Bruker) covered with an acoustic hood to minimize vibrational noise. The AFM was operated in tapping mode under ambient conditions using commercial silicon nitride cantilevers. The AFM images were acquired with a scan rate of 0.1 Hz and a resolution of 5120 x 5120 pixels per image. All images were flattened to remove background curvature using the Bruker Nanoscope 8.10 software and no further image processing was carried out. The contour lengths and the average height distributions were extracted from >95000 individually traced fibrils using FiberApp[1], an open-source tracking and analysis software written in Matlab (The MathWorks, USA).

Dynamic light scattering. DLS spectra of a 100 µM insulin fibril solution in HCl (pH 2.0) were recorded using a 3D cross-correlation dynamic light scattering spectrometer (LS instruments) equipped with a He-Ne-Laser emitting polarized light at a wavelength of 632.8 nm. Dynamic light scattering measurements were performed at room temperature at a fixed scattering angle of 90°. The spectrum represents the average of three independent measurements of 600 s each. Time correlation functions of the scattered intensity were analyzed using the CONTIN analysis algorithm[2].

Fourier transform infrared spectroscopy. FTIR spectra of lyophilized insulin fibrils were obtained by using a Varian 640 FTIR spectrometer (Varian Inc.) equipped with a Specac Diamond ATR Golden Gate single attenuated total reflection (ATR) system. Samples were scanned at room temperature over the range of 4000 to 600 cm^{-1} with a resolution of 2 cm^{-1}, averaged over 64 scans, and baseline corrected. The amide region was normalized, resolved by second-derivative analysis, and peak deconvoluted using Igor Pro 6.3.4.1 software.

Far-UV circular dichroism spectroscopy. CD spectra were measured on a Jasco J-815 spectropolarimeter (Jasco Inc.) at 25 °C using an insulin concentration of 20 µM in HCl (pH 2.0). Far-UV spectra were recorded in a 0.1 cm precision quartz cuvette (Hellma) from 200 to 260 nm with a data pitch of 0.2 nm, a bandwidth of 2 nm and a scanning speed of 20 nm min^{-1}. Spectra were background subtracted and averaged over 10 scans. The molar mean residue ellipticity θ_{mrw} was calculated as

$$\theta_{mrw} = \frac{\theta}{d \cdot c \cdot n_{aa}} \quad (1)$$

where θ is the measured ellipticity, d is the thickness of the cuvette, c is the concentration and n_{aa} is the number of residues of insulin.

Self-propagation activity assay. The self-propagation activity of the preformed fibrils was assessed in a seeded aggregation experiment. 400 µM monomeric insulin was mixed with 0, 1, 2 and 5% (related to the monomer concentration) preformed fibrils and incubated in HCl (pH 2.0) at 45 °C and 300 rpm continuous agitation in 96-well polystyrene microplates (Corning, prod. no. 3880). The aggregation time courses were monitored by following the absorbance at 370 nm using a Spectramax Paradigm microplate reader (Molecular Devices). Triplicate measurements of all conditions were performed and averaged to calculate the mean ± SD of the lag phases.

SI Figures

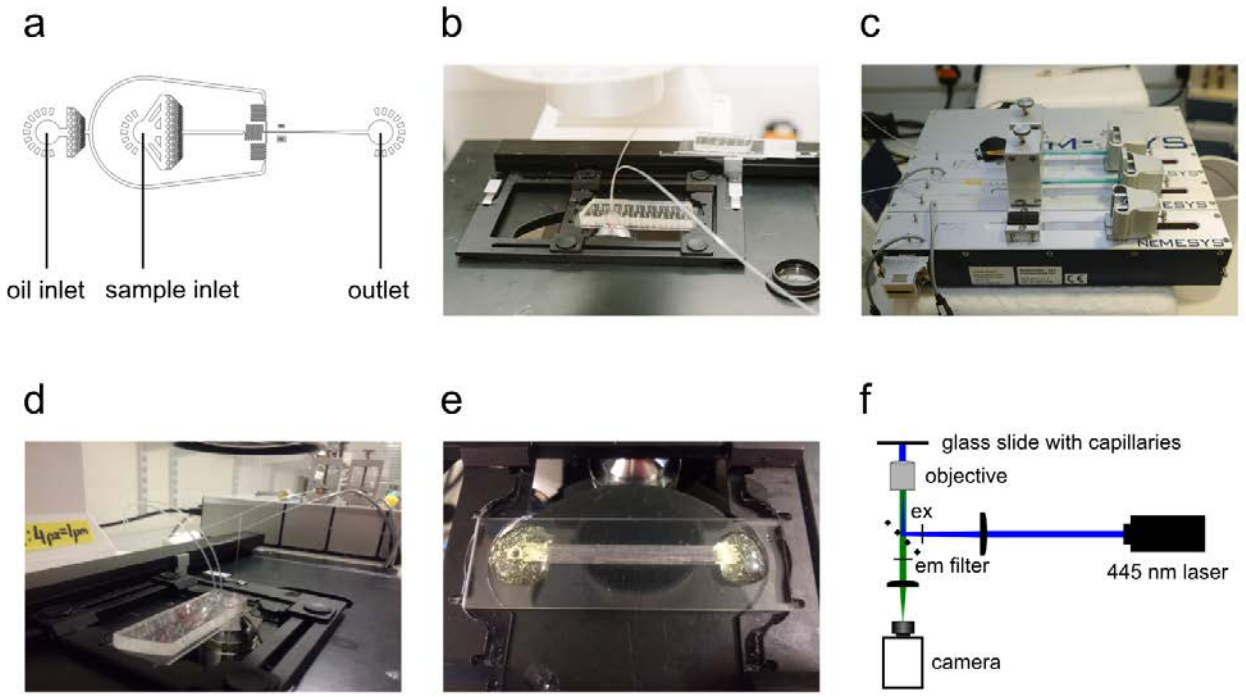

Figure S1. Experimental setup of d-AQuA. (a) Design of the microfluidic flow focusing device for droplet generation. Oil and sample inlets as well as the outlet for droplet collection are highlighted. (b) Both the oil phase and the sample are injected into the device through the respective inlets and floated through the flow focusing geometry to form stable microdroplets. (c) The flow rates of both phases are controlled using a neMESYS syringe pump fixed at 300 µL h^{-1} and 200 µL h^{-1} for the oil phase and the sample phase, respectively. (d) Stable droplets are collected into glass capillaries by plugging the capillary into the outlet and floating the droplets into the capillary. (e) The capillaries containing droplets with different fibril dilutions are sealed with wax and glued on a glass slide for amplification and imaging. (f) Schematic illustration of the laser setup used for monitoring the fluorescence intensity of the microdroplets. The glass slide with the capillaries is placed on the automated motorized stage of a fluorescent laser microscope and covered with a hotplate heated to 65 °C. Images are taken every 15 min following excitation with a 445 nm laser.

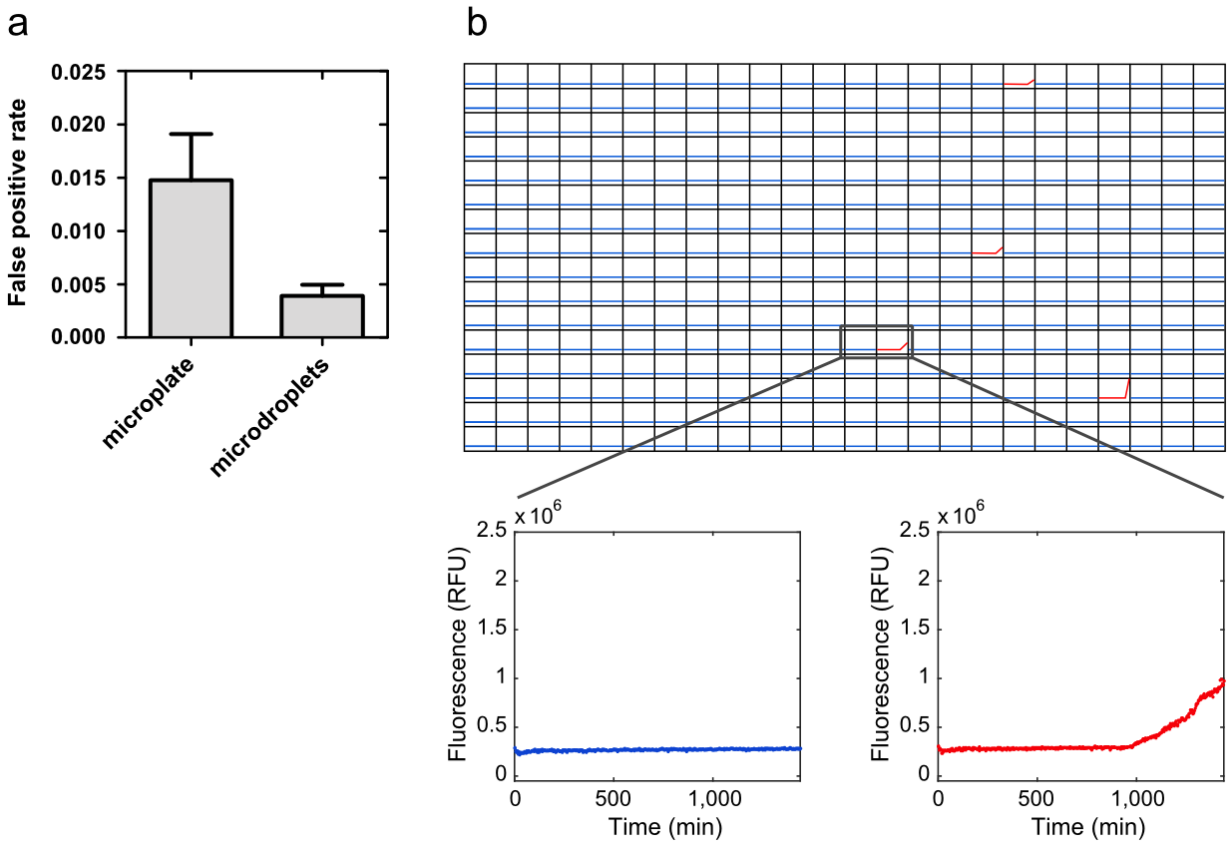

Figure S2. Comparison of the average false positive rates between the microplate assay and d-AQuA (a) The average false positive rates were (1.5 ± 0.3)% (mean ± SD, n=3) and (0.36 ± 0.09)% (mean ± SD, n=2) for the microplate assay and d-AQuA, respectively. (b) Representative time courses of 384 replicates of unseeded conversion reactions in the microplate assay monitored by ThT fluorescence. 2 μM of monomeric substrate protein was mixed with HCOOH (pH 3.0) and incubated under assay conditions. Four (indicated in red) out of 384 replicas showed positive fluorescence after 24 h of incubation, which accounts for a false positive rate of ~1%.

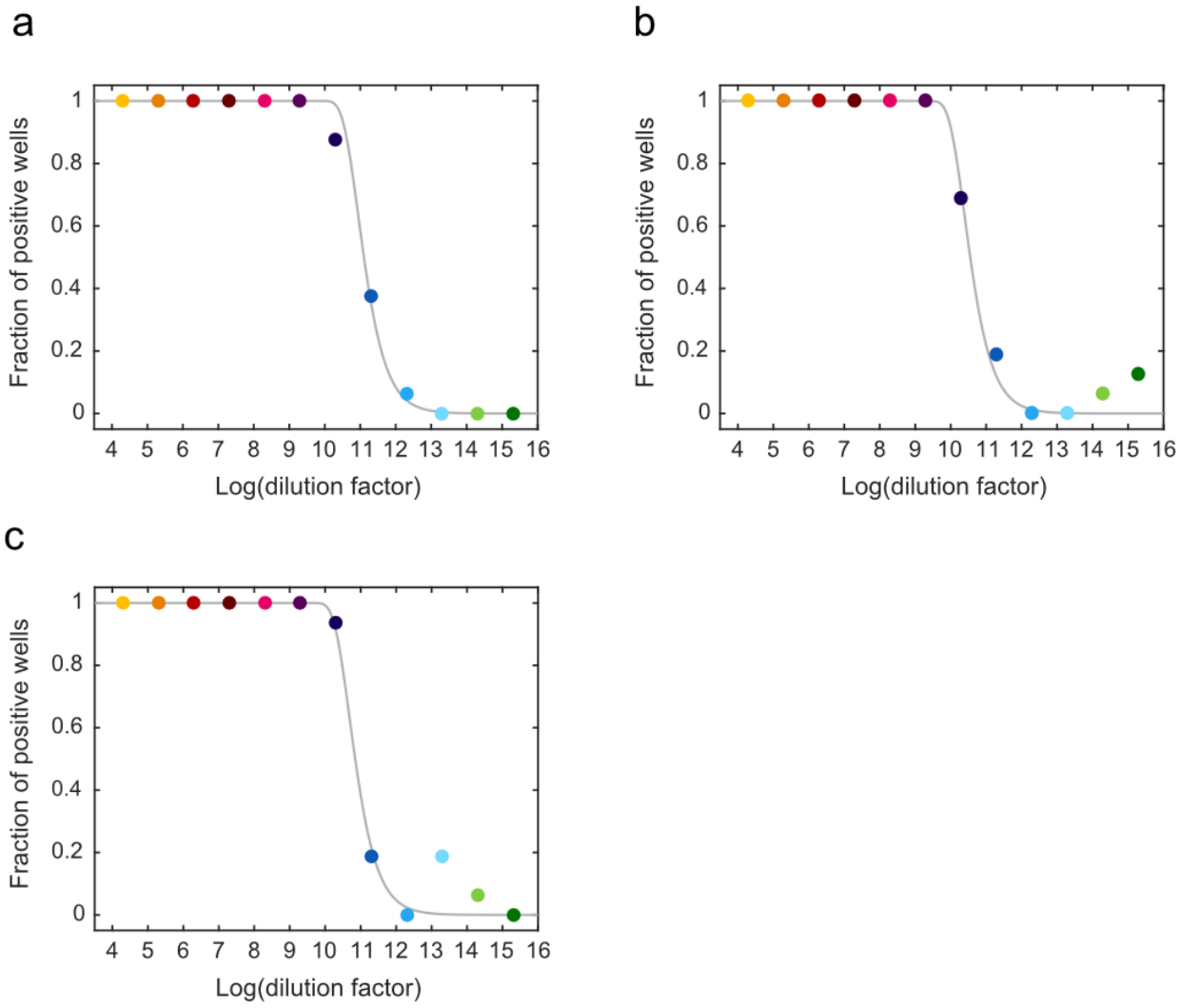

Figure S3. Poisson analysis of three individual microplate assays. A Poisson distribution was fitted to the fraction of positive wells as a function of the logarithm of the dilution factor. The three individual assays were used to calculate the mean ± SD shown in Figure 5c.

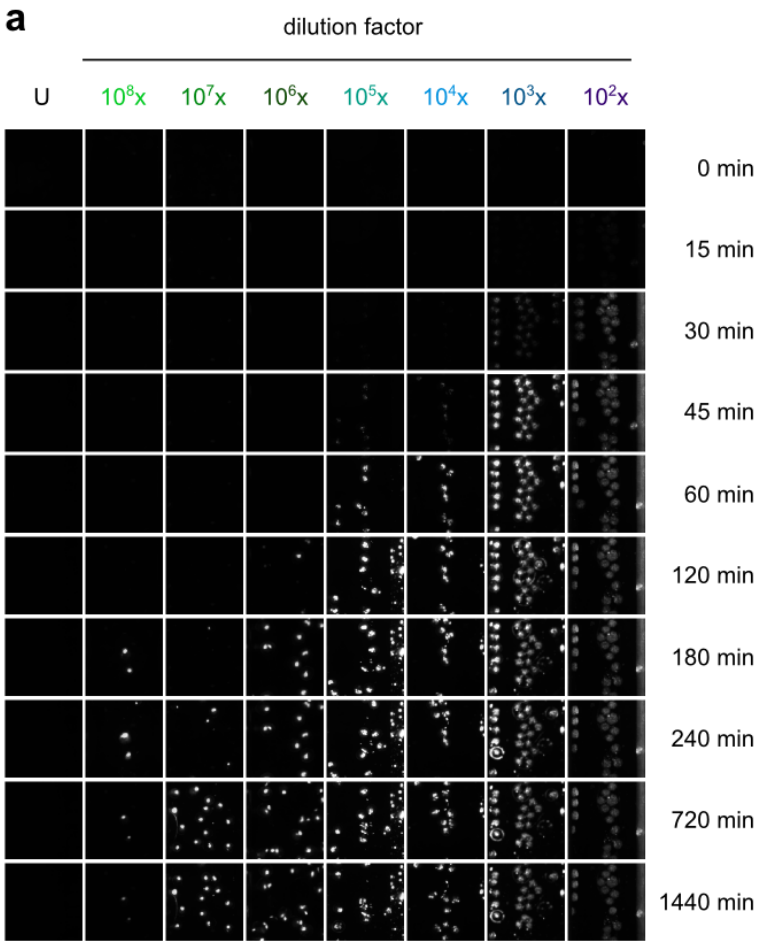

Figure S4. Raw images of Figure 3a.

REFERENCES

(1) Usov, I.; Mezzenga, R. *Macromolecules* **2015,** *48*, 1269-1280.

(2) Provencher, S. W. *Computer Physics Communications* **1982,** *27*, 213-227.