Program – Virtual UK Chaperone Club Meeting - December 18, 2020

Organizer: Patricija van Oosten-Hawle, University of Leeds

Meeting start is 14:00 UK (London)/ 9:00 EST/ 15:00 CET/ 16:00 Israel

14:05 Welcome and Opening Remarks

Session 1 - Hsp70 & co-chaperone function and biology

Session Chair: **Patricija van Oosten-Hawle (University of Leeds, UK)**

Keynote Talk

14:10 – 14:40 **Andrew Truman (University of North Carolina, Charlotte, USA)**
Understanding PTM-driven Hsp70 interactions using crosslinking mass spectrometry

14:40 – 15:05 **David Balchin (The Francis Crick Institute, UK)**
Catalysis of protein folding by Hsp70

15:05 – 15:17 **Kinneret Rozales (Technion, Haifa, Israel)**
Differential roles for DNAJ isoforms in polyQ and mutant FUS aggregation modulation revealed by chaperone network screens

15:17 – 15:32 **Theo Karamanos (University of Leeds, UK)**
Intricate structural equilibria determine DNAJB6b function: insights from solution NMR.

15:32 – 15:44 **Eliana Nachman (University of Heidelberg, ZMBH, Germany)**
Disassembly of Tau fibrils by the human Hsp70 disaggregation machinery generates small seeding-competent species

15:45 – 16:00 **Break**

Session 2 – Chaperone Structure, Function & Assembly

Session Chair: **Jacqueline van der Spuy (UCL, UK)**

16:00 – 16:25 **Rahul Samant (Babraham Institute, UK)**
Proteomic approaches to uncover new HSP90 biology

16:25 – 16:37 **Mohinder Pal (University of Sussex, UK)**
Cryo-EM structure of R2TP-TTT complex, a chaperone for mTOR assembly

16:37 – 16:49 **Josefine Vallin (University of Gothenburg, Sweden)**
Sequestration of the transcription factor STAT3 by the molecular chaperone CCT: a mechanism for modulation of STAT3 phosphorylation

16:49 – 17:01 **Bert Houben (KU Leuven, Belgium)**
Autonomous aggregation suppression by acidic residues explains why chaperones favour basic residues

17:01 – 17:13 **Dominik Saman (University of Oxford, UK)**
Quantitative description of co-assembly between human HSPB1 and HSPB5 and its evolutionary consequences

17:15 **Closing Remarks & End of Meeting**

Abstracts

Short Talks

Differential roles for DNAJ isoforms in polyQ and mutant FUS aggregation modulation revealed by chaperone network screens

Kinneret Rozales[#], Amal Younis[#]and Reut Shalgi[*]

Neurodegenerative diseases, a class of fatal diseases affecting the brain and nervous system, are often caused by mutant proteins that misfold in the cell, leading to the accumulation of protein aggregates. In order to handle misfolding and aggregation, cells have evolved a network of molecular chaperones, comprised of different families. Hsp70 is a hub in the chaperone network, which includes over 70 different co-chaperones (aka as Hsp40s/DNAJs). While several studies highlighted important roles for individual chaperones in modulation of aggregate formation (Hageman et al ,2010) or disaggregation (Nillegoda et al, 2015), the roles of each chaperone in regulation of aggregation has not been systematically examined, and the potential function of many of them in aggregation modulation is still largely unknown.

Here we decided to explore chaperones functional diversity, through the lens of pathological aggregation, by characterization and quantification of their effects on aggregation properties using the FACS-based PulSA method (Ramadzan et al. 2012). We performed a chaperone screen for modulators of two neurodegenerative disease related aggregating proteins, the Huntington's disease-related HTT-polyQ, and ALS-related mutant FUS (mutFUS).

Surprisingly, modulators of mutFUS were completely different from those of HTT-polyQ. Interestingly, different naturally occurring isoforms of DNAJ chaperones had opposing effects on HTT-polyQ vs. mutFUS aggregation. We identified a complex of the full-length (FL) DNAJB14 and DNAJB12 isoforms, which substantially alleviated mutFUS aggregation, in an HSP70-dependent manner. Their naturally occurring short isoforms were unable to form the complex, nor to interact with HSP70, and lost their ability to reduce mutFUS aggregation. In contrast, the short isoform of DNAJB12 significantly alleviated HTT-polyQ aggregation, while DNAJB12-FL aggravated HTT-polyQ aggregation. Finally, we demonstrated that full-length DNAJB14 ameliorated mutFUS aggregation compared to DNAJB14-short in primary neurons. Together, our data unraveled distinct molecular properties required for aggregation protection in different neurodegenerative diseases, and revealed a new layer of complexity of the chaperone network elicited by naturally occurring J-protein isoforms, highlighting functional diversity among the DNAJ family.

Disassembly of Tau fibrils by the human Hsp70 disaggregation machinery generates small seeding-competent species

Eliana Nachman[1], Anne S Wentink[1], Karine Madiona[2], Luc Bousset[2], Taxiarchis Katsinelos[3], Kieren Allinson[4], Harm Kampinga[5], William A. McEwan[3], Thomas R. Jahn[6], Ronald Melki[2], Axel Mogk[7], Bernd Bukau[8] and Carmen Nussbaum-Krammer[9]*

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The accumulation of amyloid Tau aggregates is implicated in Alzheimer's disease (AD) and other tauopathies. Molecular chaperones are known to maintain protein homeostasis. Here we show that an ATP-dependent human chaperone system disassembles Tau fibrils *in vitro*. We found that this function is mediated by the core chaperone HSC70, assisted by specific co-chaperones, in particular class B J-domain proteins and a heat shock protein 110 (Hsp110)-type nucleotide exchange factor (NEF). The Hsp70 disaggregation machinery processed recombinant fibrils assembled from all six Tau isoforms as well as sarkosyl-resistant Tau aggregates extracted from cell cultures and human AD brain tissues, demonstrating the ability of the Hsp70 machinery to recognize a broad range of Tau aggregates. However, the chaperone activity released monomeric and small oligomeric Tau species, which induced the aggregation of self-propagating Tau conformers in a Tau cell culture model. We conclude that the activity of the Hsp70 disaggregation machinery is a double-sided sword, as it eliminates Tau amyloids at the cost of generating new seeds.

Title: Cryo-EM structure of R2TP-TTT complex, a chaperone for mTOR assembly

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Abstract

HSP90 and the R2TP (Rvb1p-Rvb2p-Tah1p-Pih1p in yeast and RUVBL1-RUVBL2-RPAP3-PIH1D1 in humans) complex are known to be involved in the assembly and maturation of several client proteins. These include RNA polymerase II, axonemal dyneins and the mTOR and SMG1 kinases of the PIKKs family. Despite recent advances in the structural characterisation of R2TP and its connection with HSP90, it is still unknown how mTOR is recruited and activated by them.

Our current data suggest that R2TP needs the adaptor protein complex, TELO2-TTi1-TTi2 (TTT) to recruit mTOR to HSP90. In this work, we determined the first cryo-EM structure of R2TP-TTT complex at the maximum resolution of 3.4Å resolution, which shows the previously unknown structure of the TTT complex. Our structural analysis and complementary biochemistry show that TTT directly interacts with both RUVBL1-RUVBL2 and mTOR. In summary, this work provides the mechanistic insights of how mTOR is tethered to HSP90 by the R2TP-TTT complex.

Sequestration of the transcription factor STAT3 by the molecular chaperone CCT: a mechanism for modulation of STAT3 phosphorylation

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Chaperonin Containing Tailless complex polypeptide 1 (CCT or TRiC) is a molecular chaperone composed of eight individual subunits, all essential in yeast. The subunits are stacked back-to-back, forming two rings with a central folding cavity. The main folding substrates for CCT are the cytoskeletal proteins actin and tubulin. In addition, CCT has functions beyond folding, for example, CCT is involved in the assembly between the von Hippel Lindau protein and elongins. CCT can also act as a sequestering protein for the actin capping and severing protein gelsolin. CCT oligomer is very dynamic and it is now clear that individual CCT subunits can have functions, when monomeric, related to assembled cytoskeletal structures. Here we show that oligomeric CCT binds to the transcription factor STAT3 where the kinetics of binding between CCT and STAT3 differ from a CCT folding substrate. Instead of early binding to and release from CCT, small amounts of full length STAT3 accumulates on CCT in an in vitro translation/transcription assay. Upon IL-6 stimulation, STAT3 is phosphorylated, dimerises and translocates to the nucleus where it transcribes its target genes, many of them directly related to cancer. When any of the eight CCT subunits are reduced by siRNA there is an increase in tyrosine phosphorylation levels of STAT3. Reduction of CCT levels does not affect dimerization, nuclear translocation or the transcriptional activity of STAT3. This is consistent with STAT3 not being folded by CCT but instead that CCT can modulate STAT3 phosphorylation levels. We describe a model where non-phosphorylated STAT3 can be sequestered by CCT, potentially regulating STAT3 phosphorylation regulation.

Autonomous aggregation suppression by acidic residues explains why chaperones favour basic residues

Bert Houben[1,2,] Emiel Michiels[1,2,] Meine Ramakers[1,2], Katerina Konstantoulea[1,2,] Nikolaos Louros[1,2], Joffré Verniers[1,2,] Rob der Kant[1,2], Matthias De Vleeschouwer[1,2], Nuno Chicória[1,2], Thomas Vanpoucke[1,2], Rodrigo Gallardo[1,2], Joost Schymkowitz [*,1,2] and Frederic Rousseau [*,1,2]

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Many chaperones favour binding to hydrophobic sequences that are flanked by basic residues while disfavouring acidic residues. However, the origin of this bias in protein quality control remains poorly understood. Here, we show that while acidic residues are the most efficient aggregation inhibitors, they are also less compatible with globular protein structure than basic amino acids. As a result, while acidic residues allow for chaperone-independent control of aggregation, their use is structurally limited. Conversely, we find that, while being more compatible with globular structure, basic residues are not sufficient to autonomously suppress protein aggregation. Using Hsp70, we show that chaperones with a bias towards basic residues are structurally adapted to prioritize aggregating sequences whose structural context forced the use of the less effective basic residues. The hypothesis that emerges from our analysis is that the bias of many chaperones for basic residues results from fundamental thermodynamic and kinetic constraints of globular structure. This also suggests the co-evolution of basic residues and chaperones allowed for an expansion of structural variety in the protein universe.

Quantitative description of co-assembly between human HSPB1 and HSPB5 and its evolutionary consequences

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The small heat shock proteins (sHSPs) are present in all kingdoms of life and play an important role in cellular protein quality. sHSPs typically assemble into large oligomers, with many sHSPs also known to co-assemble in vivo. HSPB1 and HSPB5, also known as HSP27 and alphaB-crystallin, are expressed throughout the human body. They are both polydisperse, and are known to co-assemble, leading to a level of heterogeneity that has proved insurmountable to detailed characterisation.

Here, we present a novel integration of native tandem mass spectrometry (MS/MS) and mass photometry (MP) as complementary methods for elucidating the distribution of extremely polydisperse, heterogeneous systems, tying them together using chemical kinetics.

In the native MS/MS experiment, a systematic scan of subpopulations can be used to determine the microheterogeneity of the oligomers. To obtain a full mass envelope of the oligomers, we used MP, which enables mass determination of proteins in solution and is not beholden to the mass limitations of the MS/MS experiment.

We then use chemical kinetics modelling to tie both MS/MS and MP measurements together to obtain the full, quantitatively estimate of the abundance hundreds of different sub-stoichiometries in the HSPB1:HSPB5 mixture. We were further able to extract the energy difference between homo- and hetero-dimerization of the two proteins and found the energy difference to be very small (4.0 +- 3.6 kJ/mol of interactions). This is remarkable since protein paralog tend to self-assemble, rather than co-assemble, to acquire distinct function. Yet, HSPB1 and HSPB5 seemingly co-assemble without preference even after 400 million years from their gene duplication.

These recent developments allow us to quantitatively describe the co-assembly in highly heterogenous, polydisperse systems, and will, ultimately, allow to answer one of the important questions in the field: Is the co-assembly between various sHSPs functionally relevant? And perhaps an even more fundamental question: How does neutral evolution affect high-entropy states in proteins?

In summary, our new, integrative approach allows for a complete description of a highly polydisperse and heterogeneous system consisting of hundreds of different oligomers, thereby enabling unprecedentedly detailed biophysical insights into protein assembly, with further possible implications in the field of protein evolution.