Microfluidics for the study of biomolecular assembly

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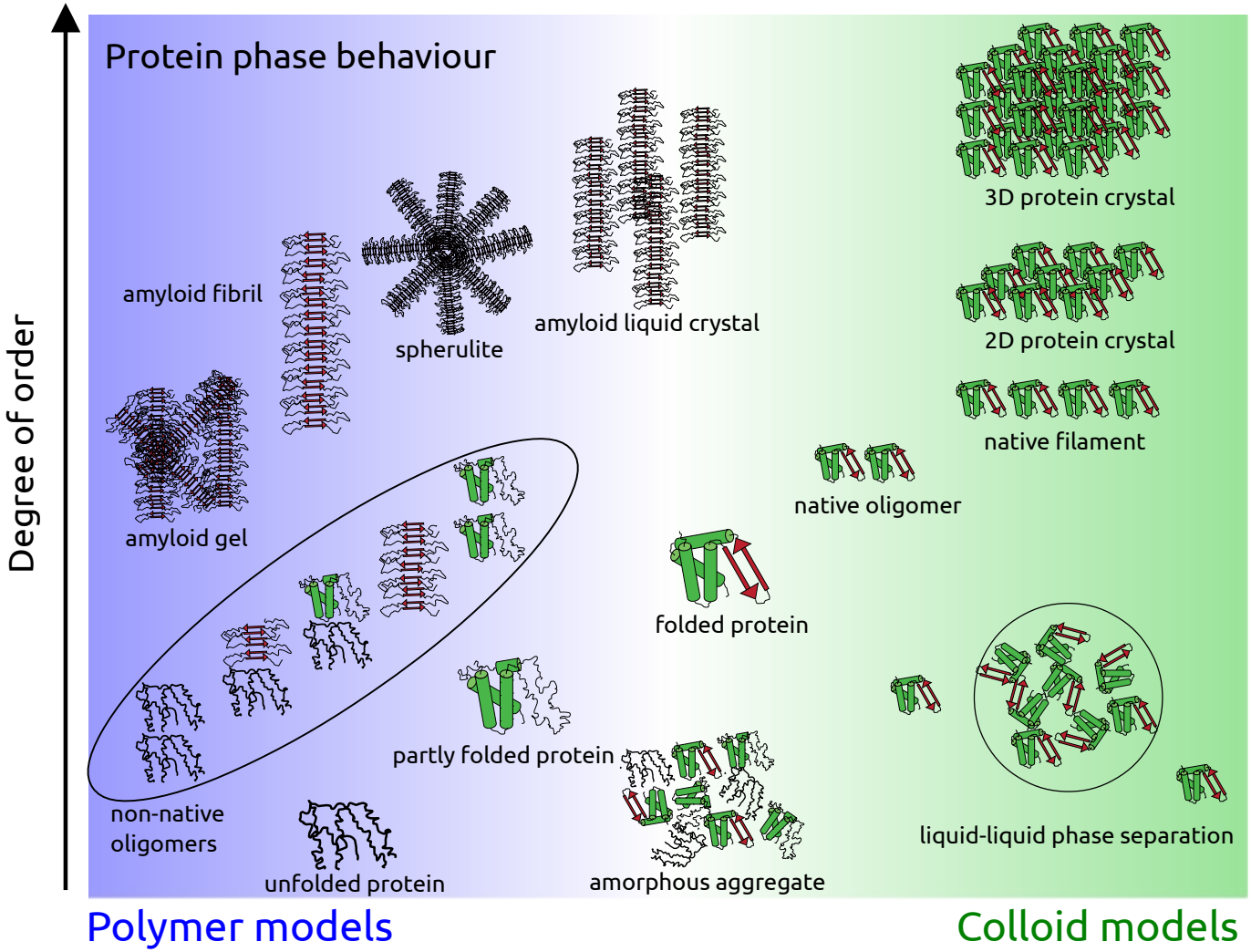
Polypeptide phase behaviour



Overarching aim: Study and control of the **thermodynamics** and **kinetics**, as well as the **mechanisms** of interconversion between the different states a polypeptide can adopt.

Buell AK, Intern. Rev. Cell. Mol. Biol. 2017

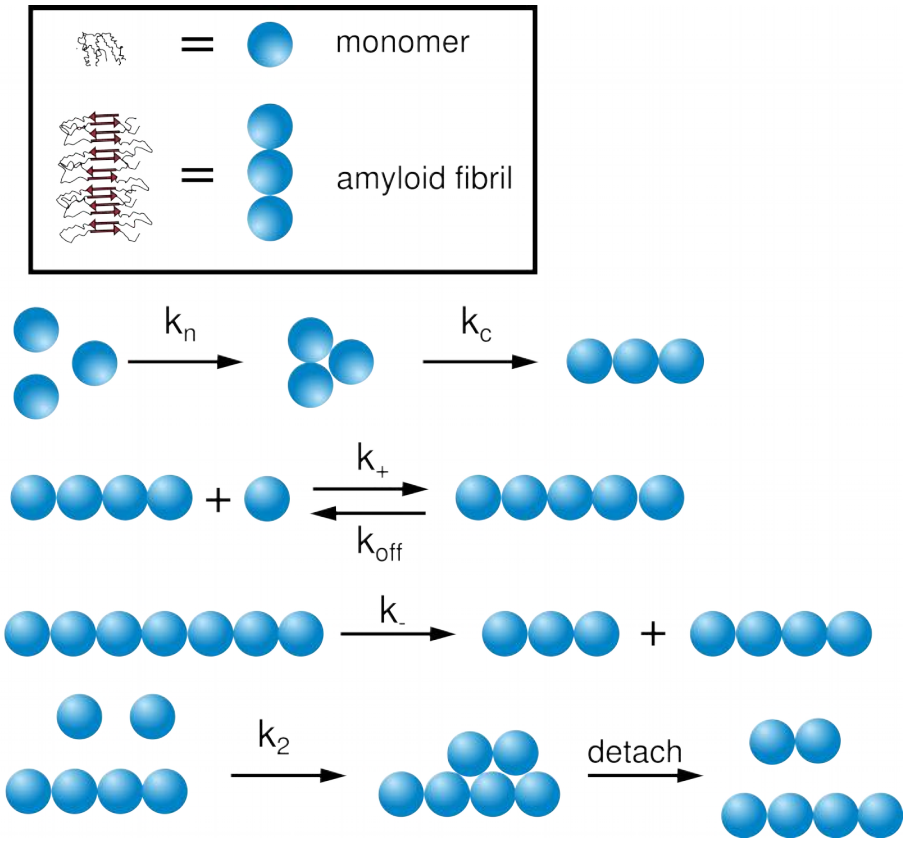
Structure of the talk

1) The energy barriers of amyloid fibril and peptide crystal growth

2) Mechanistic insight into autocatalytic secondary nucleation of α-synuclein amyloid fibrils.

3) Microfluidics and microcapillary experiments for the study of biomolecular interactions

The mechanism of amyloid fibril formation



Amyloid fibril formation from soluble protein is a multi-step process, involving steps of

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growth

and proliferation

We have devised experimental strategies that allow to determine the rate constants of the individual steps.

Buell et al., Essays in Biochemistry 2014

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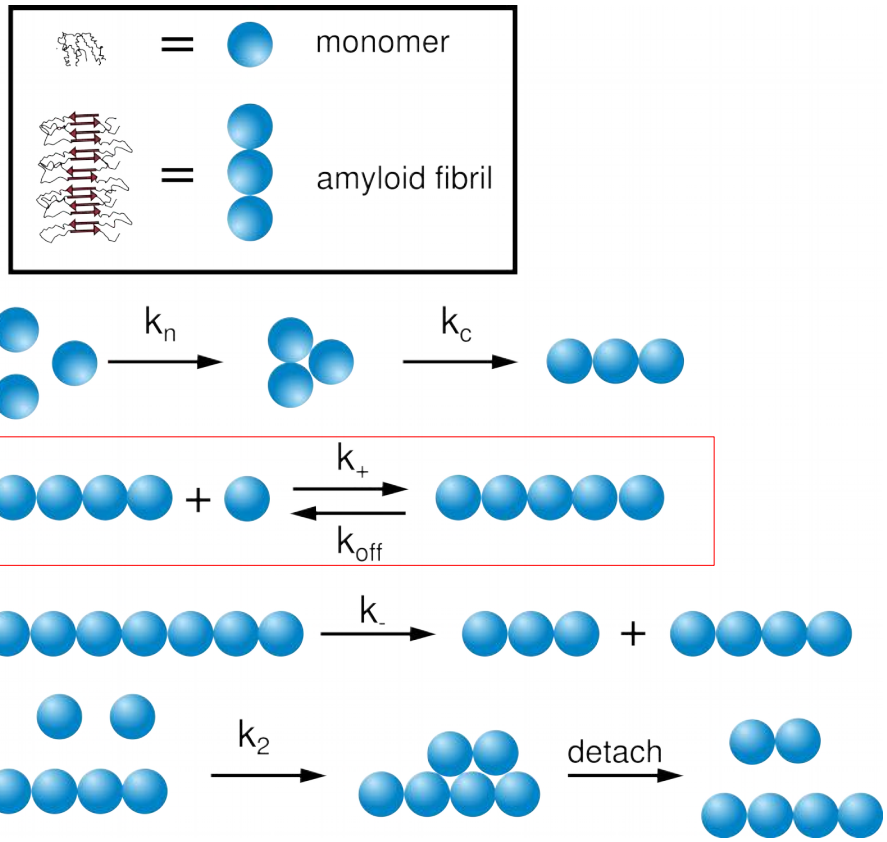

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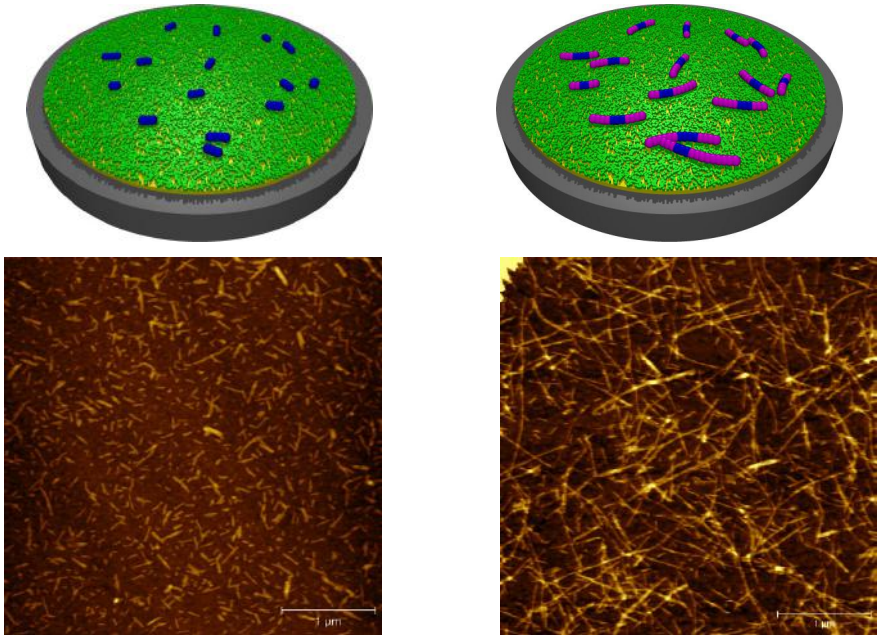
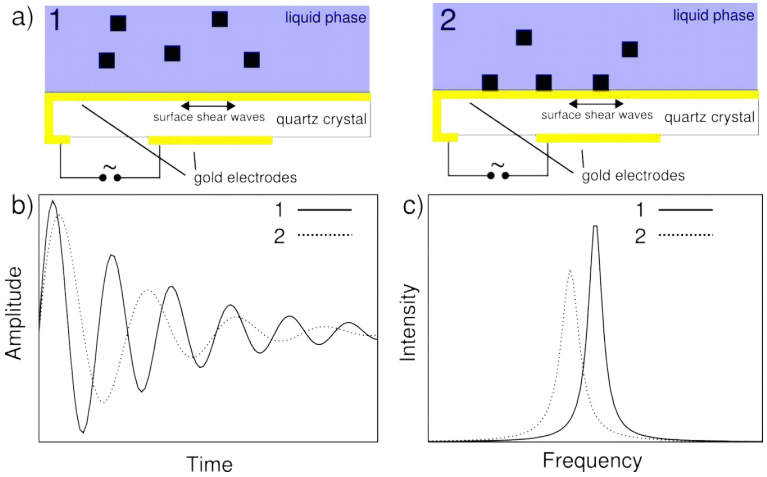
Buell et al., Essays in Biochemistry, 2014

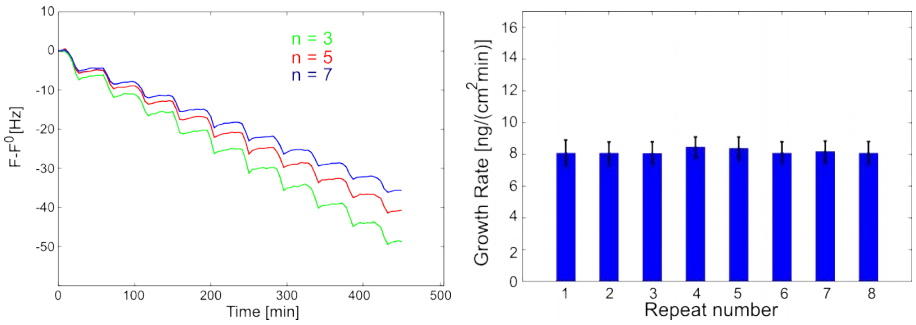
Quartz crystal microbalance measurements of amyloid fibril growth





The resonant frequency decreases and the dissipation increases when the aggregates grow.

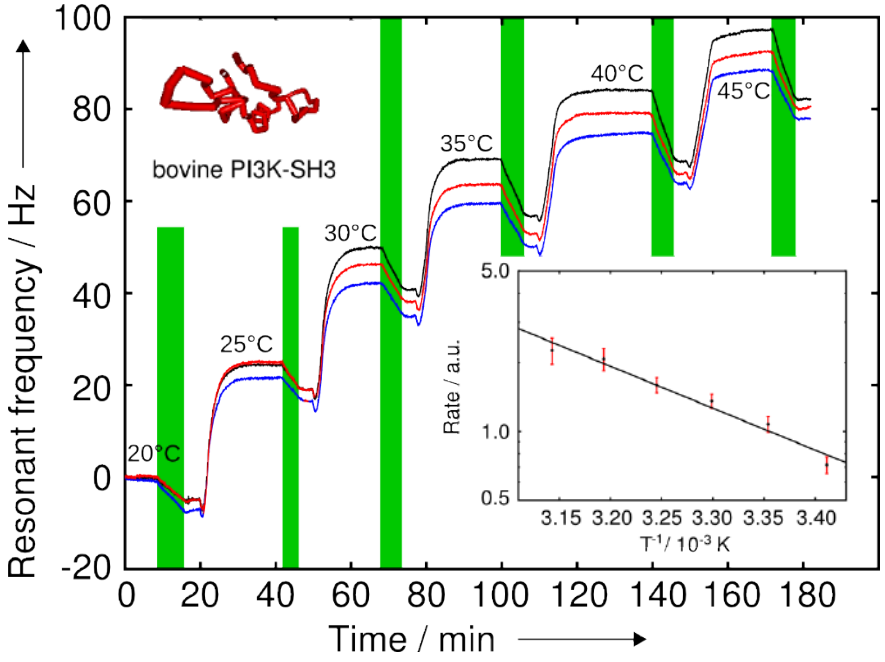
Surface-bound seed aggregates can be repeatedly incubated with soluble protein and the rate of change of frequency, and hence the growth rate, can be very accurately measured.



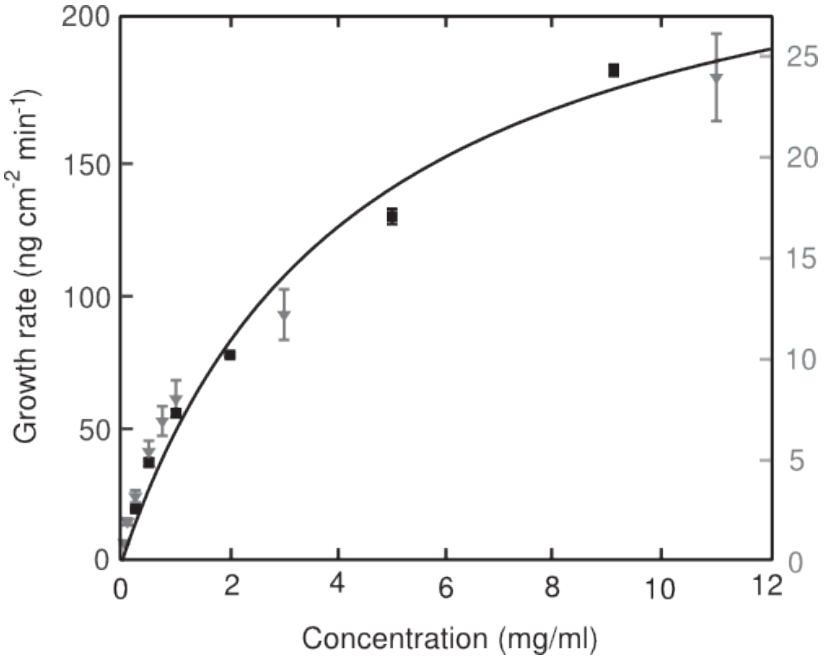
The rate of frequency change is highly reproducible upon repeated exposure to monomer.

Knowles *et al.*, PNAS 2007
Buell et al., J. Phys. Chem. B. 2010
Buell et al., Meth. in Mol. Biol., 2012

Fundamental features of amyloid fibril growth



Buell *et al.*, Angew. Chem. Int. Ed. 2012

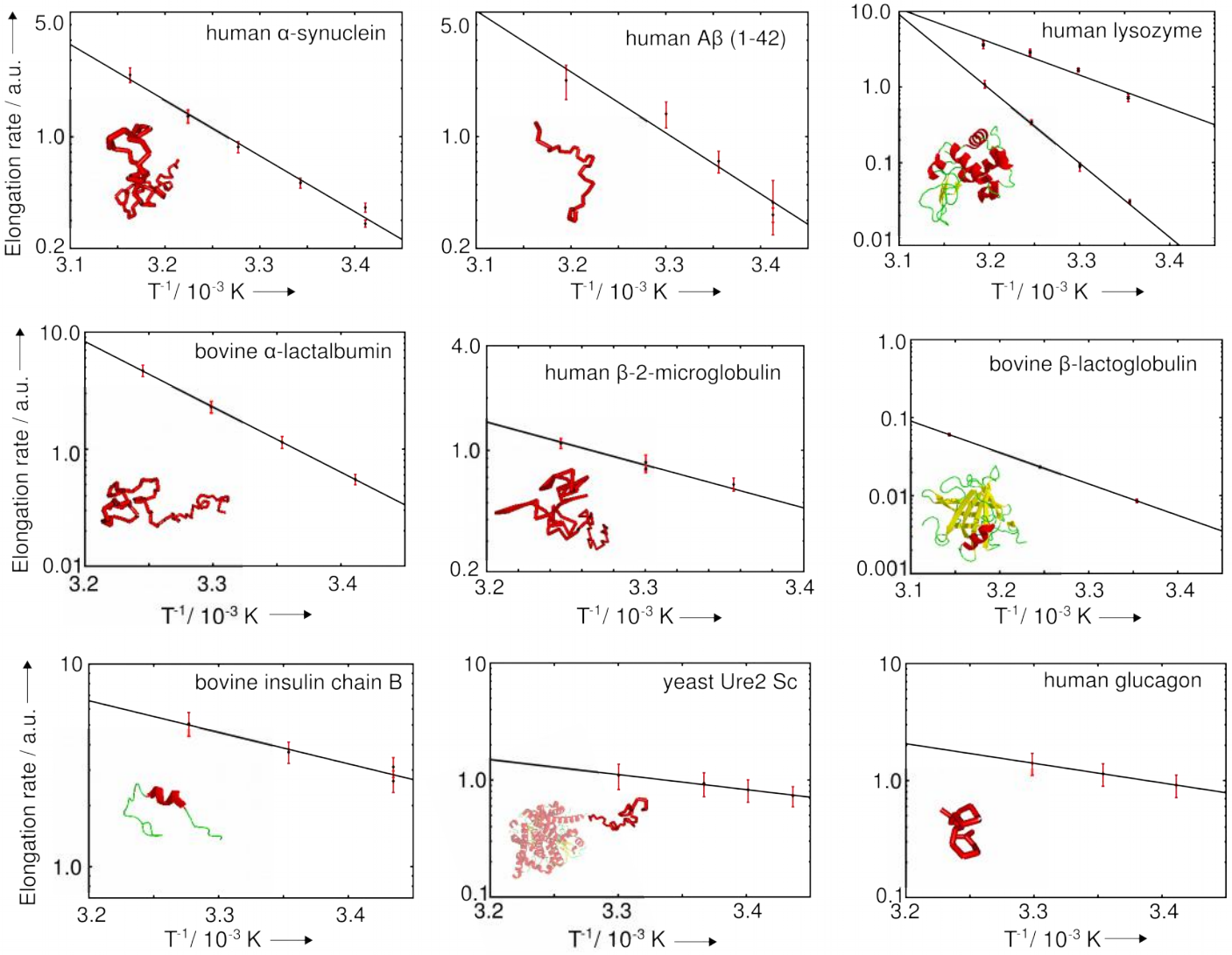


Buell *et al.*, Phys. Rev. Lett 2010

If the rate of fibril growth is measured at different temperatures, the enthalpy of activation can be determined from an Arrhenius plot.

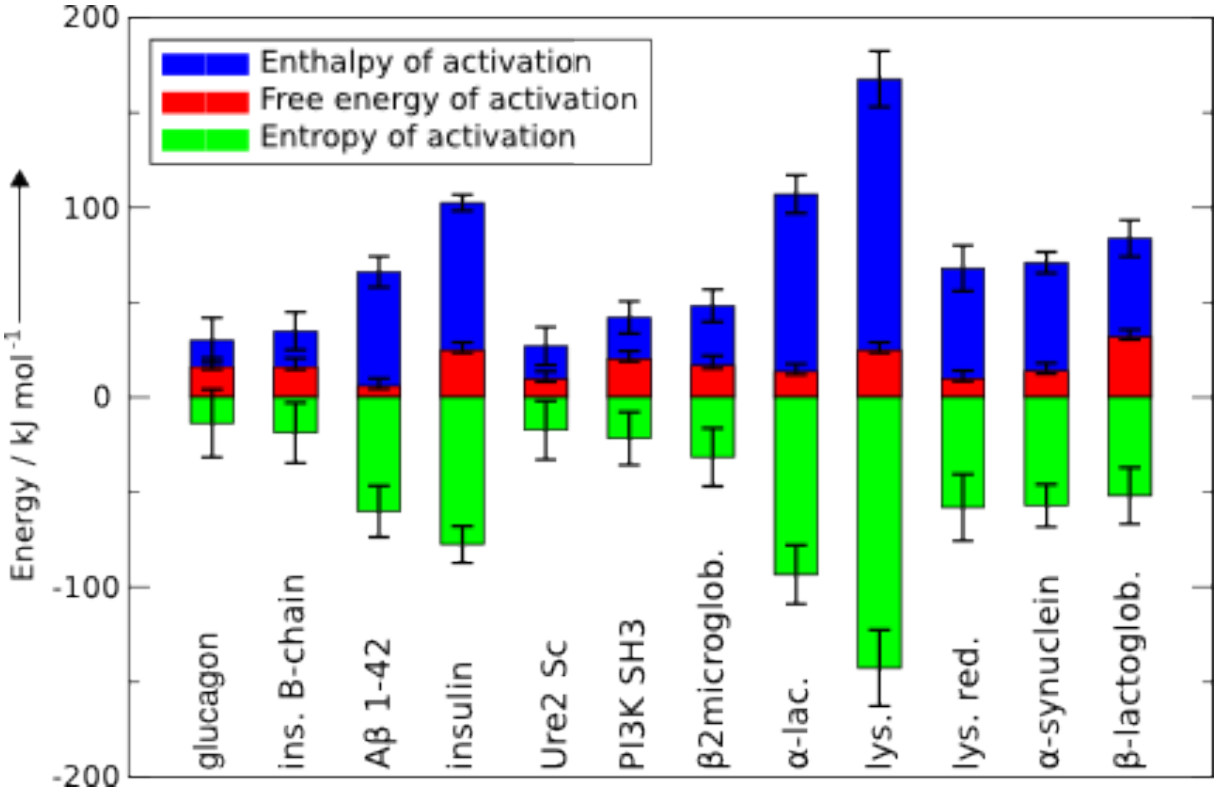
The aggregation rate as a function of monomer concentration shows saturation ('Michaelis-Menten-like'), due to the finite incorporation rate.

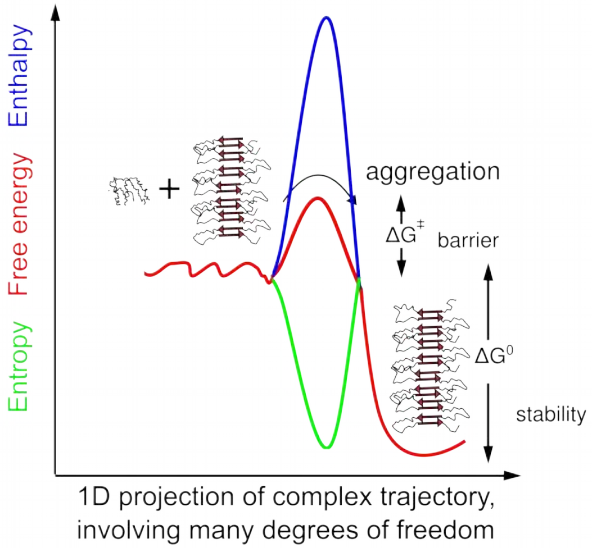
Arrhenius analysis of amyloid fibril growth



Buell et al., Angew. Chem. Int. Ed., 2012

Decomposing the free energy barriers



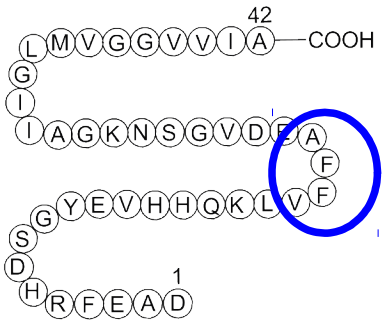


Results of the systematic kinetic analysis of amyloid fibril elongation:

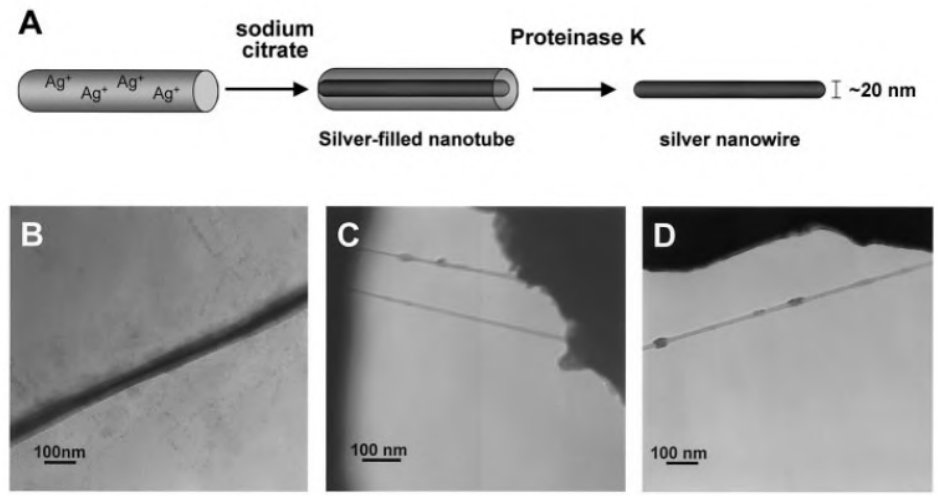
- $\Delta H^{\ddagger}$ is unfavourable in all cases (and correlates with structure and size of monomer)
- $\Delta S^{\ddagger}$ is favourable in all cases (and correlates with hydrophobicity of monomer)
- $\Delta G^{\ddagger}$ is strongly compensated

Buell et al., Phys. Rev. Lett., 2010
Buell et al., Angew. Chem. Int. Ed., 2012

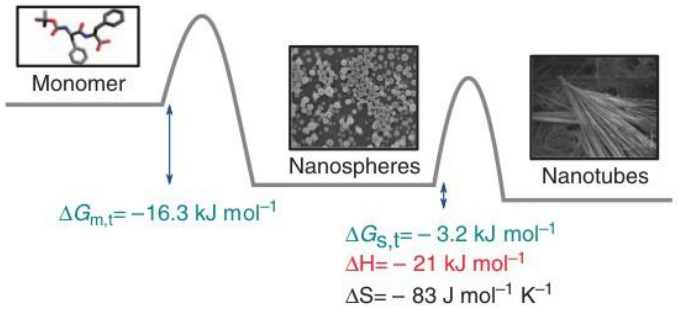
Self-assembly of diphenylalanine



Diphenylalanine is a central motif of the Aβ peptide.

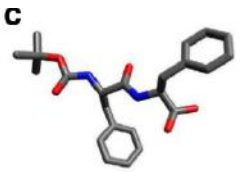


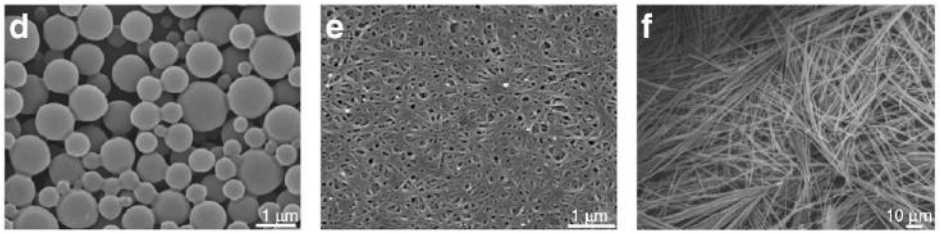
Reches and Gazit, Science 2003, 300, 625-627



Diphenylalanine and its derivatives form a variety of self-assembled structures with remarkable properties: hollow cores, piezo-electric, optical waveguide, intrinsic fluorescence, spheres with metallic stiffness, ...

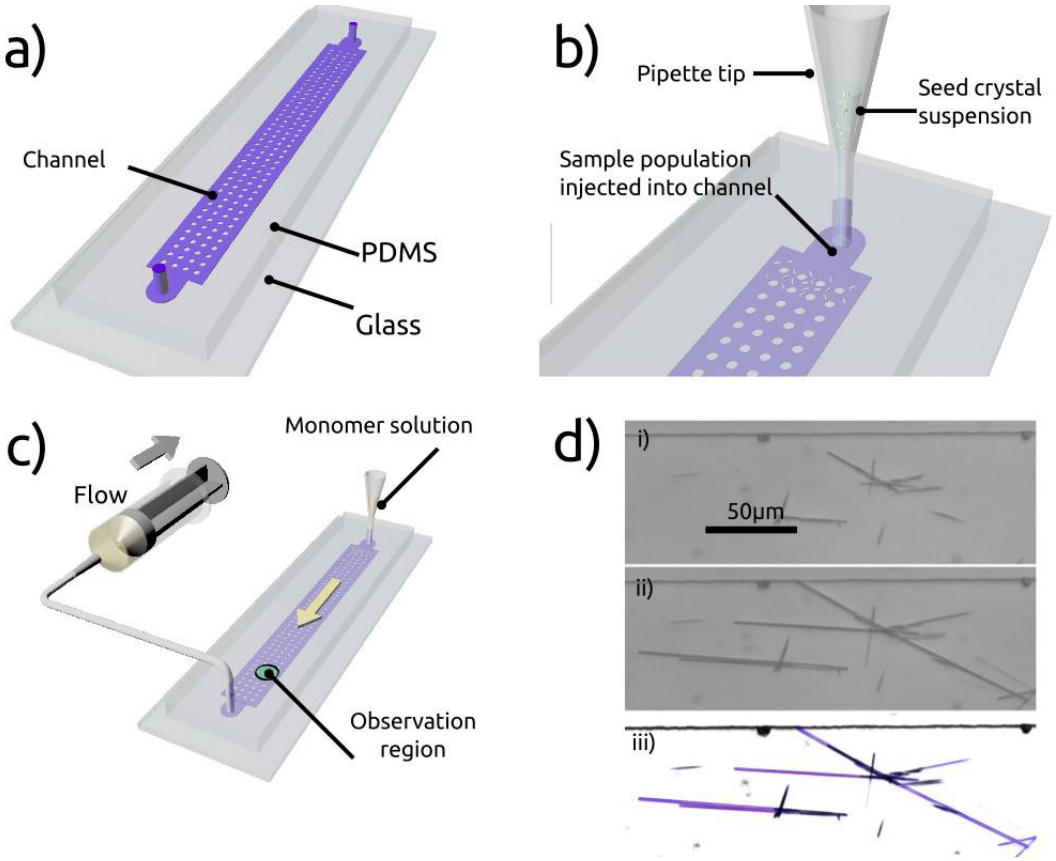
Boc-FF structures undergo Ostwald ripening.





Levin and Mason *et al.*, Nat. Comm. 2014

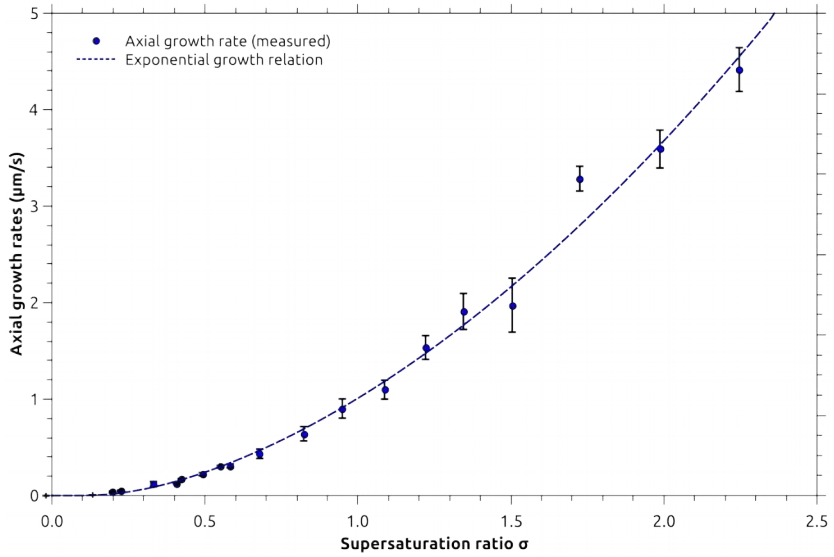
Microfluidics for the study of peptide assembly



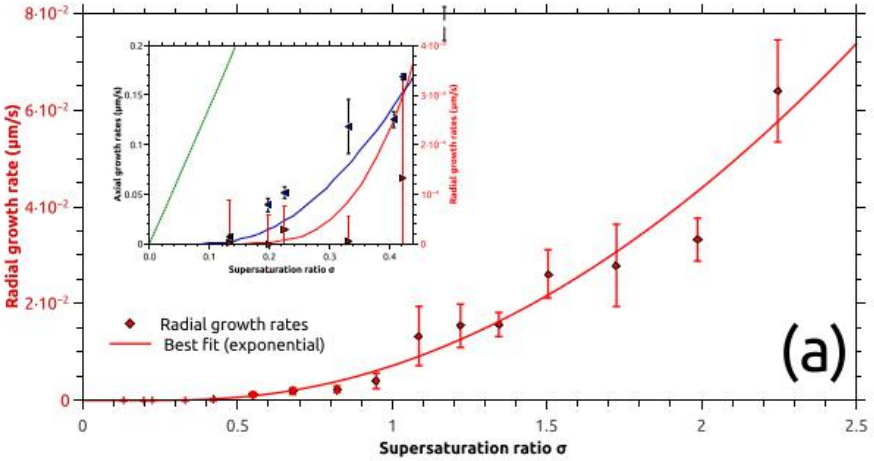
In a microfluidic flow reactor, the growth of seed crystals is monitored. The peptide concentration and hence the chemical potential can be kept constant or varied as desired.

Mason *et al.*, JACS, 2016

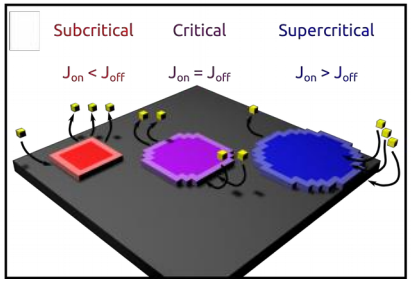
The concentration-dependence of FF crystal growth

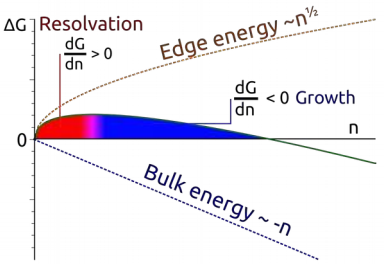


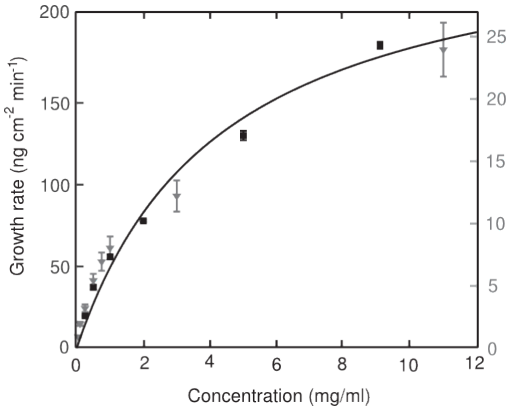
The axial growth rate depends exponentially on the FF concentration.



Significant radial growth is only observed at higher concentrations.





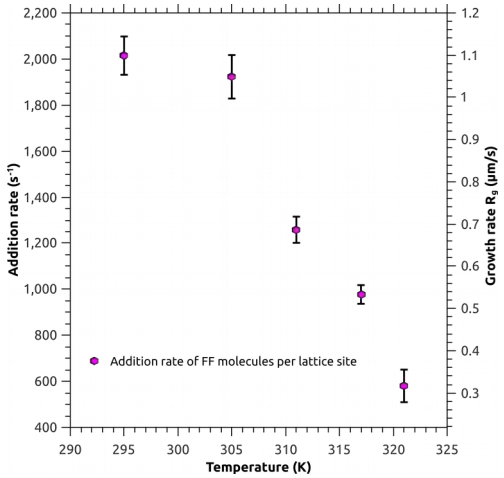


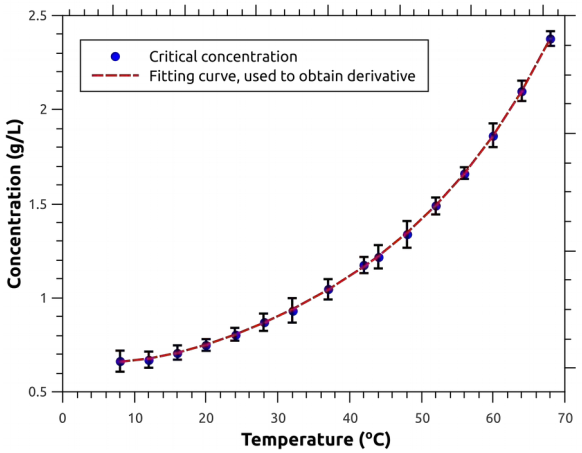
Amyloid fibrils show a sub-linear concentration dependence of the growth rate.

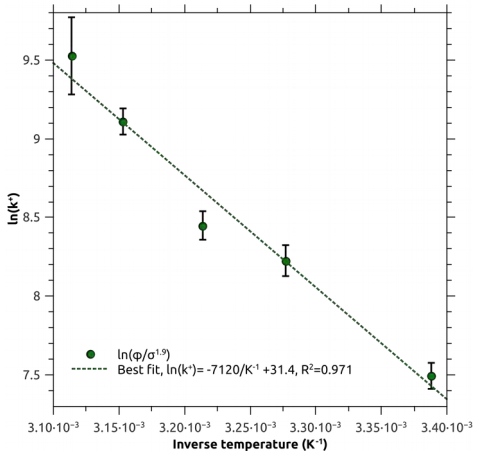
In a crystal, even growth is a nucleated process, explaining the higher concentration dependence.

Mason *et al.*, JACS 2016
Buell *et al.*, Phys Rev Lett 2010

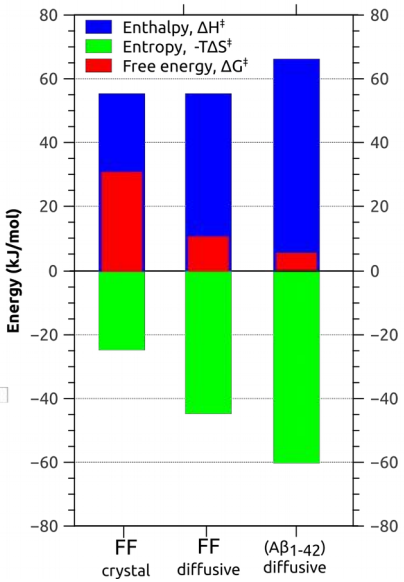
The temperature-dependence of FF crystal growth



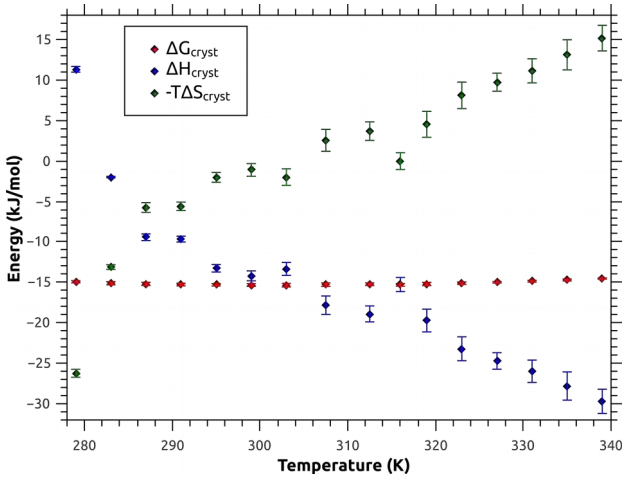




The temperature dependence of the growth rate needs to be corrected for the increased solubility at higher temperatures and hence the reduced driving force of assembly.

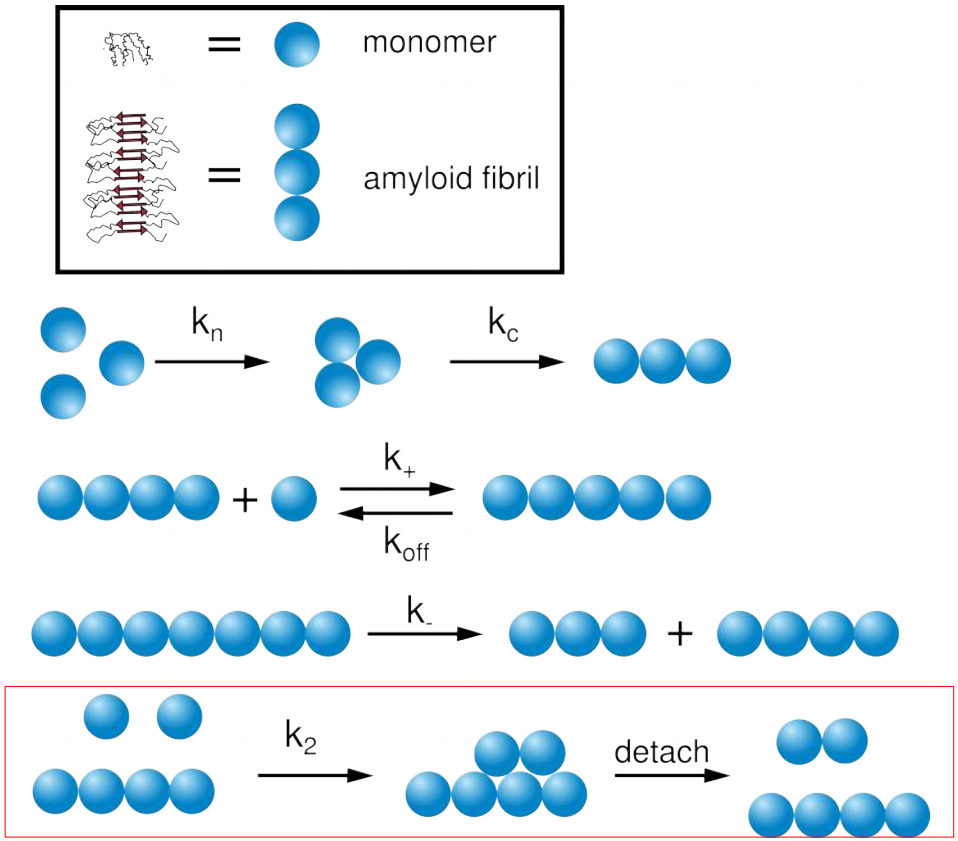


Analysis of the temperature dependence of the kinetics reveals that FF crystal growth is an activated process with a compensated barrier.



Mason *et al.*, JACS 2017

The mechanism of amyloid fibril formation



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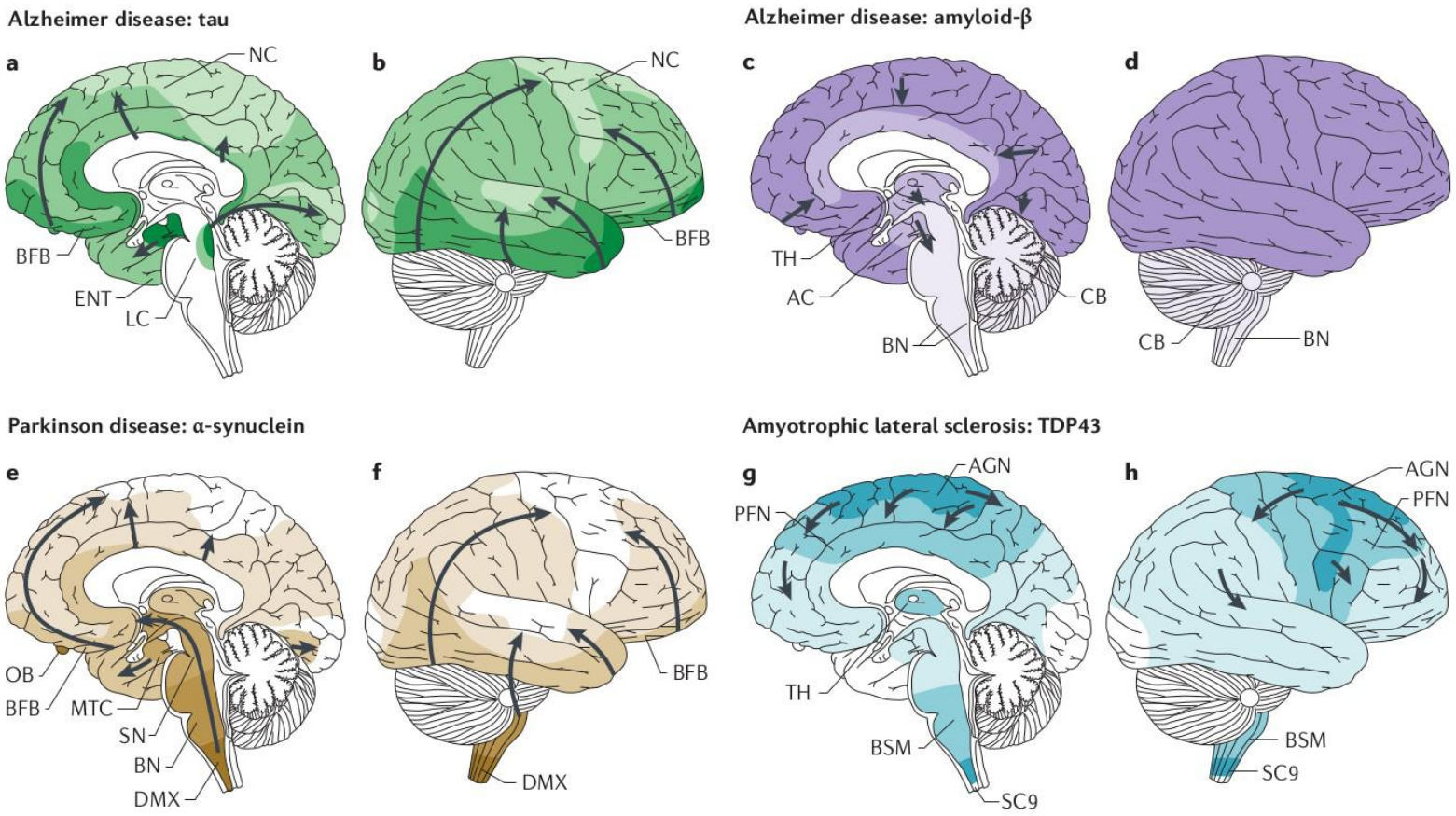
Buell *et al.*, Essays in Biochemistry, 2014, 56, 11-39

The spreading of disease pathology

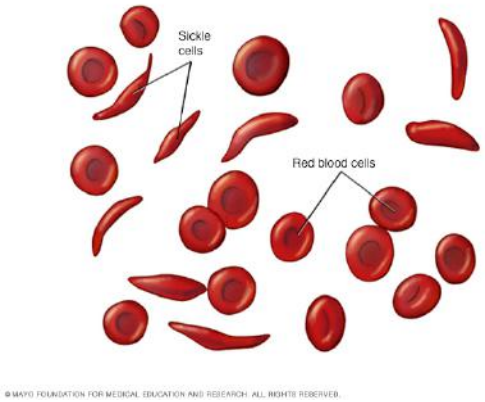


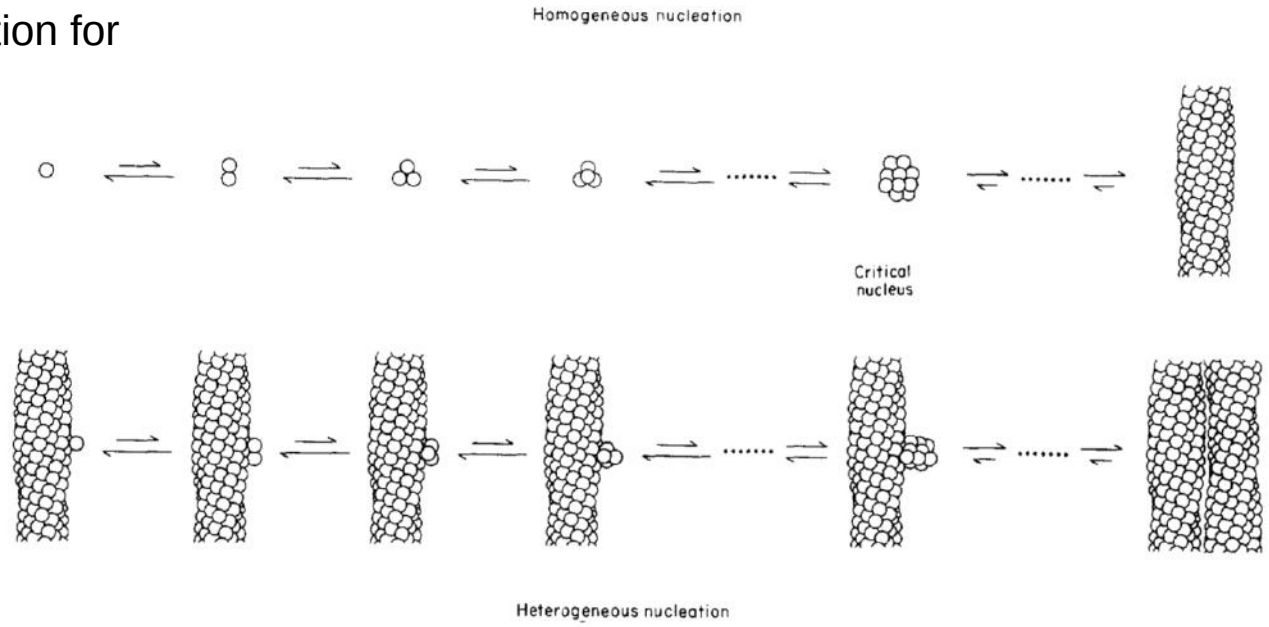
Brettschneider *et al.*, Nature Reviews Neuroscience 2015

The systematic spreading of the pathology through the CNS is one of the striking features of neurodegenerative disorders, suggesting the existence of a fibril amplification process.

What is secondary nucleation?

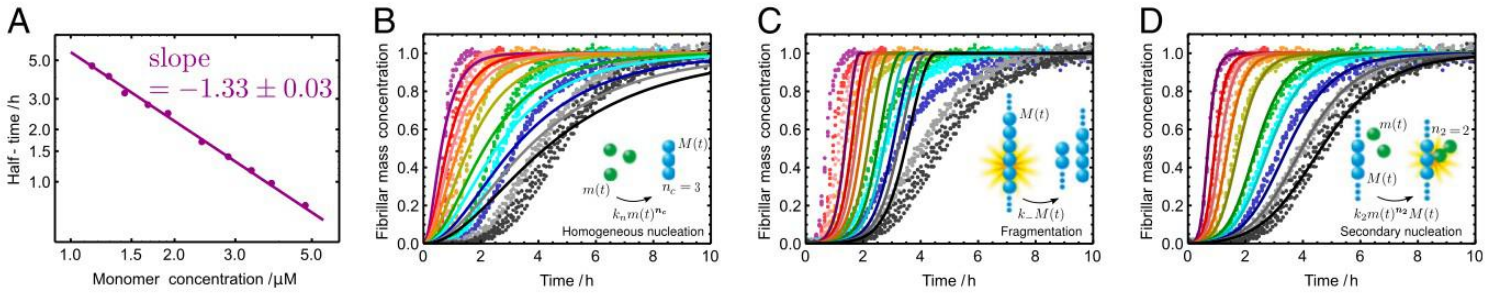
Proposed secondary nucleation for sickle haemoglobin:





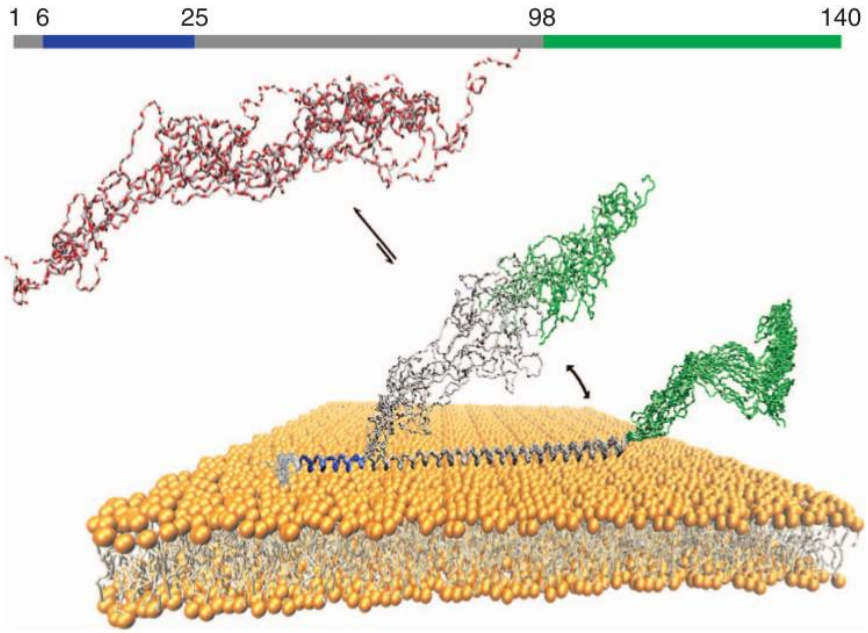
Ferrone, Hofrichter and Eaton, JMB 1985

Proposed secondary nucleation for Aβ:

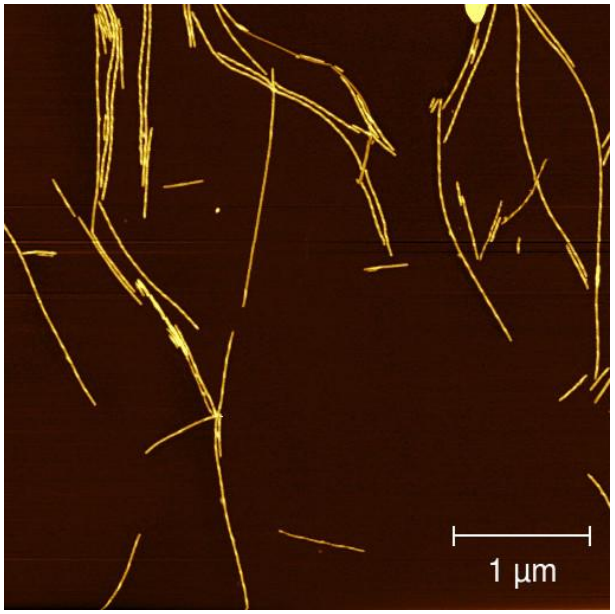


Cohen, Linse, Knowles and Dobson *et al.*, PNAS 2013

α-synuclein



Fusco *et al.*, Nature Comm. 2014

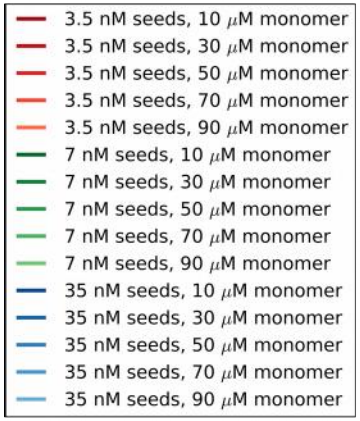


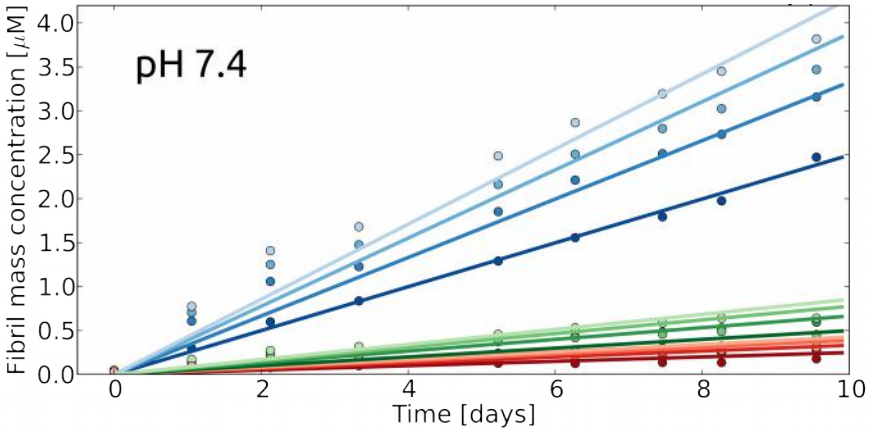
Buell *et al.*, PNAS 2014

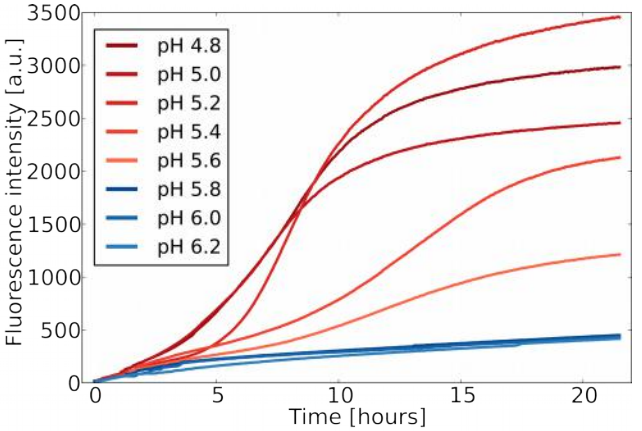
α-synuclein is disordered in solution but adopts α-helical structure upon binding to phospholipid membranes.

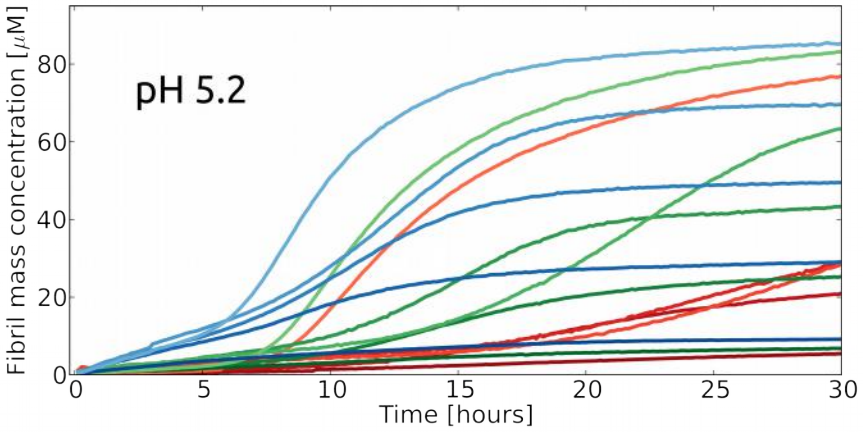
α-synuclein can form a variety of oligomeric structures (on and off-pathway to amyloid fibril formation), as well as a range of amyloid structures (“strains”), depending on the solution conditions.

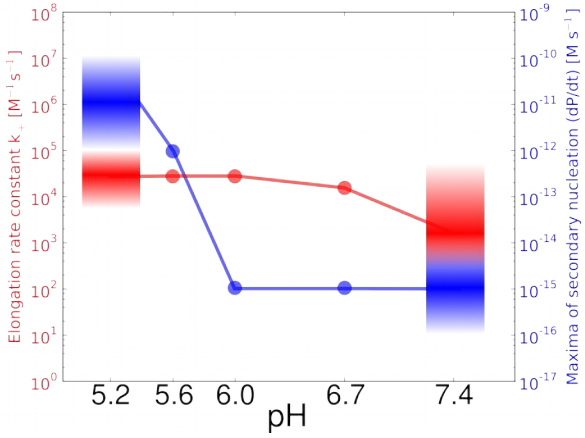
pH-dependent secondary nucleation







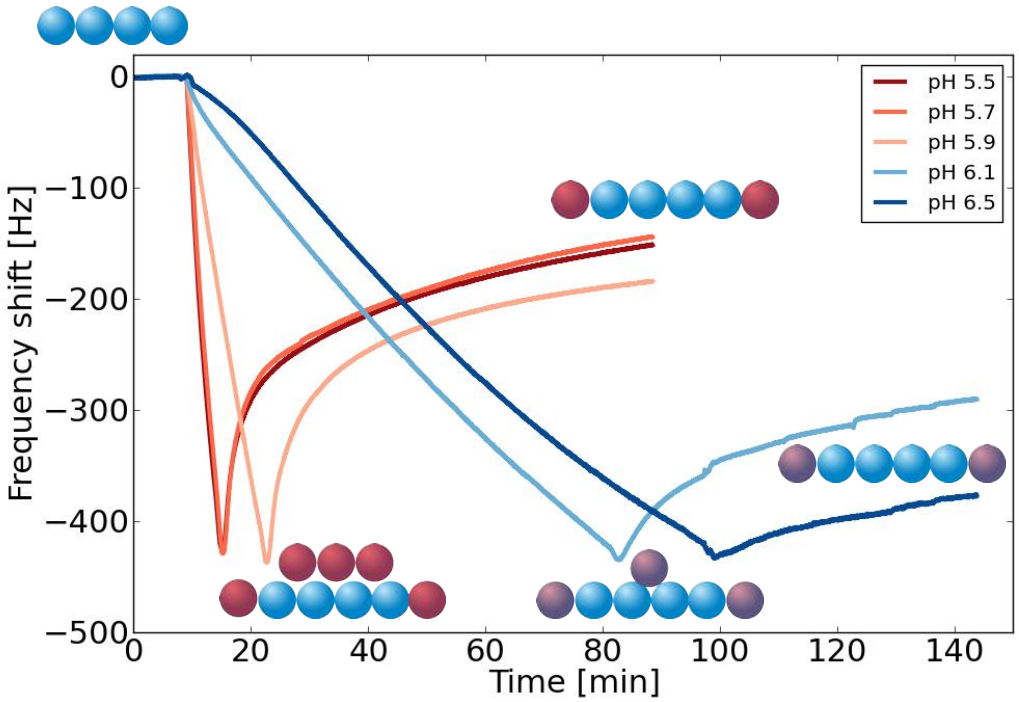


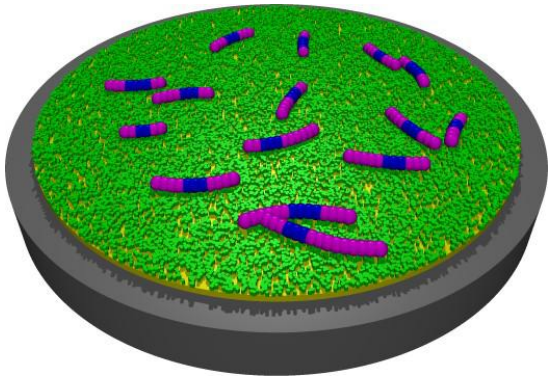


At neutral pH, α-synuclein fibrils grow, but do not proliferate under quiescent conditions. At mildly acidic pH, the fibrils show autocatalytic amplification.

Buell *et al.*, PNAS 2014

QCM experiments at different pH values



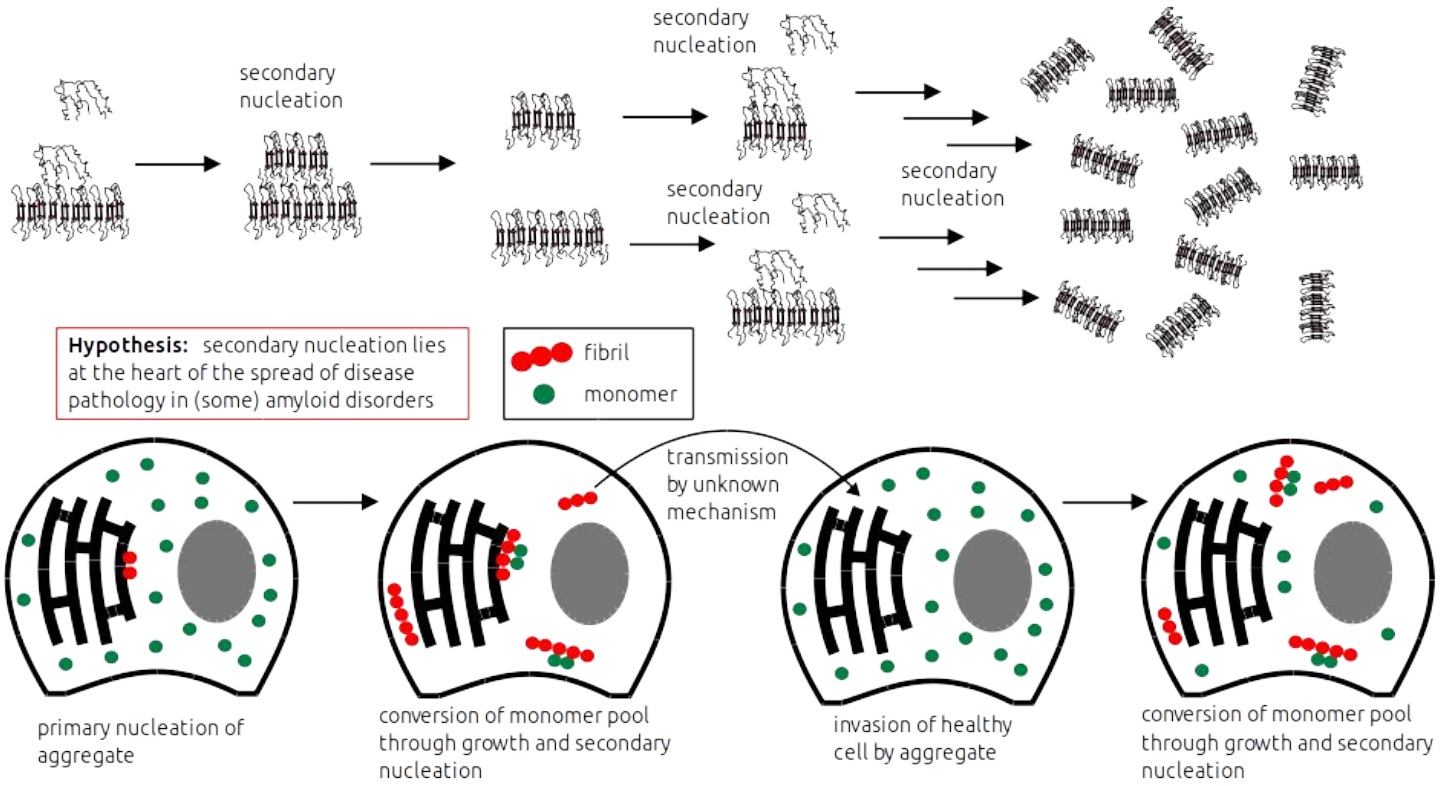


There are two modes of monomer binding to fibrils, and their relative proportion and reversibility is pH-dependent.

Monomer is able to bind to fibril ends (high affinity) and to the fibril surface (lower affinity). The former leads to growth, the latter to secondary nucleation.

Gaspar *et al.*, Quart. Rev. Biophys. 2017

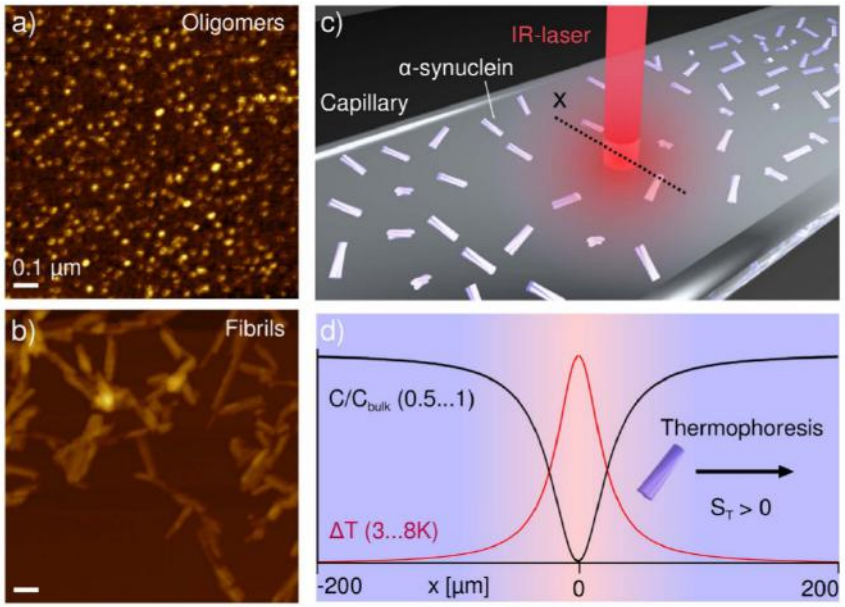
Secondary nucleation as mechanism for the spreading of aggregates?

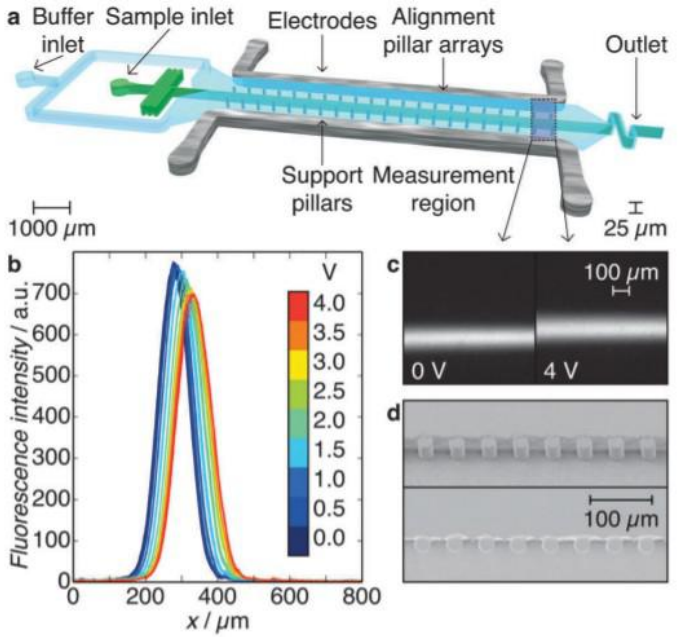


Buell AK, Intern. Rev. Cell. Mol. Biol. 2017

Secondary nucleation provides a plausible mechanism by which protein aggregates can amplify and spread *in vivo*.

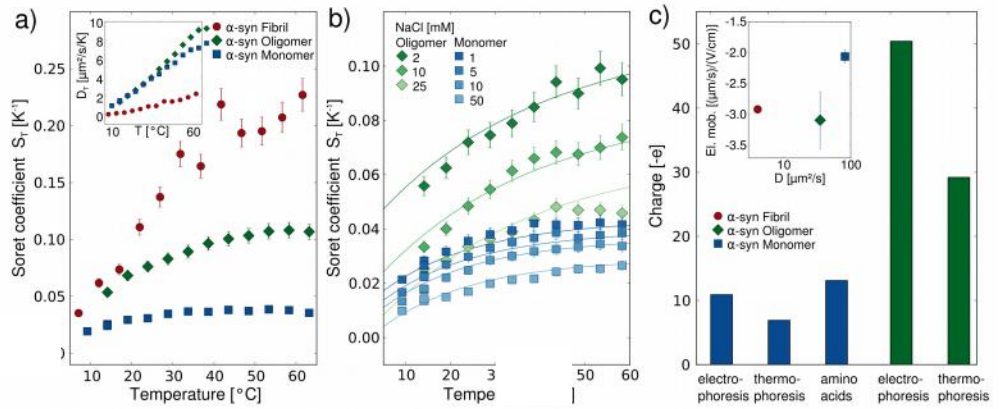
Microcapillaries for the study of biomolecular phoretic mobilities





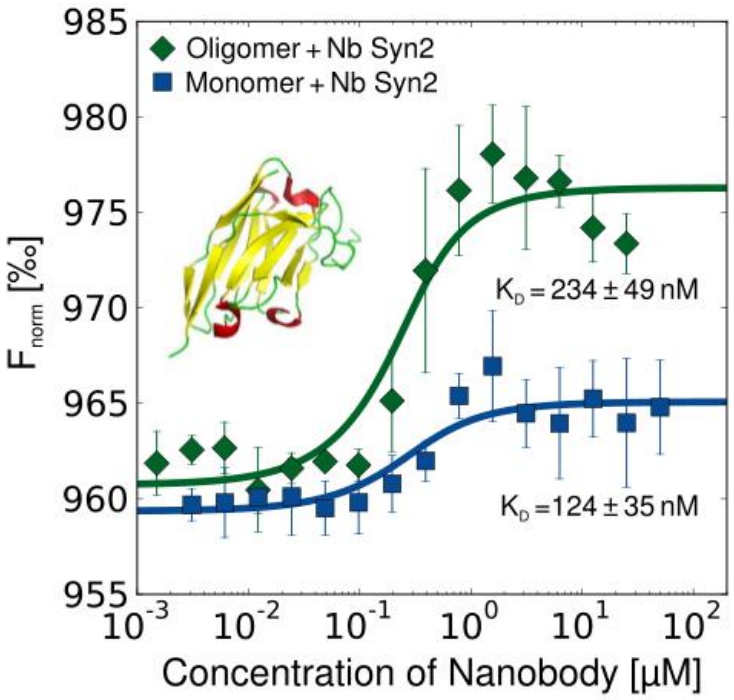
Herling *et al.*, Phys. Chem. Chem. Phys. 2015

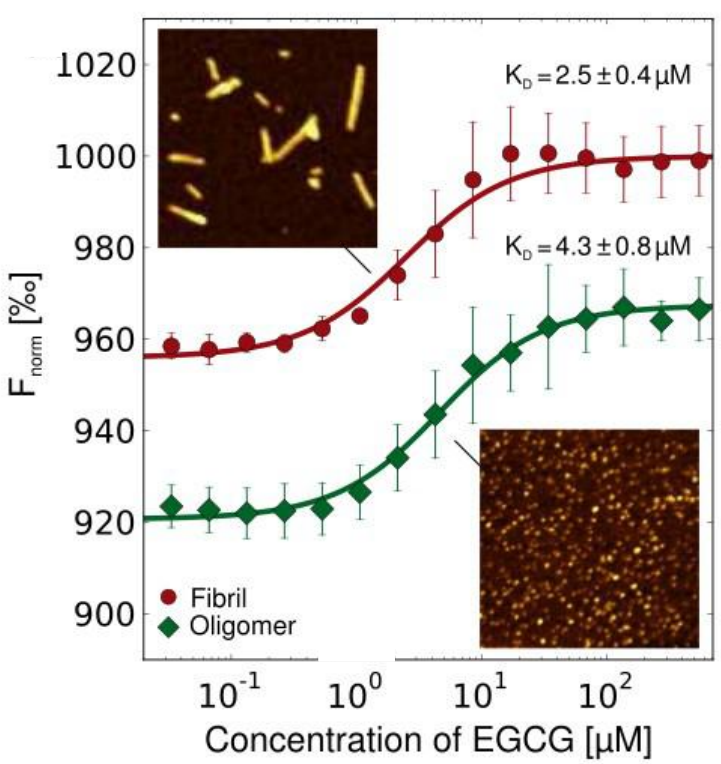
Thermophoresis in glass micro-capillaries and microfluidic free-flow electrophoresis can be used to quantify the thermophoretic and electrophoretic mobilities of protein aggregates.



Wolff *et al.*, Sci. Rep. 2016

Microcapillaries for the study of biomolecular interactions

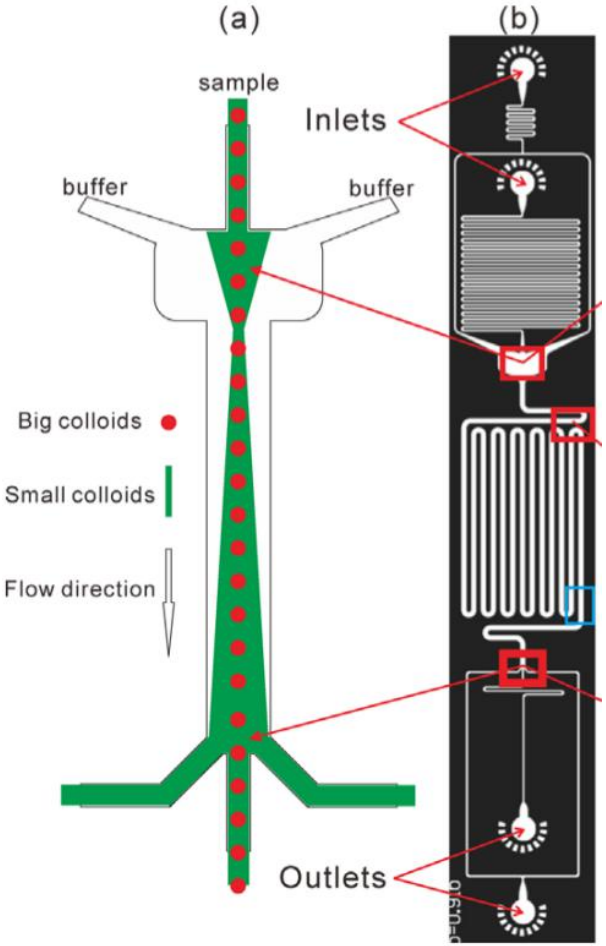


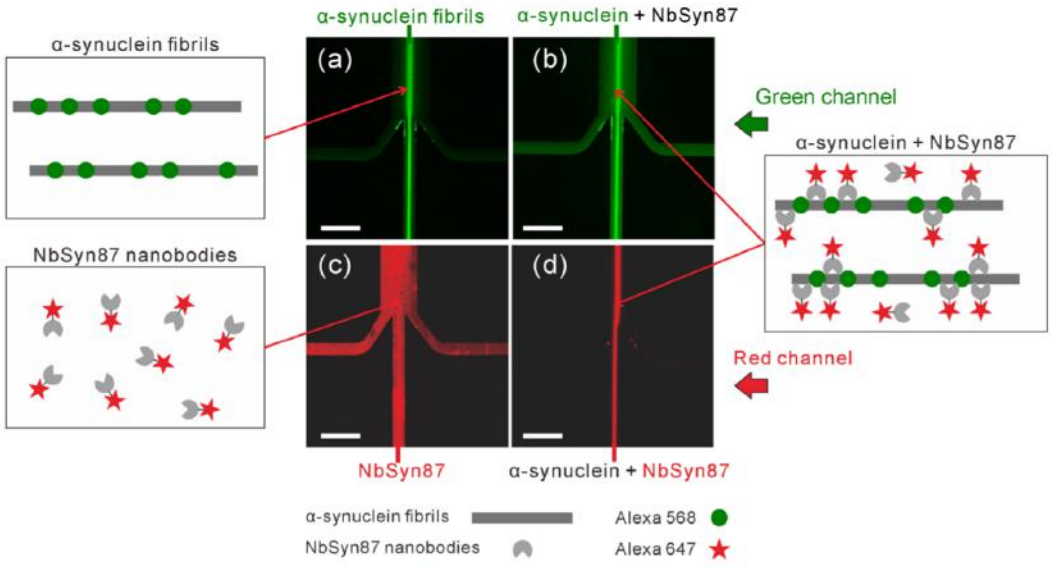


Microscale thermophoresis (MST) in glass capillaries can be used to quantify ligand binding to protein oligomers and amyloid fibrils.

Wolff *et al.*, Sci. Rep. 2016

Microfluidics for the study of biomolecular interactions



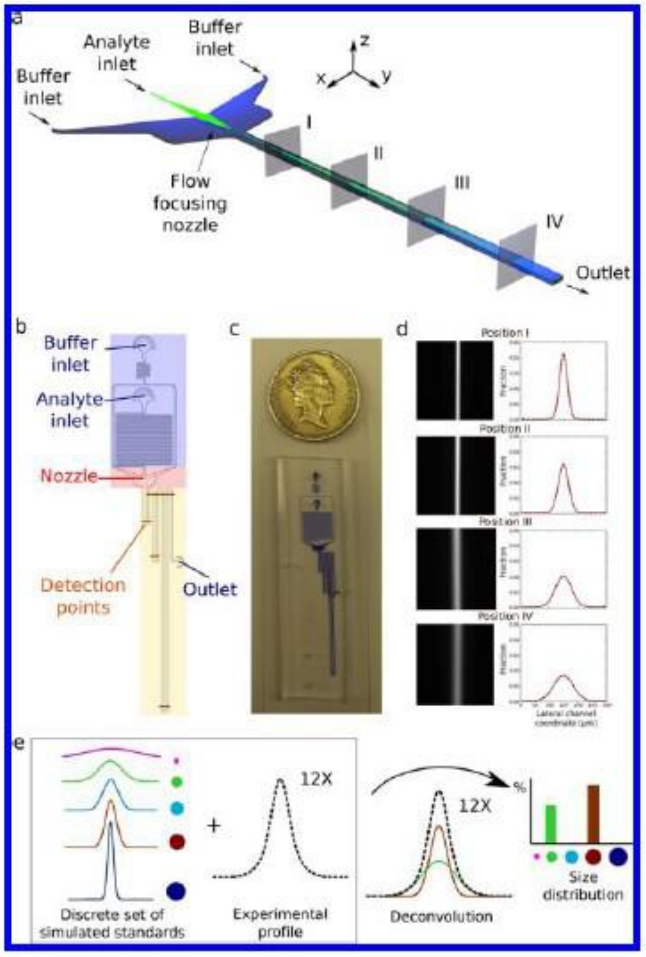


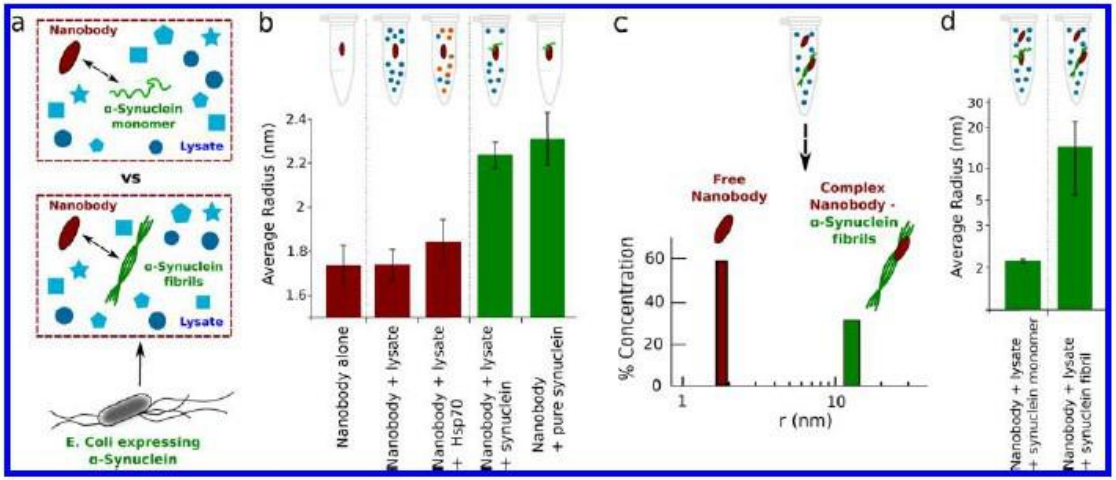
Differences in diffusion coefficient can be exploited for the identification of interactions between biomolecules and supramolecular protein aggregates.

Zhang *et al.*, ChemBioChem 2016

Microfluidic diffusional sizing

Microfluidics offers a platform for measuring binding between biomolecules through changes in diffusional behaviour.

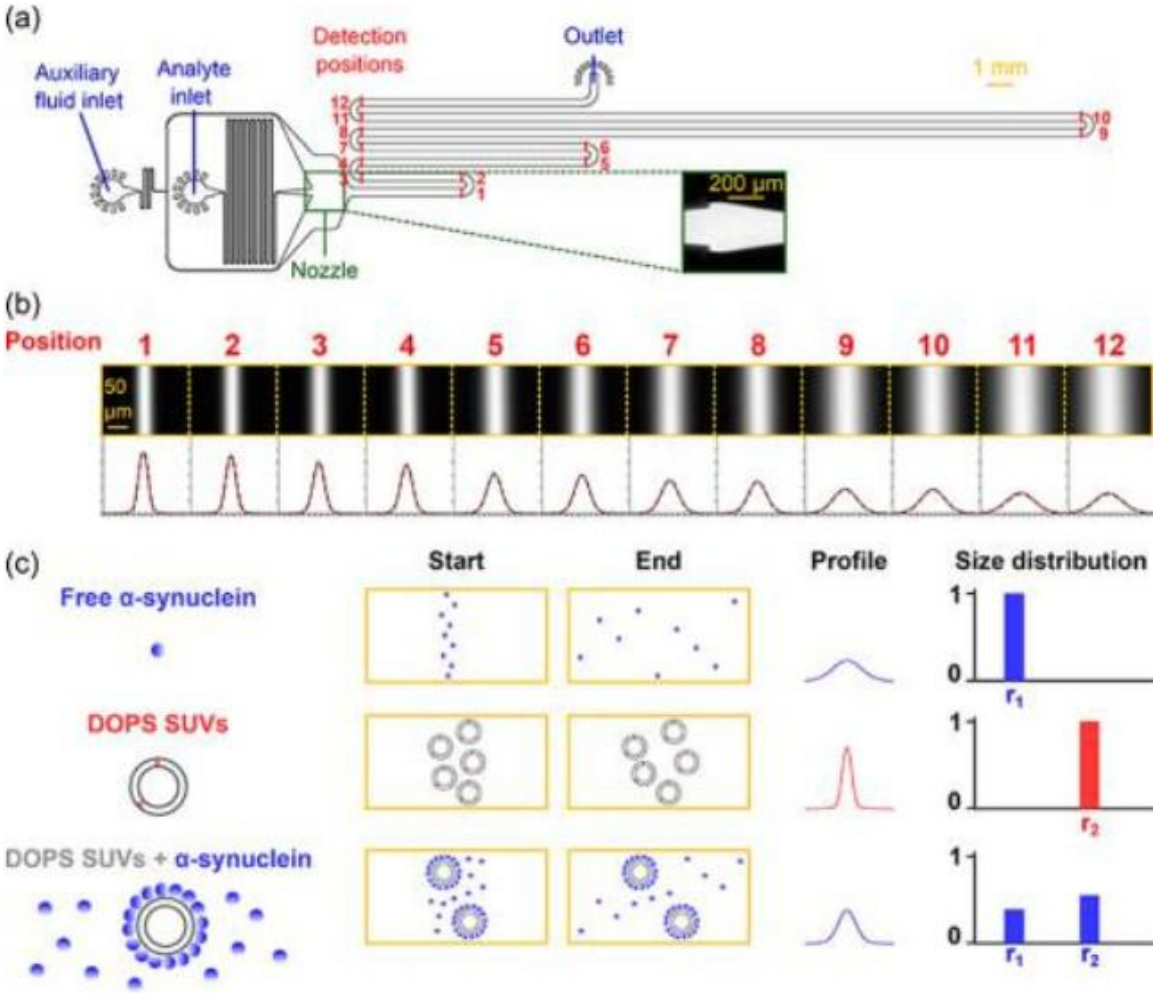


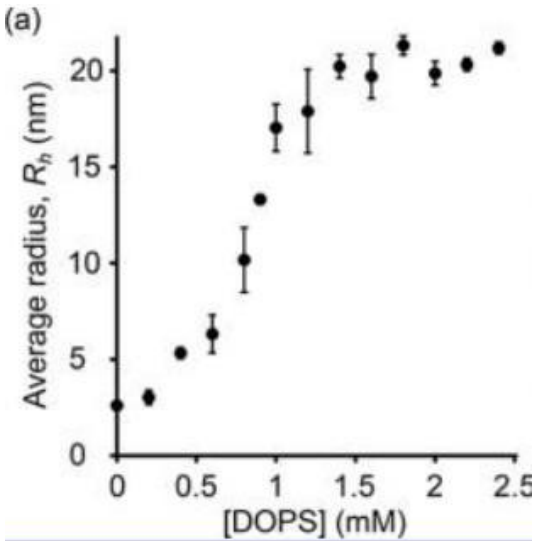


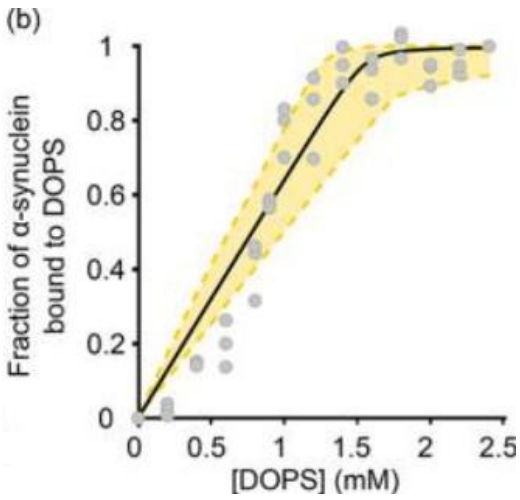
Only laminar flow is present, i.e. no turbulent mixing. Mass transport orthogonal to flow direction only through diffusion.

Arosio *et al.*, ACS Nano 2015

α-synuclein binding to lipid vesicles

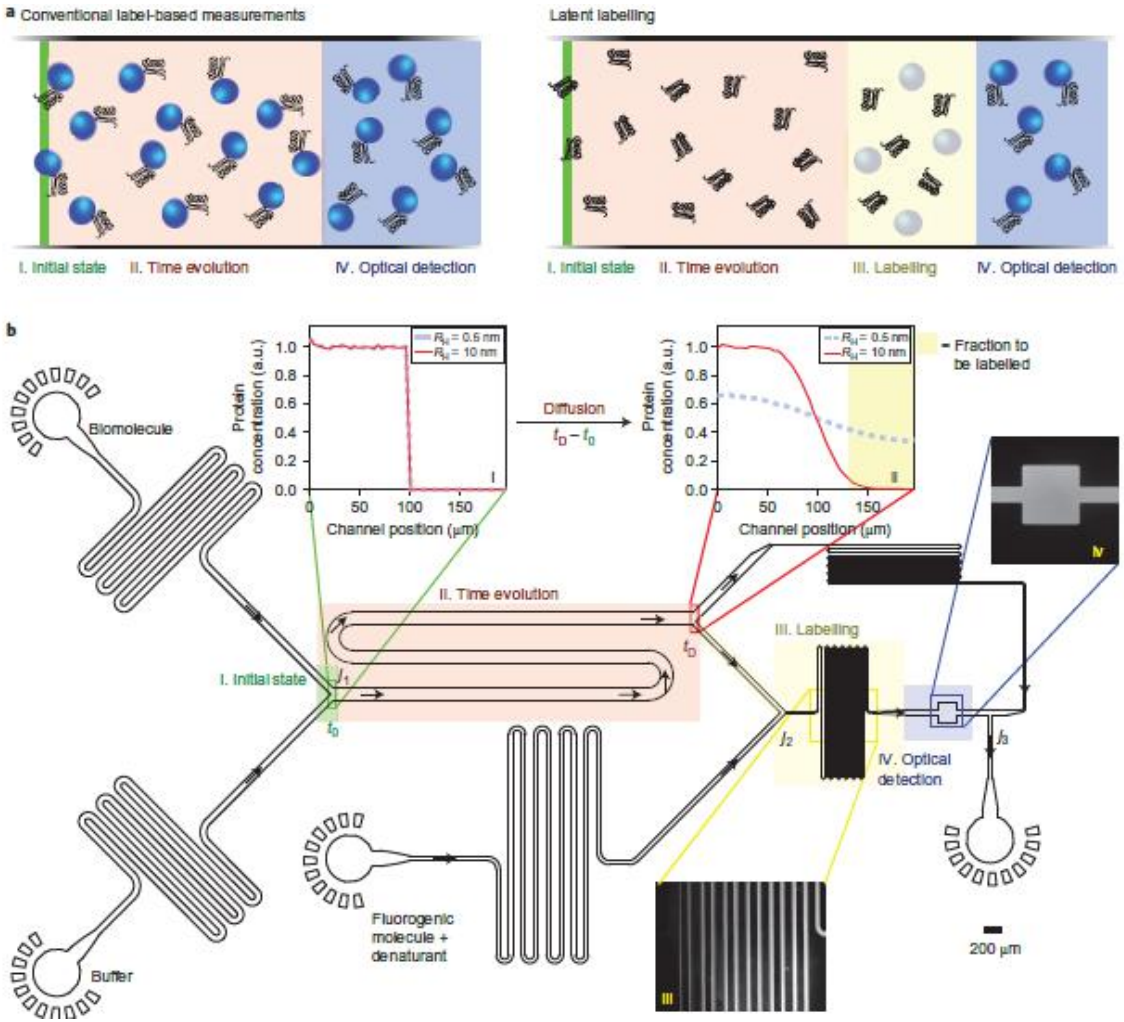






Gang, Galvagnion *et al.* Analytical Chemistry 2018

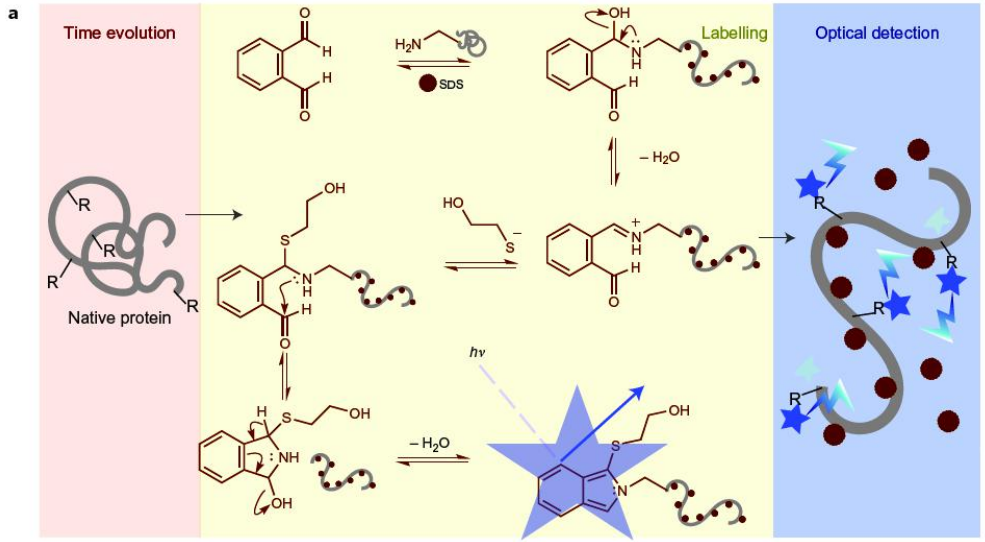
Label-free microfluidic diffusional analysis



Yates *et al.* Nature Chemistry 2015

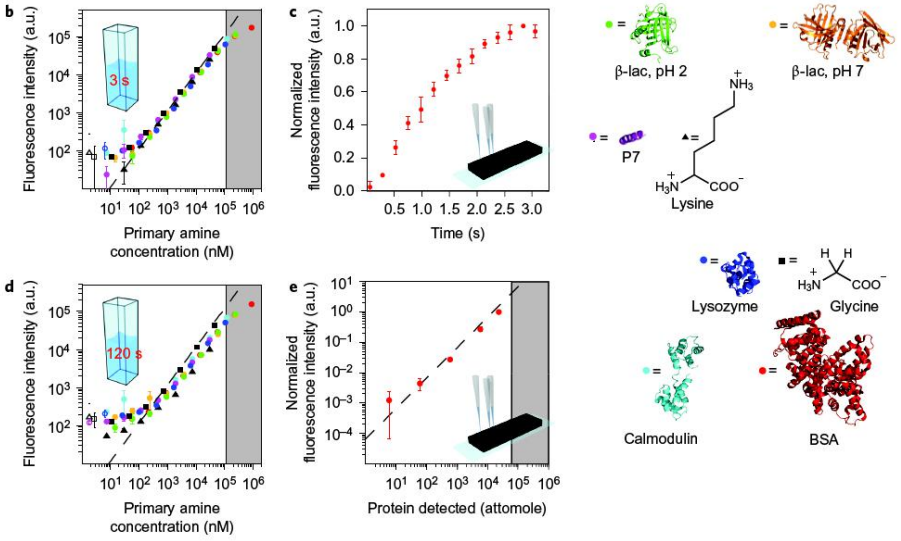
The presence of a label can change the binding behaviour. Therefore it can make sense to decouple the detection from the reaction and diffusion. After the diffusion, the protein mixture is fluorescently marked.

The labelling chemistry



The compound OPA (ortho-phalaldehyde) reacts with the protein amines and yields a fluorescent product.

Primary amines can be quantified over 5 orders of magnitude in concentration.



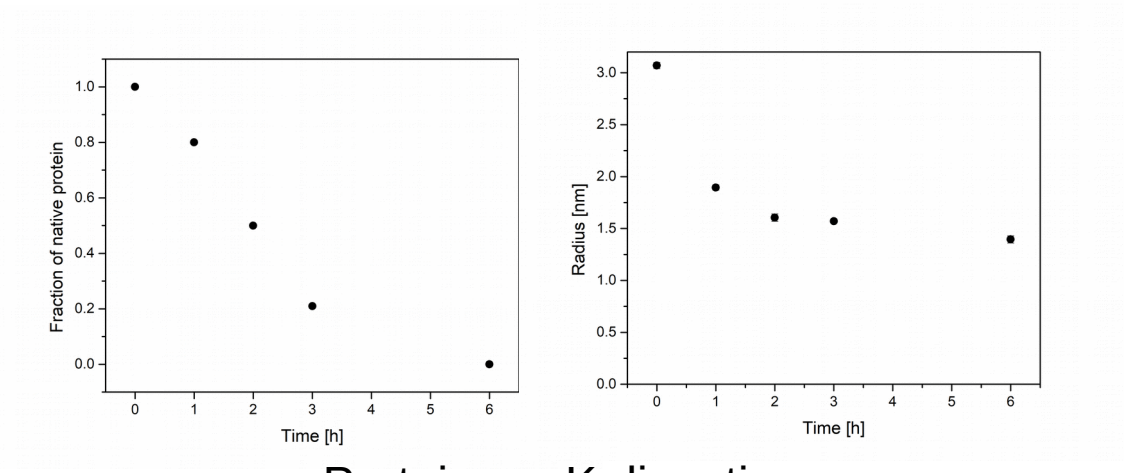
Yates *et al.* Nature Chemistry 2015

The Fluidity One instrument

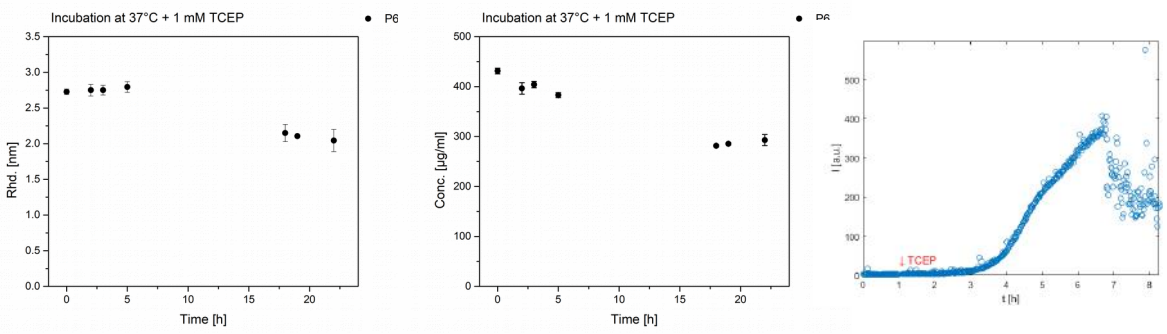
This device allows to determine the concentration and average size of protein samples simultaneously from a few microlitres of solution.

Applications of the Fluidity One

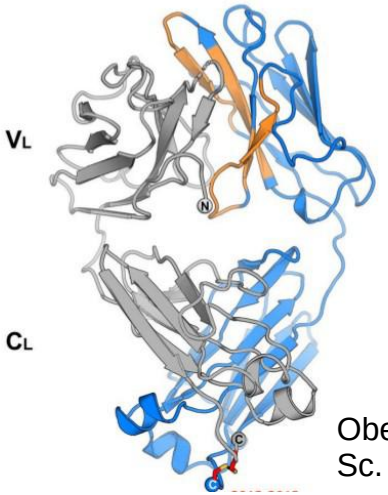
Example: biophysical and biochemical analysis of patient-derived light chain proteins



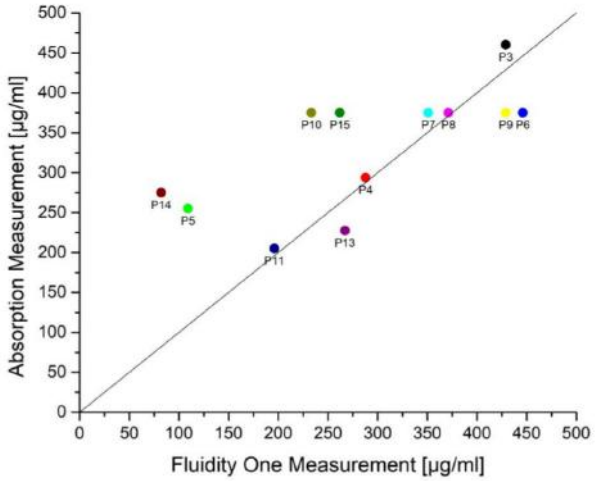
Proteinase K digestion



Aggregation induced by reducing agent



Oberti et al.
Sc. Rep. 2017



Concentration determination

Summary

The self-assembly and aggregation of peptides and proteins has relevance in biological function and malfunction as well as in biotechnology.

Microfluidics represents a powerful platform for the study and control of biomolecular interactions and assembly phenomena.

Acknowledgements

Cambridge
Prof. Christopher M. Dobson
Prof. Tuomas Knowles
Prof. Sir Mark Welland
Prof. Michele Vendruscolo
Prof. Clemens Kaminski
Prof. Franklin Aigbirhio
Dr Anne Dhulesia
Dr. Janet Kumita
Dr. Erwin de Genst
Dr. Gian Gaetano Tartaglia
Dr. Chris Waudby
Dr. Tim Guilliams
Dr. Xavier Salvatella
Dr. Neil Birkett
Dr. Céline Galvagnion
Dr. Myriam Ouberaï
Dr. Duncan White
Dr. Sam Cohen
Dr. Elin Esbjörner
Dr. Dorothea Pinotsi
Dr. Gergely Toth
Dr. Gabi Kaminski-Schierle
Dr. Yanyan Zhao
Dr. Sarah Shammas
Dr. Andela Saric
Dr. Ulyana Shimanovich
Dr. Andrea Pica
Dr. Thomas Mason
Dr. Patrick Flagmeier
Dr. James Brown
Dr. Therese Herling
Dr. Alex van der Wateren
Dr. Georg Meisl
Dr. Thomas Michaels
Dr. Yingbo Zhang
Dr. Thomas Mueller
Peter Hung
Aarti Dalsania
Shuyu Wang
Oliver Twinham
Julian Willis
Dr. Emily Fisher
Manuela Pfammatter

Lund
Prof. Sara Linse
Prof. Emma Sparr
Dr. Ricardo Gaspar

Aarhus
Prof. Daniel Otzen
Dr. Nikolai Lorenzen

Liège
Dr. Mireille Dumoulin
Dr. Céline Huynen

München
Prof. Dieter Braun
Dr. Manuel Wolff
Dr. Judith Mittag

Beijing
Prof. Sara Perrett
Dr. Yiqian Wang
Dr. Li-Qiong Xu

Tel Aviv
Prof. Ehud Gazit
Prof. Lihi Adler-Abramovich
Dr. Aviad Levin

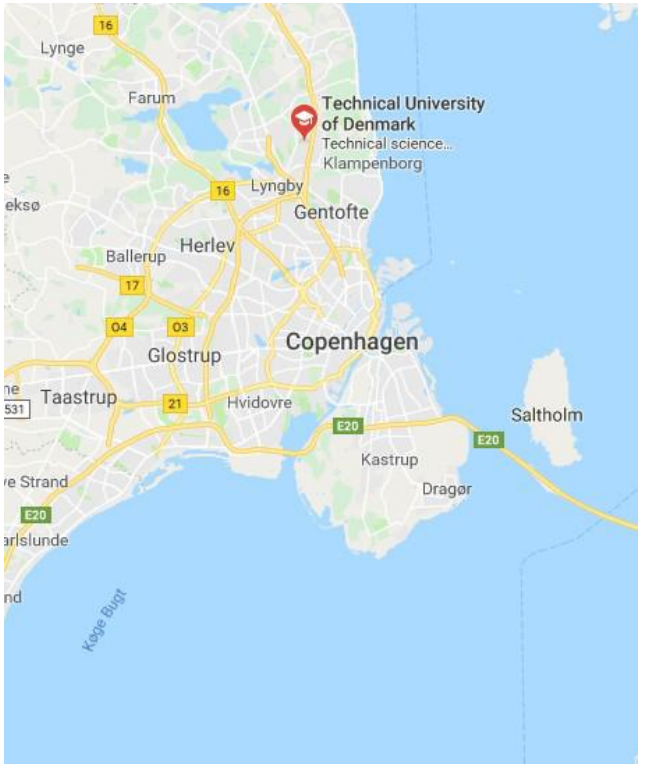
Institut Pasteur
Dr. Julia Chamot-Rooke
Dr. Mathieu Dupré

Düsseldorf
Prof. Wolfgang Hoyer
Prof. Rainer Haas
Prof. Stefan Egelhaaf
Prof. Dieter Willbold
Dr. Christina Dammers
Dr. Tamar Ziehm

Current group
Alessia Peduzzo
Nicola Vettore
Rebecca Sternke-Hoffmann
Soumav Nath
Lena Mangels
Marie Schützmann
Marcel Dickmanns

Funding: Magdalene College, EPSRC, EMBO, FEBS, Leverhulme Trust, Turnberg Foundation

Come to Copenhagen!



Start of new research group in April 2019

PhD and postdoc positions available!

Topics: Biophysics of biomolecular assembly in disease and function

Microfluidics experts (and those who want to become one) needed!