Workshop on Protein Aggregation: Biophysics & Mathematics

June 6-8, 2017 - University of Vienna, Sky Lounge, 12th floor

This workshop will focus on protein aggregation in various fields of application like amyloid diseases, actin filaments, autophagy.

The aim of this meeting is to bring together expert and young researchers in these fields working either on biophysics or in mathematical modellings. The reasonable number of talks will leave room for informal scientific discussions between the participants, which is one of the purposes of this meeting.

Confirmed participants

Vincent Béringue, Inra Jouy-en-Josas, France
Alexander Büll, University of Düsseldorf, Germany
Julia Delacour, WPI, Austria
Marie Doumic, Inria Paris & WPI, France and Austria
Angélique Egalon, Inra Jouy-en-Josas, France
Klemens Fellner, University of Graz, Austria
Frédéric Halgand, University Paris-Sud, France
Sascha Martens, University of Vienna, Austria
Sara Merino, Imperial College, United Kingdom
Mathieu Mézache, Inria Paris, France
Diane Peurichard, WPI, Vienna, Austria
Laurent Pujo-Menjouet, University of Lyon, France
Human Rezaei, Inra Jouy-en-Josas, France
Christian Schmeiser, Université of Vienna & WPI, Austria
Michael Sixt, Institute of Science and Technology, Vienna, Austria
Cassandra Terry, UCL Institute of Technology, London, United Kingdom
Magali Tournus, University of Aix-Marseille, France
Nicola Vettore, University of Düsseldorf, Germany
John Viles, Queen Mary University of London, United Kingdom
Wei-Feng Xue, University of Kent, United Kingdom
Yi Yin, Inria Paris, France
Romain Yvinec, INRA, France
Gabriele Zaffagnini, University of Vienna, France

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Tuessday, June 6th		**Wednesday, June 7th**		**Thursday, June 8th**	
		9h30	**Cassandra Terry** Structural characterisation of *ex vivo* mammalian prions	9h30	**Wei-Feng Xue** Nano-scale properties of amyloid fibril fragments
		10h10	**Laurent Pujo-Menjouet** Modelling prion dynamics: a fruitful collaboration between mathematicians and biologists	10h10	**Magali Tournus** Estimating the division rate and kernel in the fragmentation equation
		10h50	**BREAK**	10h50	**BREAK**
13h30: **Welcome of the participants** 14h: Welcome word by Christian Schmeiser professor at the university of Vienna and Wolfgang Pauli Institute		11h20	**Sascha Martens** Mechanism of p62-mediated protein aggregation in selective autophagy	11h20	**Frédéric Halgand** Prion protein conformational landscape studied by mass spectrometry and ion mobility
14h05	**Human Rezaei** Introduction of the workshop	12h	**LUNCH BREAK**	12h	**Yi Yin** Automated quantification of amyloid fibrils morphological features based on image analysis of transmission electron microscopies
14h20	**John Viles** Co-fibrillisation of truncated isoforms of Amyloid-β and ion-channel formation in Alzheimer's Disease	14h10	**Christian Schmeiser** Homeostatic regulation of actin density at the leading edge of lamellipodia	12h20	**LUNCH BREAK**
15h	**Klemens Fellner** Equilibration and Quasi-Steady-State Asymptotics of a Volume-Surface Reaction-Diffusion Model for Asymmetric Protein Localisation	14h50	**Michael Sixt** *to be announced*	13h50	**Alexander Büll** Kinetic and thermodynamic analysis of peptide self-assembly
15h40	**BREAK**	15h30	**BREAK**	14h30	**Sara Merino-Aceituno** A new flocking model through body attitude coordination
16h10	**Vincent Béringue** Small prion assemblies are involved in prion replication	16h15	**Marie Doumic** Modelling protein polymerisation: results and open questions	15h10	**BREAK**
16h50	**Romain Yvinec** Time scales in a coagulation-fragmentation model	17h15-18h15	**Poster session** A. Igel-Egalon, M. Mézache, D. Peurichard, N. Vettore, G. Zaffagnini	15h20	**Human Rezaei** Prion quasi-species and molecular basis of auto-perpetuation of Prion structural information
		19h	**social event** dinner in a "Heuriger"	16h	**CONCLUSION**

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Abstracts of talks and posters

June 6-8, 2017 - University of Vienna, Sky Lounge, 12th floor

Vincent Béringue, Inra Jouy-en-Josas, France

Small prion assemblies are involved in prion replication

Angélique Igel-Egalon[1¶], Mohammed Moudjou[1¶], Florent Laferrière[1¶], Tina Knäpple[1], Laetitia Herzog[1], Fabienne Reine[1], Hubert Laude[1], Human Rezaei[1]*, Vincent Béringue[1]*
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Mammalian prions are proteinaceous pathogens responsible for fatal, neurodegenerative disorders in human and animals. They are formed of misfolded assemblies (PrP^{Sc}) of the host-encoded cellular prion protein (PrP^{C}). In the infected species, prions replicate by seeding the conversion and polymerization of host PrP^{C}. Distinct prion strains are recognized within the same host-species, exhibiting defined PrP^{Sc} biochemical properties and stereotyped biological traits. While strain information is encoded within the conformation of PrP^{Sc} assemblies, the storage of the structural information and the molecular requirements for self-perpetuation remain uncertain. In particular, the polymerization steps and its dynamic nature remains mostly hypothetical. It is widely believed that monomeric PrP^{C} is constantly recruited within the forming aggregates allowing PrP^{Sc} fibril growth. Fibril fragmentation is supposed to provide further converting seeds, favouring prion exponential replication. Whether this proposed mechanism is versatile or strain-dependent remains to be determined, as is the real contribution of fragmentation. We have investigated this issue by analysing the dynamic of PrP^{Sc} assembling during cell-free prion amplification by protein misfolding cyclic amplification (PMCA). We show that: i) prion amplification occurs through preferential amplification of small oligomeric forms of PrP^{Sc} that can further assemble into larger aggregates; ii) disassembling rather than fragmentation sustains the self-perpetuation of the process, iii) different prion strains exhibit similar amplification dynamic. Thus, prion replication may proceed through an assembly/disassembly process.

Alexander K. Buell, Institute of Physical Biology, University of Düsseldorf, Germany

Kinetic and thermodynamic analysis of peptide self-assembly

In this talk I will discuss various aspects of the kinetics and thermodynamics of the self assembly of peptides into amyloid fibrils and crystals. I will present a theoretical framework that allows to determine free energy barriers and entropies from kinetic data of amyloid fibril growth [1,2]. I will contrast the kinetic behaviour of longer, amyloid forming sequences with that of aromatic dipeptides that form crystals, rather than amyloid fibrils [3,4].
Furthermore, I will present the phenomenon of autocatalytic secondary nucleation, whereby new amyloid fibrils nucleate on the surface of existing ones [5,6]. In particular, I will show how this

phenomenon manifests itself in kinetic measurements of protein aggregation, and how biosensing can be used to explore its molecular origin [6,7].

[1] A. K. Buell, J. R. Blundell, C. M. Dobson, M. E. Welland, E. M. Terentjev, and T. P. Knowles, Phys. Rev. Lett. **104**, 228101 (2010).
[2] A. K. Buell, A. Dhulesia, D. A. White, T. P. J. Knowles, C. M. Dobson, and M. E. Welland, Angew. Chem. Int. Ed Engl. **51**, 5247 (2012).
[3] T. O. Mason, T. C. T. Michaels, A. Levin, E. Gazit, C. M. Dobson, A. K. Buell, and T. P. J. Knowles, J. Am. Chem. Soc. **138**, 9589 (2016).
[4] T. O. Mason, A. Levin, C. M. Dobson, E. Gazit, T. P.J. Knowles and A. K. Buell, JACS **under revision**, (n.d.).
[5] A. K. Buell, C. Galvagnion, R. Gaspar, E. Sparr, M. Vendruscolo, T. P. J. Knowles, S. Linse, and C. M. Dobson, Proc. Natl. Acad. Sci. **111**, 7671 (2014).
[6] R. Gaspar, G. Meisl, A. K. Buell, L. Young, C. F. Kaminski, T. P. J. Knowles, E. Sparr, and S. Linse, Q. Rev. Biophys. **50**, (2017).
[7] A. Šarić, A. K. Buell, G. Meisl, T. C. T. Michaels, C. M. Dobson, S. Linse, T. P. J. Knowles, and D. Frenkel, Nat. Phys. **12**, 874 (2016).

Marie Doumic, Inria Paris & Wolfgang Pauli Institute, France & Austria

Modelling protein polymerisation: results and open questions

Mathematical modelling of protein polymerisation is a challenging topic, with wide applications, from actin filaments in myocytes (muscle tissues) to the so-called *amyloid* diseases (e.g. Alzheimer's, Parkinson's or Creuzfeldt-Jakob's diseases). In this talk, we will give an overview of recent results for both deterministic - where statistical mechanical fluctuations arising from intrinsic noise are negligible - and stochastic approaches, envisaged as giving complementary insights on the still largely mysterious intrinsic mechanisms of polymerisation. A data assimilation approach is developed in parallel of more specific methods for fragmentation estimation.

The results we will present are partly joint work with A. Armiento, J. Calvo, S. Eugène, M. Escobedo, P. Moireau, B. Perthame, H. Rezaei, P. Robert, M. Tournus and W.F. Xue.

Klemens Fellner, University of Graz, Austria

Equilibration and Quasi-Steady-State Asymptotics of a Volume-Surface Reaction-Diffusion Model for Asymmetric Protein Localisation

The protein Lgl (Lethal giant larvae) is part of a conserved protein complex, which is responsible for the asymmetric localisation of cell-fate determinants, for instance, in Drosophila SOP precursor cells.
We formulate continuum models, which consider the phosphorylated and the unphosphorylated conformations of Lgl within the cell cytoplasm and on the cell cortex. After presenting illustrative numerical simulations, we prove first the equilibration of the underlying complex-balance volume-surface reaction-diffusion system and perform further a rigorous quasi-steady-state-approximation in a fast-reaction limit.

Franca Fraternali, King's College London, United Kingdom

Disentangling Prion Oligomers Assemblies by Atomistic Molecular Dynamics Simulations

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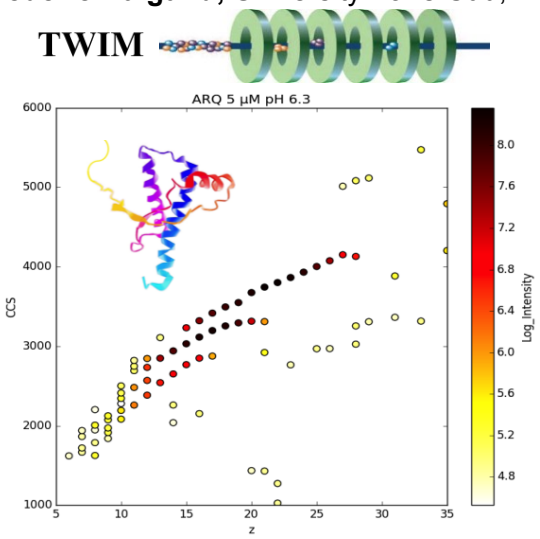
Prions are self-replicating infectious protein conformations capable to form oligomers leading to amyloid fibrils. Recent experimental findings demonstrated that the helical domain H2H3 of ovine prion protein (OvPrP) is the minimal portion of PrP capable of prion activity and it is involved in the oligomerization process (1). Molecular Dynamics (MD) simulations gave mechanistic insights into the possible early conversion of $OvPrP^{C}$ into a beta-rich $OvPrP^{Sc}$ (H2H3-$OvPrP^{Sc}$) (2). Starting from those outcomes, we present results from multiple replicas simulations of 18 units of the beta-rich fragment in solution to provide a possible model for the aggregation of H2H3-$OvPrP^{Sc}$. We elucidate the process by a kinetic model of the simulated aggregation process as determined from a Markov State Model (MSM), derived from the atomistic simulations.
Thanks to this abstraction model we have been able to describe the time evolution of the aggregation process and the average connectivity among H2H3-$OvPrP^{Sc}$ in the observed oligomers. We are able to identify the "core" of the final aggregation states obtained from 11 replicas and distinguish possible pathways of aggregation. We show that the most important residues for the stabilization of the observed aggregation states coincide with the ones determined experimentally to play a key role in the aggregation process of H2H3-$OvPrP^{Sc}$. (1,2)

REFERENCES

(1) Chakroun N, Prigent S, Dreiss CA, Noinville S, Chapuis C, Fraternali F and Rezaei H. The oligomerization properties of prion protein are restricted to the H2H3 domain. *FASEB J.* 24 3222–3231 (2010).
(2) Chakroun N, Fornili A, Prigent S, Kleinjung J, Dreiss CA, Rezaei H, Fraternali F. Decrypting Prion Protein Conversion into a beta-Rich Conformer by Molecular Dynamics. J Chem Theory Comput. 2013 9(5):2455-2465.

Frédéric Halgand, University Paris-Sud, France



Prion protein conformational landscape studied by mass spectrometry and ion mobility.

Guillaume van der Rest, Human, Rezaei, Frédéric Halgand, Université Paris Sud, Laboratoire de Chimie Physique

Prion protein is involved in deadly neurodegenerative diseases. Its pathogenicity is linked to its structural conversion (a-helix to b-strand transition). However, recent studies suggest that prion protein can follow a plurality of conversion pathways which hints towards different conformers that might coexist in solution. We therefore decided to screen the ovine and human PrP monomers using ion mobility coupled to mass spectrometry following electrospray ionization. After a short presentation of ion mobility for studying ionized proteins in the gas phase, we will briefly discuss issues with the collision cross section calibration procedure that we have encountered when using travelling wave ion mobility. We will also discuss the development of an automated data extraction pipeline for which we developed a Python/Qt script base interface. Infusion of monomeric PrP solutions have shown that at least three PrP conformers are observed in the gas phase. PrP monomers are known to lead to the formation of oligomeric species in specific conditions (temperature, pH and buffer), which are not compatible with mass spectrometry. We have therefore developed a size-exclusion chromatography IMS-MS setup with the aim to study the oligomers produced in these conditions. The development of this SEC-IMS-MS methodology will be presented as well as its application for calibration with standard protein complexes. Although we did not achieve resolution of the large (O1 ~36-mer) oligomeric species, optimization of the experimental parameters led to the observation of the small (O3) oligomeric species. One key observation in this process was that the abundance of the gas phase monomeric conformers changed upon the oligomerization process. First results allow us to interpret this as an effect of monomer concentration on the ratio of conformers present in solution, which is observed only in specific buffer conditions.

Angélique Igel-Egalon, INRA Jouy-en-Josas, France

Depolymerization instead of fragmentation spreads the replication unit of prion assemblies

Angélique Igel-Egalon[1], Mohammed Moudjou[1], Alexandra Busley[1], Laetitia Herzog[1], Fabienne Reine[1], Charles-Adrien Richard[1], Tina Knäpple[1] Vincent Béringue[1*] and Human Rezaei[1*]
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The prion phenomenon is based on autonomous structural information propagation towards single or multiple protein conformation changes. During this last decade the prion concept referring the transmission of structural information has been extended to several regulation systems and pathologies including Alzheimer and Parkinson's diseases.
Despite intensive investigation, the molecular basis of structural information transmission remains obscure. Templating (i.e. secondary nucleation as vector of structural information) has been proposed as origin of autocatalytic structural information perpetuation. However, the templating process does not consider the spreading process which consists in an exponential amplification of structural information. Active fibril fragmentation (AFF) constitutes a solution for exponential spreading and amplification of the structural information as strongly suggested in fungi prions (Shorter and Lindquist, Mol Cell, 2006).
In the present work, we demonstrate that mammalian Prion assemblies (PrP^{Sc}) are constituted from an oligomeric elementary brick called suPrP. We show that in physiological conditions Prion assemblies are in equilibrium with suPrP. The existence of such equilibrium as simple depolymerization/condensation process is sufficient to spread the replicative unit through the release of suPrP, followed by its Brownian diffusion and condensation into PrP^{Sc} and discards the requirement of fragmentation for prion spreading.

Sascha Martens, Max F. Perutz Laboratories (MFPL), University of Vienna, Austria

Mechanism of p62-mediated protein aggregation in selective autophagy

Autophagosomes are double membrane-bound organelles that are formed *de novo* during a process called autophagy. Autophagosomes mediate the bulk degradation of cytoplasmic material such as aggregated proteins, dysfunctional or surplus mitochondria and intracellular pathogens. Autophagy is conserved from yeast to human and has been shown to protect the organism from conditions such as starvation, neurodegeneration and infectious diseases. During autophagosome formation initially small membrane structures termed isolation membranes are formed. These isolation membranes expand and thereby gradually enclose cytoplasmic cargo. Finally, isolation membranes close to give rise to mature autophagosomes. After their formation autophagosomes fuse with lysosomes within which their inner membranes and the contents are degraded. Autophagy has the ability to selectively capture and subsequently degrade aggregated and ubiquitinated proteins. This is mediated by the p62 cargo receptor, which is required for the aggregation of these proteins into larger structures. These structures then serve as templates for autophagosome formation. I will present our results from a fully reconstituted system, which enabled us to dissect the interplay between p62 and ubiquitin positive proteins during protein aggregation in selective autophagy.

Sara Merino-Aceituno, Imperial College, London, United Kingdom

A new flocking model through body attitude coordination

We present a new model for multi-agent dynamics where each agent is described by its position and body attitude: agents travel at a constant speed in a given direction and their body can rotate around it adopting different configurations. Agents try to coordinate their body attitudes with the ones of their neighbours. This model is inspired by the Vicsek model. The goal of this talk will be to present this new flocking model, its relevance and the derivation of the macroscopic equations from the particle dynamics. In collaboration with Pierre Degond (Imperial College London) and Amic Frouvelle (Université Paris Dauphine).

Mathieu Mézache, Inria Paris and Univ. Pierre et Marie C, France

An oscillatory kinetic model for the Prion aggregation process.
From Belousov-Zhabotinsky reaction to a Prion polymerisation/depolymerisation chemical system.

We investigate the oscillatory behaviour of the PrP protein during the polymerization/depolymerization process. In order to modelize this oscillatory process, we study a simplified Belousov-Zhabotinsky reaction from a kinetic point of view. This simplified oscillatory system of chemical reactions allows us to introduce a modified Becker-Döring system where the trajectories oscillate. A key to have a closed oscillatory polymerization/depolymerization system is to consider different specices of polymers and monomers. We finally present several system where the numerical simulations show a more or less sustained oscillatory behaviour.

Laurent Pujo-Menjouet, University of Lyon, France

Modelling prion dynamics: a fruitful collaboration between mathematicians and biologists

In a previous work by Alvarez-Martinez et al. (2011), the authors pointed out some fallacies in the mainstream interpretation of the prion amyloid formation. It appeared necessary then to propose an original hypothesis able to reconcile the in vitro data with the predictions of a mathematical model describing the problem. The model presented here, has been developed accordingly with the hypothesis that an intermediate on-pathway leads to the conformation of the prion protein into an amyloid competent isoform thanks to a structure, called micelles, formed from hydrodynamic interaction. Experimental data have been compared to the prediction of our model leading to a new hypothesis for the formation of infectious prion amyloids. In the last part, we will introduce a new model describing another dangerous liaison: the interaction between prion proteins and Abeta peptides that may lead to Alzheimer's disease.

Human Rezaei, Inra Jouy-en-Josas, France

Prion quasi-species and molecular basis of auto-perpetuation of Prion structural information.

Davy Martin[1], Joan Torrent i Mas[1], Stéphanie Prigent[1], Mathieu Mezache[2], Marie Doumic-Jauffret[2], Vincent Béringue[1] and Human Rezaei[1*]

1. National Institute for Agricultural Research (INRA), Pathological Macro-assemblies and Prion Pathology group (MAP[2]), UR892, Virologie Immunologie Moléculaires, Jouy-en-Josas, 78350-F, France

2. Sorbonne Universités, Inria, UPMC Univ Paris 06, Lab. J.L. Lions UMR CNRS 7598, Paris, France

The prion phenomenon is based on autonomous structural information propagation towards single or multiple protein conformational changes. Since this last decade the prion concept referring to the transmission of structural information has been extended to several regulation systems and pathologies including Alzheimer and Parkinson's diseases. The unified theory in Prion replication implies structural information transference (SIT) from the prion to a non-prion conformer through a mechanism also called improperly, with regards to biophysical considerations "seeding" phenomenon. Therefore considering prion replication as a structural information transduction from a donor (i.e. template) to an acceptor (i.e. substrate) through a transduction interface a new questioning arises: what are molecular mechanisms of the auto-perpetuation of the Prion structural information and its faithfulness?

Considering the Prion propagation as more or less faithful perpetuation of structural information, in the present work, we explored the concept of prion quasi-species (*i.e.* existence of prion heterogeneous assemblies) and highlighted the existence of prion network, which has an autopoietic behaviour (auto-replicative). Our observations strongly suggest that specific criteria in term of: protein structure, delay-process and thermo-kinetics should be collated before a system become dissipative and autopoietic.

Christian Schmeiser, University of Vienna and Wolfgang Pauli Institute, Austria

Homeostatic regulation of actin density at the leading edge of lamellipodia

Some recent contributions to the modeling of the polymerization and depolymerization of actin filaments will be reviewed. Some results of the embedding of these models into the Filament Based Lamellipodium Model will be presented.

Cassandra Terry, MRC Prion, UCL Institute of Technology, London, United Kingdom

Structural characterisation of ex vivo mammalian prions.

Cassandra Terry[a] Adam Wenborn[a] Nathalie Gros[a] Jessica Sells[a] Susan Joiner[a] Laszlo L.P. Hosszu[a] M. Howard Tattum[a] Silvia Panico[b] Daniel K. Clare[b], John Collinge[a], Helen R. Saibil[b] and Jonathan D.F. Wadsworth[a]*
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b, Institute of Structural and Molecular Biology, Department of Biological Sciences, Birkbeck College, University of London, Malet Street, London WC1E 7HX, UK

Prions cause lethal neurodegenerative diseases in mammals, including scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt–Jakob disease (CJD) in humans. Mammalian prions are hypothesised to be fibrillar or amyloid forms of prion protein (PrP) which self-propagate by means of seeded protein polymerisation but structures observed had not been definitively correlated with infectivity and the three-dimensional structure of prions remained unknown. We developed new methods to obtain pure preparations of intact prions from mouse brain[1] and showed that pathogenic PrP is assembled into rod-like assemblies (PrP rods) that faithfully transmit prion strain-specific phenotypes when inoculated into mice. We have utilised the precision of cell culture prion infectivity assays to define the physical relationship between PrP rods and prion infectivity and used electron tomography to define their architecture. Our 3D analysis[2] demonstrates that *ex vivo* infectious PrP rods from different strains observed have a common hierarchical assembly comprising twisted pairs of short fibres with repeating substructure which are markedly different to non-infectious PrP fibrils generated *in vitro*.

References

1. A. Wenborn, C. Terry, N. Gros, S. Joiner, L. D'Castro, S. Panico, J. Sells, S. Cronier, J. Linehan, S. Brandner, H.R. Saibil, J. Collinge, J.D.F Wadsworth, *Sci. Rep*. *A novel and rapid method for obtaining high titre intact prion strains from mammalian brain,* 2015, **5**, 10062.
C. Terry, A. Wenborn, N. Gros, J. Sells, S. Joiner, L.L.P Hosszu, M.H. Tattum, S. Panico, D.K. Clare, J. Collinge, H.R. Saibil, J.D.F Wadsworth. *Open Biology. Ex vivo mammalian prions are formed of paired double helical prion protein fibrils*, 2016, **6**, 160035.

Magali Tournus, University of Marseille, France
Estimating the division rate and kernel in the fragmentation equation.

We consider the pure fragmentation fragmentation equation
and address the question of estimating the fragmentation parameters (division rate and fragmentation kernel) from measurements of the size distribution at various times.
Under the assumption of a polynomial division rate and a self-similar fragmentation kernel, we use the well-known asymptotic behaviour of the solution to guarantee the well-posedness of our inverse problem and provide a representation formula for the fragmentation kernel.
The tools used are the Mellin transform and the Wiener-Hopf method.
Motivations for studying this problem and applications to amyloid fibril breakage will be described in the talk of W.F. Xue.

Nicola Vettore, Institute of Physical Biology, University of Düsseldorf, Germany
Temperature dependence of amyloid fibril stability studied through equilibrium denaturation

Nicola Vettore and Alexander K. Buell, Institute of Physical Biology, University of Düsseldorf

Amyloid fibrils are thermodynamically very stable [1], but the origin of their enhanced stability with respect to the native state has not yet been elucidated in molecular detail. The high stabilities of amyloid fibrils render the study of their equilibrium behaviour challenging.
One way to approach this issue, in direct analogy to the study of protein folding equilibria is denaturation with commonly used denaturants, such as GdmCl or Urea. A theoretical framework to extract from such measurements the free energy difference between the fibril state and the soluble state, based on Oosawa's linear polymerisation model, was proposed in [2].
Here we present experimental results of amyloid fibril equilibrium denaturation measured via capillary fluorescence over a wide range of temperatures. The data highlight how the influence of temperature seems of primary importance not only for the kinetics of fibril formation, but also for the thermodynamic stability of the fibrillar structures. We will also present our attempts to describe the temperature-dependence of fibril stability within a general thermodynamic framework.

[1] A. J. Baldwin, T. P. J. Knowles, G. G. Tartaglia, A. W. Fitzpatrick, G. L. Devlin, S. L. Shammas, C. A. Waudby, M. F. Mossuto, S. Meehan, S. L. Gras, J. Christodoulou, S. J. Anthony-Cahill, P. D. Barker, M. Vendruscolo, and C. M. Dobson, J. Am. Chem. Soc. **133**, 14160 (2011).
[2] T. Narimoto, K. Sakurai, A. Okamoto, E. Chatani, M. Hoshino, K. Hasegawa, H. Naiki, and Y. Goto, FEBS Lett. **576**, 313 (2004).

John H Viles, Queen Mary, University of London, United Kingdom**,** j.viles@qmul.ac.uk
Co-fibrillisation of truncated isoforms of Amyloid-β and ion-channel formation in Alzheimer's Disease

Amyloid-β peptide (Aβ) isoforms of different lengths and aggregation propensities coexist *in vivo*. These different isoforms are able to nucleate or frustrate the assembly of each other. N-terminal truncated $A\beta_{(11-40)}$ and $A\beta_{(11-42)}$ make up one fifth of plaque load yet nothing is known about their interaction with full-length $A\beta_{(1-40/42)}$. Here we show that in contrast to C-terminal truncated isoforms which do not co-fibrillise, deletions of ten residues from the N-terminus of Aβ have little impact on its ability to co-fibrillise with the full-length counterpart. As a consequence N-terminal truncated Aβ will accelerate fibre formation and co-assemble into short rod-shaped fibres with its full-length Aβ counterpart. Furthermore we show Cu^{2+} forms a very tight tetragonal complex with truncated $A\beta_{(11-40)}$ with a femtomolar affinity. These observations have implications for the assembly kinetics, morphology and toxicity of all Aβ isoforms.
The process by which amyloid-β (Aβ) disrupts synaptic activity, and causes neuronal cell death in Alzheimer's disease remains poorly understood. A potential mechanism of toxicity is in the ability of Aβ to form, membrane-spanning ion channels. However, there has been a mismatch between the channel forming properties of Aβ isoforms, 40 and 42 amino acids long, and their known relative pathogenicity. We observe ion channel formation by

oligomeric $A\beta_{42}$, but also show $A\beta_{40}$ does not form ion channels in cellular membranes. This makes a strong link between ion channel formation and the pathology of Aβ isoforms. Molecules that block these ion channels may represent therapeutic targets.

[1] Ion Channel Formation by Amyloid-β42 Oligomers but not Amyloid-β40 in Cellular Membranes DC Bode, MD Baker, JH Viles* (2017) *J of Biol Chem* 292, 1404-1413

[2] Truncated Amyloid-β (11-40/42) from Alzheimer's Disease Binds Copper2+ with a Femtomolar Affinity and Influences Fibre Assembly J D Barritt, J H. Viles* (2015) *J of Biol Chem*, 290, 27791-27802

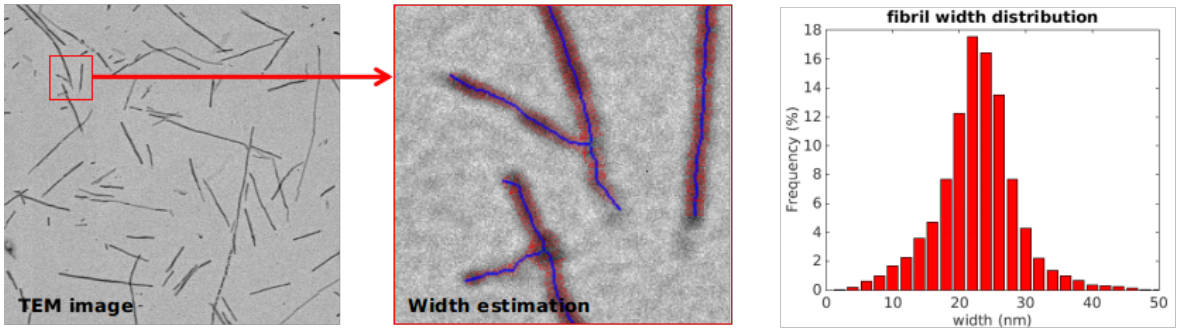
[3] The Rapid Exchange of $Zinc^{2+}$ Enables Trace Levels to Profoundly Influence Amyloid-β Misfolding and Dominates Assembly Outcomes in Cu^{2+}/Zn^{2+} Mixtures C J Matheou, N D Younan, J H Viles* (2016) *J Mol Biol* 428, 2832-2846

Wei-Feng Xue, University of Kent at Canterbury, United Kingdom
Nano-scale properties of amyloid fibril fragments

A number of devastating human disorders, for example Alzheimer's disease (AD), Hungtington's diseases, type 2 diabetes and transmissible spongiform encephalopathies (TSEs), are associated with the abnormal folding and assembly of proteins. The net result of this misfolding is the formation of large insoluble protein deposits and small toxic and transmissible protein particles in a state called amyloid. What are the molecular mechanisms that govern the amyloid fibrils' potential to seed the formation of new aggregates, to propagate the amyloid state as prion particles, and to damage cells in amyloid-associated diseases? We have developed AFM imaging approaches that are capable of resolving the fibril particle concentrations, their length distributions, as well as their toxic and infective potential to cells. With these approaches, we have shown that the disease-associated properties of amyloid can be linked to small nano-sized amyloid particles created through the breakage of amyloid fibrils. The approaches we have developed offer new opportunities to determine, quantify, and predict the course and the consequences in amyloid assembly of cytotoxic, infectious as well as functional amyloid systems.

Yi Yin, Inria Paris and Univ. Pierre et Marie Curie, France
Automated quantification of amyloid fibrils morphological features based on image analysis of transmission electron microscopies
Yi Yin*, [1], Stéphanie Prigent[1], Joan Torrent, Dirk Drasdo[1], Human Rezaei, and Marie Doumic[1]
*1. INRIA Paris, and Sorbonne Universités UPMC Univ. Paris 6, Laboratoire Jacques-Louis Lions, Paris, France, * yi.yin@inria.fr*
Protein aggregation into fibrils is a key process in amyloid diseases and also in other biological processes. The quantification of fibrils' morphology and molecular structures is urgently needed in understanding of the key mechanisms and properties of fibrils.
In this study, we propose an automated image analysis procedure to extract and quantify fibril morphological features from transmission electron microscopy (TEM) images. Fibrils are segmented by a 'maximum entropy' thresholding method and then the 'fast marching' skeletonization is applied to detect the fibril centerlines. The individual information of each fibril is gathered based on the fibril segmentation and extracted centerline, including the length (following the curvature of the fibrils, which are rarely straight lines), the varying width

along the length, the curvature, as well as the number, position and length of branches. The intricate overlapping and branching structures are identified based on the angles between fibril segments.
The proposed method was tested on experiments on the prion protein (PrP), which also allows us to explain in detail the parameters needed for the image analysis. Our method has high estimation accuracy (e.g. width estimation as shown in the figure). The results from different mutants of the PrP protein fibrils showed the potential of the method in fibrils classification through a statistical analysis.



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Time scales in a coagulation-fragmentation model}

This work is motivated by protein aggregation phenomena in neurodegenerative diseases. A key observation of *in-vitro* spontaneous polymerization experiments of prion protein is the large variability of the so-called 'nucleation time', which is experimentally defined as the lag time before the polymerization of proteins truly starts (typically several hours in a 10-20 hours experiment).

In this context, we study a stochastic version of a well-known nucleation model in physics, namely the Becker-Döring model [1]. In this model, aggregates may increase or decrease their size one-by-one, by capturing or shedding a single monomer particle. We will present numerical and analytical investigation of the nucleation time defined as a first passage time problem [2, 3].

Finally, we will present limit theorem techniques to study the link from the discrete size Becker-Döring model to a continuous size version (the Lifshitz-Slyozov model), which may be of importance to study large size aggregates formation. For general coefficients and initial data, we introduce a scaling parameter and show that the empirical measure associated to the Becker-Döring system converges in some sense to the Lifshitz-Slyozov equation when the scaling parameter goes to 0. When the aggregation is favorable, we derive a mean-field transport PDE limit together with an entrant boundary condition, leading to an effective reduced dynamical model [4]. When the aggregation is initially unfavorable, we shed light on metastable behavior and phase transition phenomena.

[1] E. Hingant, R. Y., arXiv:1609.00697 (2016).
[2] R. Y., M. R. D'Orsogna, and T. Chou. J. Chem. Phys., 137:244107, (2012).
[3] R. Y., S. Bernard, E. Hingant, L. Pujo-Menjouet, J. Chem. Phys., 144(3):034106, (2016).
[4] Julien Deschamps, Erwan Hingant, R.Y., arXiv:1605.08984 (2016).