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The multiple facets of the Hsp90 machine

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The Ninth International Conference on the Hsp90 Chaperone Machine concluded in October 2018, in Leysin, Switzerland. The program highlighted findings in various areas, including integrated insight into molecular mechanism of Hsp90, cochaperones, and clients' structure and function.

Heat shock protein-90 (Hsp90) is a molecular chaperone critical for the folding, stability, and activity of client proteins [1]. Hsp90 and its orthologs, including bacterial HtpG, mitochondrial TRAP1 and endoplasmic reticulum Grp94, exist as dimers, hydrolyze ATP, and cycle between distinct conformational states. Hsp90 preferentially binds proteins in near native states facilitating their remodeling for protein interactions and signaling. At the 9th International Conference on the Hsp90 Chaperone Machine approximately one-third of the attendees shared their data on Hsp90 structure and function through short talks (Figure 1). Here, we distill and summarize their findings.

Keynote Speaker

To open the meeting, the keynote speaker Paola Picotti (Institute of Molecular Systems Biology, Zurich, Switzerland), presented a recently developed mass spectrometry method that enables analysis of protein structural changes on a proteome-wide scale in complex biological extracts. The approach can detect subtle alterations in secondary structure content, larger scale movements such as domain motions, and more pronounced transitions between folding states and aggregation events [2]. Hsp90 chaperone machinery appears to be a good candidate for further analysis by this method.

Hsp90 Dynamics

Carefully orchestrated conformational changes in Hsp90 are essential for its association with cochaperones and client proteins. David Agard (University of California, San Francisco, California, USA) provided the first atomic details for the Hsp90/Hsp70/HOP/glucocorticoid receptor (GR) client-loading complex. Unexpectedly, this heterocomplex contains two Hsp70s both in the ADP-bound state, one of which appears to be involved in the delivery of GR, while the other supports the cochaperone HOP. GR is largely intact, but like in the Hsp90:Cdc37:kinase complex [3], part of GR is unfolded, and threaded through the lumen of a partially closed Hsp90 dimer, where it interacts with a newly revealed client binding site on HOP. Together this suggests a common mechanism for Hsp90:client recognition.

Stefan Rüdiger (Utrecht University, the Netherlands) revealed that Hsp70 binds clients through highly hydrophobic regions that provide protection from misfolding. Subsequently, Hsp90 breaks this interaction and allows clients to self-fold into a native state. Cochaperones are not necessary for folding, but mainly work to slow this process. Thorsten Hugel (University of Freiburg, Germany) described cooperation between nucleotide and the two amino-terminal ATP-binding pockets in an Hsp90 dimer using multicolor, single-molecule Förster resonance energy transfer (FRET). This novel technique adds an additional dimension that allowed for the discovery that ATP and Aha1 independently, but synergistically, promote closing of the nucleotide pocket, but work antagonistically to affect subsequent reopening [4]. Katarzyna Tych from Matthias Rief's Lab (Technische Universität München (TUM), Munich, Germany) described the dynamics of Hsp90 carboxy-terminal dimerization using single molecule optical tweezers. Interestingly, Hsp90 carboxy-terminal association has three dissociation rates that are controlled by the presence of ATP, which stabilizes this interaction by eliminating the weakest

interaction state.

Vinay Dahiya from Johannes Buchner' group (TUM, Munich, Germany) described the chaperoning mechanism of Hsp70 and Hsp90 for "the guardian of the genome," p53. Hsp70 together with Hsp40 unfolds and inactivates p53. The Hsp70 NEF, Bag1 supports the release of p53 from Hsp70 and, in coordination with HOP, Hsp90 and ATP promotes the folding of p53 and restores its DNA binding activity. Shannon Doyle from Sue Wickner's Lab (NCI, NIH, Bethesda, Maryland, USA) showed that Hsp90 and Hsp70 directly interact in both *Escherichia coli* (*E. coli*) and yeast. The region of interaction on Hsp90 involves residues in the middle-domain that also interact with several cochaperones and clients, while the interaction region on Hsp70 utilizes residues in the J-protein binding region of Hsp70 [5].

Hsp90 Phosphorylation and Regulation

Hsp90 and its cochaperones are subject to post-translational modifications (PTMs) including phosphorylation. Ioannis Gelis (University of South Florida, Tampa, Florida, USA) demonstrated that during the chaperone cycle, Hsp90 phosphorylation occurs in a cochaperone regulated manner. This is exemplified by the Hsp90/Cdc37 heterocomplex, in which the phosphorylation of Cdc37 primes Hsp90 for phosphorylation of tyrosine 197, and controls disassembly of the client recruitment complex. Thus, Hsp90 cochaperone complexes are highly dynamic and highly regulated at many steps throughout the chaperone cycle [6].

Matthias Mayer (Universität Heidelberg, Germany) revisited previous works on PTMs of Hsp90 and how they fine tune Hsp90 function. His data based on yeast growth assays revealed that some phosphomimetic mutations of Hsp90 grow more efficiently than the wild-type Hsp90 and more potently activate steroid hormone receptors, apart from GR. This supports the idea that

PTMs can tune Hsp90 specificity for chaperoning specific clients, even those that evolved from the same gene.

Structure and function of Hsp90 Orthologs

Dan Gewirth (Hauptman-Woodward Institute, Buffalo, New York, USA) presented data on the structure of the Pre-amino (Pre-N) domain of Grp94, the endoplasmic reticulum (ER) Hsp90, along with functional data showing its role in client protein maturation. The Pre-N domain regulates the ATPase activity of the chaperone and appears to functionally substitute for cochaperones of Hsp90, which are missing in the ER [7].

Olivier Genest (CNRS, Aix Marseille University, France) showed that Hsp90 is essential under heat stress in the aquatic bacterium *Shewanella oneidensis* [8]. He found that TilS, a tRNA modifier, is a client of Hsp90 and that an interplay takes place between folding by Hsp90 and degradation by the protease HslUV to finely regulate the level of TilS.

Modulation of Hsp90 by Cochaperones

The chaperone function of Hsp90 is tightly regulated by cochaperones. Pierre Goloubinoff (University of Lausanne, Switzerland) proposed Hsp70 as the major cochaperone of Hsp90. Deletion studies in *E. coli* demonstrate that, unlike the deletion of Hsp70/Hsp40, which upregulated many chaperones and proteases while suppressing metabolic and respiratory enzymes, deletion of *E. coli* Hsp90 did not dramatically impact protein levels. This work also revealed that Hsp90 promotes the degradation of aggregation-prone clients of Hsp70/Hsp40 through the HsIUV protease.

Kaushik Bhattacharya from Didier Picard's group (University of Geneva, Geneva,

Switzerland) addressed the question of the importance of the HOP protein in eukaryotes [9]. He found that the complex Hsp70-HOP-Hsp90 physically interacted with the proteasome. Although in the absence of HOP the proteasome activity is reduced, he showed that proteostasis is maintained by a "super-chaperone" complex minimally consisting of Hsp90, Hsp70, a J-protein and a nucleotide exchange factor, resembling the chaperone complex found in prokaryotes. Michael Reidy from Dan Masison's group (NIDDK, NIH, Bethesda, Maryland, USA) reported that Sti1 (HOP) has two distinct functions for yeast Hsp90. The first function is to connect Hsp70 to Hsp90 by binding directly to both chaperones. The second function is to facilitate transfer of clients from Hsp70 to Hsp90 by priming Hsp90 for client loading.

The R2TP complex is a cochaperone of Hsp90 that includes RuvBL1 and RuvBL2, two AAA+ proteins involved in cancer progression [10]. Walid Houry (University of Toronto, Toronto, Canada) developed a screen to find inhibitors of the RuvBL2 protein. He successfully identified a compound that inhibits RuvBL2. Chris Prodromou (University of Sussex, Brighton, UK) described the 3D structure of the yeast and human R2TP complex that was recently solved using X-ray crystallography and single-particle EM [11]. These structures revealed the importance of the Hsp90 cochaperone complex as a hexamer-ring and that in yeast, binding of a single Tah1 coupled to Pih1 could promote Rvb1p-Rvb2p ATPase activity. Philippe Meyer (Sorbonne Université, CNRS, Paris, France) presented the crystal structure of the human RPAP3 in complex with PIH1D1, important cochaperones in the R2TP complex that regulate Hsp90 activity. This work revealed that the TPR2 domain in RPAP3 recruits Hsp90, which stimulates Hsp90 ATPase activity in synergy with Aha1. This stimulation activity is abolished if the amino-terminus of RPAP3 is deleted.

Markus Zweckstetter (DZNE, Göttingen, Germany) described the 3D structure of Hsp90

in complex with misfolded transthyretin, which was similar to the binding previously described for tau [12]. The impact of cochaperones on Hsp90 structure was also described using the example of FKBP51, which binds to all three domains of Hsp90 with varying affinities and results in reduced ATPase activity by stabilizing an open conformation of the amino-terminal domain. When bound to Hsp90, the catalytic site of FKBP51 remains accessible supporting the idea that Hsp90 likely acts as a scaffold to support the functional interaction of misfolded proteins with cochaperones. Mehdi Mollapour (SUNY Upstate Medical University, Syracuse, New York, USA) described the cooperative function of new cochaperones, Tsc1 and FNIP1/2. They work cooperatively to decelerate Hsp90 chaperone cycle and play a role in the chaperoning of both kinase and non-kinase clients [13]. He also showed how phosphorylation, SUMOylation and ubiquitination of the cochaperone Protein Phosphatase-5 (PP5) regulate its activation, substrate binding and degradation in cancer.

Moonlighting Functions of Hsp90

Several newly emerged moonlighting functions of Hsp90 at the nucleus and plasma membrane were revealed at this meeting. Brian Freeman (University of Illinois, Urbana, Illinois, USA), using an elegant single cell approach with a fluorescently marked DNA locus, demonstrated how Hsp90 is involved in chromosome motion within interphase cells. He showed that Hsp90 and p23 keep ARP-containing chromatin remodelers in a dynamic state allowing them to interact with target gene promoters, in turn directing actin polymer formation and chromosome motion.

Gergely Lukacs (McGill University, Montreal, Canada) showed that both eukaryotic (Hsc70 and Hsp90) and prokaryotic chaperone (DnaK) systems can reshape the conformational energetics of mutant CFTR channel final fold towards that of the wild-type at the single molecule level and in cells. This mechanism has implications in the regulation of metastable ABC-transporters and other membrane proteins. Dragana Vidovic, from Ineke Braakman's group (Utrecht University, The Netherlands) reported that Hsp90 is required for a crucial step in folding of CFTR. More specifically Hsp90 is involved in folding of the CFTR nucleotide-binding domain. Wild-type CFTR in the presence of compromised Hsp90 function is folded like the pathogenic mutant CFTR ΔF508. Michael Heider and Vanesa Fernandez-Saiz from Florian Bassermann's Lab (TUM, Munich, Germany) described Cereblon (CRBN), the target of immunomodulatory drugs (IMiDs), as a novel regulator of the Hsp90-Aha1 axis that mediates maturation of transmembrane proteins such as CFTR. They also showed that Hsp90-CRBN interaction was abrogated upon IMiD-treatment, thus explaining CRBN-dependent IMiD induced destabilization of its transmembrane clients.

Shiran Dror from Anat Ben-Zvi's group (Ben-Gurion University of the Negev, Beer Sheva, Israel) presented his work on the role of Hsp90 in myosin assembly in *C. elegans*. He

showed that knockout or overproduction of Hsp90 and some of its cochaperones resulted in loss of motility phenotypes due to myosin filament disorganization. Looking at chaperone localization patterns in the sarcomere, he proposed that Hsp90 machinery is important for regulating myosin translocation across the sarcomere allowing the formation of proper filaments of myosin. Yu-Chun Wang from Patrik Verstreken's lab (VIB, Leuven, Belgium), showed a previously unexpected role of Hsp90 in membrane remodeling. *In vitro* and *in vivo* evidence indicated that Hsp90 interacts with membranes via an amphipathic helix, deforms the membranes and allows exosome release. This activity can be inhibited by Hsp90 inhibitors [14].

Christine Queitsch (University of Washington, Seattle, Washington, USA) described that the effects of Hsp90 on the evolutionary rate of protein kinases is comparable to effects of gene expression and protein interactions. She showed that a single mutation can render a non-client transcription factor Hsp90-dependent and that Hsp90-dependent *de novo* mutations do not account for most Hsp90-dependent phenotypes.

Hsp90 in Maladies

Hsp90 is involved in various maladies including cancer, neurodegenerative diseases, and pathogenic infections. Laura Blair (University of South Florida, Tampa, Florida, USA) demonstrated that Aha1 increases tau aggregation. In contrast, cyclophilin 40 (CyP40) disrupts tau fibrils reducing neurotoxicity *in vivo* [15]. These findings provide strong rationale to develop therapeutics for tauopathies by targeting molecular chaperones. Aaron Voigt (RTWH Aachen University, Germany) demonstrated that mitochondrial Hsp90 TRAP1 is an important player in Parkinson's disease (PD) and his data suggest that enhancing TRAP1 abundance or activity in neurons might be an avenue for future PD therapies.

Oliver Krämer (Johannes Gutenberg University Mainz, Germany) presented his work on the involvement of Hsp90 and the histone deacetylase 6 (HDAC6) in acute myeloid leukemia (AML) cells. He showed that a combination of inhibitors directed towards HDAC6 and Hsp90 triggered apoptosis of AML cells, providing new promising ways to treat AML. Ramona Schulz-Heddergott (University Medical Center of Göttingen, Germany) described that stabilization of a cancer-relevant Hsp90 client p53-R248Q contributes to STAT3 hyper-activity. Vice versa, Hsp90 inhibition by 17AAG leads to degradation of the p53 mutant and thus, down-regulation of p-STAT3. This data suggests that the cancer-relevant Hsp90 client p53 mutants represent an actionable drug target for treatment with Hsp90 inhibitors.

Stephanie Diezmann (University of Bristol, Bristol, UK) described how Hsp90 and lack of the cochaperone Sti1 (HOP) enables phenotypic variation via loss-of-heterozygosity and aneuploidy in the ameiotic human fungal pathogen *Candida albicans.* She proposed the environmentally responsive chaperone Hsp90 as a novel mechanism for the creation of genetic diversity in *C. albicans*. Harriet Mok from Jason Mercer's group (University College London, UK) highlighted the importance of Hsp90 in the life cycle of a poxvirus family member, vaccinia virus (VACV). Using microscopy and virus-specific assays, she showed that multiple isoforms of Hsp90 are required during late stages of vaccinia infection including successful genome release into the host cytoplasm and replication.

Joachim Clos (Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany) discussed the translatome changes in *Leishmania donovani* following Hsp90 inhibition. This protozoan parasite showed increased synthesis of proteins that are involved in oxidative stress protection, proteolysis, chromatin assembly, and chaperoning following Hsp90 inhibition. These results underscore the importance of Hsp90 in the control of the parasite's life cycle.

Hsp90 in the Extracellular Environment

The extracellular molecular chaperone Hsp90 (eHsp90, released or surface-bound) is responsible for chaperoning clients outside the cell. Wei Li (University of Southern California, Los Angeles, California, USA) provided a remarkable overview of the roles of extracellular Hsp90α in repairing injured tissues and in supporting tumorigenesis. During wound healing, secreted Hsp90α interacts with the extracellular domain of a receptor (LRP-1) that is stabilized by Hsp90β inside the cell. Hsp90α protects cells from hypoxia-triggered apoptosis and promotes cell motility to close the wound. Surprisingly, the activity of Hsp90α is independent of its ATPase activity and can be fulfilled by a short region of Hsp90α of only about 115 amino acids, called fragment-5 [16]. These properties of secreted Hsp90α have been taken advantage of by tumor cells to gain invasion and metastasis, representing an alternative target for anti-tumor therapeutics. Natasha Boel from Adrienne Edkins' group (Rhodes University, Grahamstown, South Africa) investigated the role of Hsp90 in the dynamics of the fibronectin (FN) matrix. She showed that Hsp90 interacts with FN, and that inhibition of Hsp90 by novobiocin resulted in FN turnover via a LRP-1 receptor mediated response [17].

Patricija van Oosten-Hawle (University of Leeds, Leeds, UK) explained how a proteotoxic stress perceived in one tissue induces a response in other tissues leading to molecular chaperone activation via a pathway termed transcellular chaperone signaling (TCS). Using *C. elegans*, she showed that the zinc finger transcription factor PQM-1 is involved in mediating TCS [18]. Importantly, she found that activating Hsp90 by TCS reduced the formation of toxic amyloid beta aggregates.

Small molecule inhibitors of Hsp90

Hsp90 inhibitors that target the ATP binding pocket in the amino-terminal domain are currently evaluated in cancer patients. Brian Blagg (University of Notre Dame, Notre Dame, Indiana, USA) presented the design and development of two new scaffolds that selectively inhibit the Hsp90ß isoform [19]. As a result of these studies, new isoform-dependent substrates were identified that can be selectively modulated via inhibition of Hsp90ß. In addition, it was observed that selective inhibition of Hsp90ß induces the degradation of HSF1, and thus, there is no induction of the heat shock response. Initial studies suggest that Hsp90ß-selective inhibition may overcome some of the obstacles encountered with the pan-Hsp90 inhibitors that have struggled in clinical trials. Timothy Haystead (Duke University, Durham, North Carolina, USA) presented data on tethered inhibitors of Hsp90 amino-terminal domain causing aggregation and reinternalization of the Hsp90 protein such that it accumulates intracellularly at high concentration. This observation led to development of a series of imaging agents for the detection of early metastatic disease as well as imaging of inflammatory responses to immune challenge.

Understanding Hsp90 Function Through Artificial Intelligence

William E. Balch (The Scripps Research Institute, La Jolla, California, USA) used a new Gaussian Process-based machine learning approach referred to as Variation Spatial Profiling (VSP) to show that diversity at the population level is sensitive to the buffering capacity of the proteostasis program. Based on Spatial CoVariance (SCV), genetic diversity contributing to misfolding disease found in the population can be displayed as high-dimensional phenotype landscapes. Application of these technologies reveal a map of the specific sequence-to-function-

to-structure features of the client protein fold that are responsive to Hsp70-Hsp90 chaperone/cochaperone management at molecular resolution [20]. Gennady Verkhivker (Chapman University School of Pharmacy, Irvine, California, USA) described the step-wise fashion by which the Hsp90-Cdc37 heterocomplex recognizes client kinases through differential stabilization of kinase lobes. This work revealed that Hsp90 binds after Cdc37 and helps shed light on unique dynamics signatures of protein kinase clients/nonclients that dictate chaperone addiction and explain divergences in the regulatory mechanisms among structurally similar kinases.

Recognition of Colleagues in the Hsp90 Field

For the past sixteen years two leaders in the field, Didier Picard (University of Geneva, Switzerland) and Johannes Buchner (TUM, Munich, Germany), have organized the Hsp90 conference biannually. At this 9th International Conference, they were recognized for their valuable contribution and outstanding service to the Hsp90 field and community. They were both acknowledged and thanked for their continuous support of students, post-doctoral fellows and new group leaders (Figure 1).

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Figure legend

Figure 1. Above- Participants of the Ninth International Conference on the Hsp90 Chaperone Machine. Below- Brian Freeman (far left) and Mehdi Mollapour (far right) presented recognition trophies to the meeting organizers, Johannes Buchner, Didier Picard (middle). Photos by Abhinav Joshi.



