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PROTEIN PHASE TRANSITIONS IN AGEING AND AGE-RELATED DISEASES: FROM ATOMIC RESOLUTION TO CELLULAR SOLUTIONS

LES CHANGEMENTS DE PHASE DES PROTEINES AU COURS DU VIEILLISSEMENT ET DES MALADIES ASSOCIEES AU VIEILLISSEMENT : DE LA STRUCTURE ATOMIQUE AUX SOLUTIONS CELLULAIRES

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Rapport sur la Conférence

Conference Report

General description of the conference

The Jacques Monod conference *« protein phase transitions in ageing and age-related diseases: from atomic resolution to cellular solutions »* took place in Roscoff from October 17-October 21, 2022. The president was Prof. **Ellen NOLLEN** (Groningen, The Netherlands) and the vice-president was **Ronald MELKI** (Fontenay-aux-Roses Cedex, France). The number of participants was 61 in total, which included:

- 22 invited speakers (21 with tenure positions), who were selected based on their track records and recent discoveries in the areas of protein quality control, protein misfolding and protein phase transitions in relation to ageing and age-related diseases. Of the speakers, 45% were female.
- 39 applicants were selected based on abstracts and CV (10 PI, 11 postdocs and 18 PhD students).

The meeting participants were from 13 different countries and represented scientists of different career stages as listed in the table below.

Country	Invited Speakers	Scientists (application)	Students (application)
Belgium		2	
Denmark		1	2
France	7	3	3
Germany	3	5	1
India			2
Israel	1	1	1
Italy	1	1	
Lithuania			2
Netherlands	2	2	5
Spain		1	
Switzerland	1	2	1
UK	2	1	1
USA	5	2	
Total	22	21	18

The meeting involved 11 sessions of oral presentation. After an opening reception and dinner, the meeting started with an evening session on ***protein phase transitions in aging and age-related diseases*** with an introduction by the meeting chairs followed by a in person keynote lectures by Ulrich HARTL and virtual keynote lecture by Cynthia KENYON (60 minutes each). All other sessions involved a mix of invited speaker (25 minutes + 5 minutes discussion) and selected short talks (12 minutes + 3 minutes of discussion). A total of 8 oral talks were selected based on the subject, quality, scientific advance, CV, and fit with the meeting sessions. Of the speakers, 50 % was female.

The meeting included two poster sessions of in total 31 posters and each session half of the posters was presented. Sessions IV and VIII included poster flash talks of in total 17 poster presenters that were selected based on abstract and CV to present a 3 minute poster pitch. 50% of the poster flash talks was given by female participants.

Mrs Nathalie BABIC organized all practical aspects of the meeting. Her continuous support has been instrumental to the success of the meeting. The technical support and the logistics offered by the Biological Station in Roscoff were highly appreciated, as well as the accommodations in several hotels in Roscoff and the quality and variety of food served at the Gulf Stream Hotel. CNRS and the registration fees provided the main funding for the conference. Furthermore, the organizers received a substantial financial support from Neuratris, Lundbeck, Hevolution, Teva and EMBO.

At the end of the meeting, Ronald MELKI (Fontenay-Aux-Roses, France) and Serena CARRA (Modena, Italy) were nominated as chairman and vice-chairman for the next conference application.

Report on scientific aspects

The evening opening session was chaired by Ronald **MELKI** (Fontenay-Aux-Roses, France) and Ellen **NOLLEN** (Groningen, The Netherlands), the organizers of the meeting. The first keynote lecture was from Ulrich **HARTL** (Munich, Germany) who introduced the field and addressed questions pertaining to molecular causes of selective vulnerability of certain cells to aggregation-prone proteins. He hypothesized a role for prion states, which was based on evidence from polyQ-rich protein aggregation studies in yeast. Hartl's talk was followed by a keynote lecture from Cynthis **KENYON** (Online, San Francisco, USA) who presented insights from research in C. elegans on the mechanistic link between aging and age-related proteinopathies. She showed physiological roles of protein aggregation, for example as an egg-maturation signal after fertilization. Such physiological mechanisms of aggregation may help to elucidate biological causes for disease-associated phenomena.

Session "Protein quality control (Part I)", the next day, was chaired by Monica **DRISCOLL** (New Jersey, USA and Zoher **GUEROUI** (Paris, France). Bernd **BUKAU** (Heidelberg, Germany) talked about Hsp70/Hsp40 chaperone mediated disaggregation of

fibrillar alpha-synuclein, providing evidence for different susceptibilities of different amyloid polymorphs. Sander **TANS** (Amsterdam, The Netherlands) followed by presenting single molecule approaches to determine mechanisms of ClpB disaggregation and proteasome substrate prioritization, revealing molecular strategies and rate limiting steps. Andreas **MARTIN** (Berkeley, USA) continued by discussing substrate processing by the proteasome and the Cdc48/p97/VCP protein unfoldase. He presented quantitative and "metric" insights on how CdC48/p97/VCP prepares substrates for proteasomal degradation. Jessica **TITTELMEIER** (Heidelberg Germany) addressed the toxic consequences of alpha-synuclein disaggregation mechanisms. She provided evidence in C. elegans that disaggregation by Hsp110 enhanced toxicity and transmission of alpha-synuclein seeds, revealing protective aspects of aggregation and highlighting that additional mechanisms are required for resolving toxicity.

Session "Protein quality control (Part II) was led by Olga **CORTI** (Paris, France) and Ulrich **HARTL** (Munich, Germany). Reut **SHALGI** (Technion, Israel) started the session with a talk on differences in chaperone-mediated regulation of physiological and pathological condensation. She brought evidence for specificity and adaptation of chaperone isoforms to different aggregate types. Next, Mark **HIPP** (Groningen, The Netherlands), presented work on Cdc48/p97/VCP and its role in ubiquitylated Tau fibril disaggregation and clearance. He showed, among others, that locking disaggregation by Cdc48/p97/VCP and its co-factors Ufd1, NPLOC4 & NSFL1C blocked the production of seeding-competent tau species, suggesting a role for this machinery in pathology spread in Tauopathies. Ambre **SALA** (Evanston, USA), explored protein quality control from an organismal level, showing that reproductive and metabolic cues, in particular, lipid metabolism, were important for proteostasis and resistance to stress.

Janine **KIRSTEIN** (Bremen, Germany) and Terence **STRICK** (Paris, France) chaired the session "Aging in proteinopathies". Luc **DUPUIS** (Strasbourg, France) started with a virtual presentation on FUS functions in different cells. He showed that FUS conditional truncation in adult mice is sufficient to trigger diverse ALS-related abnormalities ranging from abnormal electric activity, gene expression to behavioral changes. David **RUBINSZTEIN** (Cambridge, United Kingdom), continued to discuss functions of CdC48/p97/VCP together with beclin in proteinopathies highlighting a dual role in promoting autophagy and proteasomal regulation. Ellen **NOLLEN** (Groningen, The Netherlands), talked about a phenomics screen in C. elegans to identify systemic mechanisms of behavioral consequences of proteotoxicity, revealing reversible circuit imbalances and maladaptation as a driving force. The session ended with selected poster pitches by Nadeen **AKAREE**, Vishnu **BALAJI**, Tessa **BERGSMA**, Marta **CECCON**, Lale **GÜNGÖRDÜ**, Carmen Noemi **HERNANDEZ CANDIA**, Nikolaos **LOUROS**, Shemin **MANSURI** and Daniel **SEGAL**, to highlight part of the posters in the poster session that followed.

The third day started with a session on "Phase separation and protein aggregation" chaired by the junior scientist Sofia **LÖVESTAM** (Cambridge, UK) and Daniel F. **JAROSZ** (Stanford, USA).

Magdalena **POLYMENIDOU** (Zurich, Switzerland) started with an EMBO Young Investigator lecture on pathophysiological TDP-43 transitions and novel RNA targets with key roles in

human disease, addressing the molecular mechanisms that trigger TDP-43 aggregation. Using a set of TDP-43 mutants and variants mimicking post-translational modifications, she provided evidence that monomers condensate in the cytosol and the nucleus and that RNA binding is key, because without RNA, condensates start aggregating. After comparing protein condensation to clouds formation, Zoher **GUEROUI** (Paris, France) showed results on protein condensation in cells with fluorescently tagged model proteins. He showed that alpha-synuclein condensates fuel aggregation into amyloid fibrils. Serena **CARRA** (Modena, Italy) presented data showing that RNA binding proteins go from the nucleus to the cytosol under stress conditions and accumulate in stress granules. After stress, they move back to the nucleus. She further showed that stress triggers polysome disassembly with truncated protein products or DRIPS release, which then accumulate in stress granules. Her data suggested that the ALS/FTD-associated RNA-binding protein FUS is involved in resolving the stress granules, because when mutated, DRIPS are less well resolved. Martin **LENZ** (Paris, France) took a lively in-silico modeling approach to reveal the organizing principles of protein aggregation, showing that amyloid formation results from geometrical frustration, thus, providing new explanations for amyloid formation in cells and organisms.

Session "Amyloid strains" was moderated by Serena **CARRA** (Modena, Italy) and Martin **LENZ** (Paris, France). Sofia **LÖVESTAM** (Cambridge, United Kingdom) reported on a high-throughput Cryo-EM structure-determination approach to visualize amyloid polymorphs of the protein tau purified from patients developing different tauopathies brains and compared them to full-length and truncated tau fibrillar polymorphism made de novo. Ronald **MELKI** (Fontenay-Aux-Roses, France) addressed how amyloid polymorphs can induce cell- and disease specific pathology. He provided evidence for differential binding of alpha-synuclein neuronal cell population and proposed a structural-molecular basis for different synucleinopathies. Tetiana **SERDIUK** (Zurich, Switzerland) presented structural proteomics data on disease-specific α-synuclein strains. Her experimental approach not only reveals that polymorphisms can be distinguished by proteomic barcodes but also by distinct interactomes. Salvador **VENTURA** (Barcelona, Spain) ended the session showing Cryo-EM structures of functional amyloids of the RNA-binding ribonucleoprotein hnRNPDL, a missense mutation associated to limb-girdle muscular dystrophy-3. Alternative splicing leads to lack of the exon encoding the amyloid core, suggesting a mechanism that regulates functional diversity. The results suggest that the disease-causing missense mutation impair hnRNPDL's function by affecting its fibrillation properties.

After a lively and dynamic tour of the island Batz, the meeting resumed with the session "Phase separation and protein aggregation", chaired by Sandrine **HUMBERT** (Grenoble France) and Andreas **MARTIN** (Berkeley, USA), Frederic **ROUSSEAU** (Leuven, Belgium), talked about heterotypic amyloid interactions and their effect on amyloid assembly, Daniel F. **JAROSZ** (Stanford, USA) reported on quantitatively mapping proteome-wide protein condensate self-assembly and self-templating in youth, age, and disease, using human and killifish proteomes. These features appeared wide-spread and while beneficial in early development and stress conditions, may contribute to aging and disease.

Next day's first session on "cellular and organismal regulation", was chaired by Magdalena **POLYMENIDOU** (Zürich, Switzerland) and David **RUBINSZTEIN** (Cambridge, UK). Olga **CORTI** (Paris, France) opened the session by sharing results on mitochondrial quality control in Parkinson's disease. Using the pH-sensitive mitochondria-targeted fluorescent protein Mito-Rosella, her group visualized mitophagy and showed that this process was impaired in Parkinson-disease relevant mutant cells. Sandrine **HUMBERT** (Grenoble, France) followed by addressing the effects of the aggregation-prone mutant huntingtin early in life, revealing an early influence on brain development. Her data suggest that developmental consequences of mutant huntingtin may explain part of the age-related disease manifestations later in life, pointing at a currently often overlooked aspect of proteinopathies. Janine **KIRSTEIN** (Bremen, Germany) presented on mutant huntingtin aggregation suppression and disaggregation from a structural perspective, showing the domain requirements of the co-chaperone DNAJB1 to mediate these processes. Gea **CEREGHETTI** (Zürich, Switzerland) ended the session showing that amyloid formation and disassembly is used by the pyruvate kinase enzyme in yeast and human to respond to changing metabolic states. Understanding this physiological reversible aggregation process may allow a better comprehension of pathological protein aggregation.

In the Session "Vectors and modifiers", chaired by Reut **SHALGI** (Technion, Israel) and Sander **TANS** (Amsterdam, The Netherlands), Terence **STRICK** (Paris, France), talked about single molecule approaches to study tau fibril formation, Monica **DRISCOLL** (New Jersey, USA) introduced exophers, an exocytosic process that cells use to release trash, including aggregated proteins, from cells and provided support for evolutionary conserved mechanisms using neurons in C. elegans as a model system.

The penultimate session of oral presentations on 'Cellular and organismal regulation', was chaired by Ellen **NOLLEN** (Groningen, The Netherlands) and Bernd **BUKAU** (Heidelberg, Germany). The session took off with selected poster pitches by Rasmus Krogh **NORRILD**, Leonard **PIROSKA**, Virginie **REDEKER**, Elena **TANTARDINI**, Brent **VISSER**, Willianne **VONK**, Sina **WITTMANN**, and **YAN** Xiao. Then Ina **VORBERG** (Bonn, Germany), talked about the link between retrovirus derepression and prion-like spreading of aggregated seeds. Tim **BARTELS** (London, United Kingdom) focused his talk on data revealing the phase transition of alpha-synuclein and involvement of lipids in this process. He showed that in Lewy bodies, very little alpha-synuclein is insoluble. He provided evidence from correlative light and EM microscopy that vesicle clusters plus lipid droplets constitute Lewy bodies. This session was followed by a poster session.

The final session of the meeting on 'Pharmacological regulation and conclusions' was chaired by Ellen **NOLLEN** (Groningen, The Netherlands)/ Ronald **MELKI (**Fontenay-Aux-Roses, France), was an online lecture by Jeffery **KELLY** (La Jolla, USA), who took the audience along a journey of structural discoveries on Transthyretin aggregation in systemic amyloidosis and the use of molecular processes at action he discovered for the development of therapeutics interventions.

Conclusion and discussion

Postponing the meeting rather than having an online event in times of Covid turned out to be a wise decision. The relatively small group of attendants from a broad range of scientific backgrounds related to the topic of the meeting allowed for intense, animated, and stimulating discussions. Several new concepts emerged during the meeting that could be important for further advancing the field, which included:

- While pathological aggregates can be disassociated by molecular chaperones, the products are not necessarily beneficial, and may even lead to further progression of pathology.
- Based of structural modeling approaches, amyloid formation is likely the only option aggregation-prone proteins have when the conditions become unfavorable for their proper folding or disposal.
- Amyloids of the same aggregation-prone protein do not always look alike. Amyloid polymorphs are associated with specific disease, their severity, and cell specificity of pathology. Once formed under polymorph specific conditions, their characteristics propagate faithfully.
- Protein condensation is a common stage preceding aggregation of several disease-related aggregation-prone proteins, including alpha-synuclein and TDP-43. These processes appear to be driven by cellular conditions, prion states, RNA, lipids and stress granule formation. Aggregation associated with these condensates appears to be linked to a reduction in their reversibility, for example in case of disease-associated mutant amyloidgenic proteins.
- The propagation of amyloid pathology is also influenced by retroviral activation, suggesting that interference and synergy between different pathological conditions can aggravate proteinopathies.
- An alternative disposal strategy of exopher formation could rescue cells from irreversible pathology, but whether this will improve organismal health remains to be established.
- Beneficial aspects of amyloid formation, for example during fertilization and responses to stress, reveal cellular mechanisms of reversibility, which may help elucidating and modulating pathological aggregation.
- Continued development and use of protein structure analysis tools, such as single molecular approaches or high throughput Cryo-EM studies, and cell and animal models, will be essential to further elucidate the properties and modifying factors that distinguish physiological from pathological condensation, and their progression from liquid-like to solid states, of aggregation-prone proteins.
- The development from structure to therapy for transthyretin exemplifies the integration of the structural, cellular and animal modeling approaches as presented and united in

this meeting, and shows that such approaches may offer solutions for other proteinopathies as well.

Overall, we feel that the meeting was a success and has accomplished its goals by bringing together an interdisciplinary group of scientists that actively engaged in discussions on new concepts and directions in the field. We would recommend keeping future meetings small, because is key to enhance connections and exchanges, facilitate the access of young scientists to input from leaders in the field, and a highly opened, interactive and sharing atmosphere during all sessions.

La Conférence Jacques Monod « Les changements de phase des proteins au cours du vieillissement et des maladies associées au vieillissement: de la structure atomique aux solution cellulaires » s'est tenue à Roscoff du 17 au 21 Octobre 2022. La présidente était la Professeure Ellen NOLLEN (Groningen, Pays-Bas) et le Vice-Président le Docteur Ronald Melki (Fontenay-aux-Roses, France). Les participants étaient au nombre de 61. 22 conférenciers (dont 21 titulaires et 45% de femmes) étaient invités, sélectionnés sur la base de leurs travaux et de leurs découvertes récentes dans les domaines du contrôle de la qualité des protéines, du mauvais repliement des protéines et la transition de phase des protéines en relation avec le vieillissement et les maladies liées à l'âge. 39 participants ont été sélectionnés à travers les résumés et les CV qu'ils ont soumis (10 participants étaient des chercheurs sénior, 11 étaient des postdoctorants et 18 étaient des doctorants). Les participants à la conférence venaient de 13 pays différents et représentaient des scientifiques à différents stades de leurs carrières.

La conférence a comporté 11 sessions de présentations orales. Après une réception d'ouverture et un dîner, la réunion a débuté par une séance en soirée sur les transitions de phase protéiques dans le vieillissement et les maladies liées à l'âge avec une introduction par la Présidente et le vice-Président de la conférence suivie d'une conférence plénière en présentiel par Ulrich HARTL et d'une conférence virtuelle par Cynthia KENYON (60 minutes chacun). Toutes les autres sessions impliquaient des orateurs invités (25 minutes + 5 minutes de discussion) et de courts exposés sélectionnés sur résumé (12 minutes + 3 minutes de discussion). Au total, 8 communications orales ont été sélectionnées en fonction du sujet, de la qualité, de l'avancée scientifique, du CV et de l'adéquation avec les thématiques de la conférence. Les femmes ont représenté 50% des intervenants. Deux sessions de présentations par affiches totalisant 31 affiches ont de plus été organisées tout comme deux sessions de présentations éclair de 3 minutes ou 17 participants ont pu présenter leurs travaux. 59% des présentations éclair ont été assurés par des femmes.

Mme Nathalie BABIC a pris en charge tous les aspects pratiques de la réunion. Son soutien continu a joué un rôle déterminant dans le succès de la réunion. L'accompagnement technique et la logistique offerte par la Station Biologique de Roscoff ont été très appréciés, ainsi que l'hébergement dans plusieurs hôtels de Roscoff et la qualité et la variété de la restauration servie à l'Hôtel Gulf Stream. Le CNRS et les frais de participation ont représenté l'essentiel du financement de la conférence. En outre, les organisateurs ont reçu un soutien financier substantiel de Neuratris, Lundbeck, Hevolution, Teva et l'EMBO.

Plusieurs questions et concepts ont émergé lors des intenses discussions de chaque session, qui devraient être approfondis pour améliorer l'avancement de la recherche dans le domaine. Les plus remarquables sont ici résumées :

- Si les agrégats pathologiques peuvent être dissociés par des chaperons moléculaires, les produits ne sont pas nécessairement bénéfiques, et peuvent même conduire à une progression ultérieure de la pathologie.
- Sur la base d'approches de modélisation structurale, la formation d'amyloïde est probablement la seule option dont disposent les protéines sujettes à l'agrégation lorsque les conditions deviennent défavorables à leur repliement ou à leur élimination appropriée.
- Les amyloïdes d'une même protéine sujette à l'agrégation ne se ressemblent pas toujours. Des polymorphes sont associés à des maladie distinctes, à leur gravité et à la spécificité cellulaire de la pathologie. Une fois formés dans des conditions spécifiques, leurs caractéristiques se propagent fidèlement.
- La condensation des protéines est une étape courante précédant l'agrégation de plusieurs protéines, notamment l'alpha-synucléine et le TDP-43. Ces processus semblent être initiés par les conditions cellulaires, les propriétés intrinsèques des protéines, l'ARN, les lipides et la formation de granules de stress. L'agrégation associée à ces structures semble être liée à une réduction de leur réversibilité.
- La propagation de la pathologie amyloïde est également influencée par l'activation rétrovirale, ce qui suggère que les interférences et les synergies entre différentes pathologies peuvent aggraver les protéinopathies.
- Une stratégie alternative d'élimination de ces agrégats pourrait sauver les cellules d'une pathologie irréversible, mais il reste à établir si cela améliorera la santé de l'organisme.
- Les aspects bénéfiques de la formation d'amyloïde, par exemple lors de la fécondation et des réponses au stress, révèlent des mécanismes cellulaires de réversibilité, qui peuvent aider à élucider et à moduler l'agrégation pathologique.
- Le développement et l'utilisation d'outils d'analyse de la structure des protéines, tels que des approches reposant sur des molécules uniques ou des études Cryo-EM à haut débit, ainsi que des modèles cellulaires et animaux, seront essentiels pour comprendre davantage les propriétés et les facteurs qui distinguent la condensation physiologique de la condensation pathologique, ainsi que la progression de l'état liquide à solide des protéines sujettes à l'agrégation.
- La combinaison approches structurales, cellulaires et animales, telles que présentées lors de cette réunion et illustrées par la transthyretine démontrent de remarquables avancée dans la compréhension protéinopathies.

Dans l'ensemble, nous pensons que la conférence a été un succès et a atteint ses objectifs en réunissant un groupe interdisciplinaire de scientifiques qui se sont activement engagés dans des discussions sur de nouveaux concepts et orientations dans le domaine. Nous recommandons de limiter les conférences futures en taille, car c'est essentiel pour faciliter les connexions et les échanges, l'accès des jeunes scientifiques aux leaders dans le domaine et créer une atmosphère très ouverte, interactive et de partage pendant toutes les sessions.

Ronald MELKI (Fontenay-Aux-Roses, France) et Serena CARRA (Modène, Italie) ont été nommés président et vice-président pour porter une future conférence portant sur la même thématique à l'issue de la Conférence.