The multiple facets of the Hsp90 machine

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 insights into the 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 (ER) Grp94, exist as dimers, hydrolyze ATP, and cycle among distinct conformational states. Hsp90 preferentially binds proteins in near-native states and facilitates their remodeling for protein interactions and signaling. At the Ninth International Conference on the Hsp90 Chaperone Machine, approximately one-third of the attendees shared their data on Hsp90 structure and function through short talks (shown in group photograph). Here, we distill and summarize their findings.

Keynote speaker

To open the meeting, the keynote speaker, Paola Picotti (Institute of Molecular Systems Biology), 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]. The 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 this protein's association with cochaperones and client proteins. David Agard (University of California, San Francisco) provided the first atomic details of the Hsp90–Hsp70–HOP–glucocorticoid receptor (GR) client-loading complex. Unexpectedly, this heterocomplex contains two Hsp70s, both in the ADP-bound state: one appears to be involved in the delivery of GR, and the other supports the cochaperone HOP. The GR is largely intact, but, as in the Hsp90–Cdc37–kinase complex[3], part of the 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, these results suggest a common mechanism for Hsp90–client recognition.

Top, participants in the Ninth International Conference on the Hsp90 Chaperone Machine. Bottom, Brian Freeman (far left) and Mehdi Mollapour (far right) presented recognition trophies to the meeting organizers, Johannes Buchner and Didier Picard (middle). Credit: Abhinav Joshi

Stefan Rüdiger (Utrecht University) 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) described cooperation between nucleotides and the two N-terminal ATP-binding pockets in an Hsp90 dimer, by using multicolor single-molecule Förster resonance energy transfer. This novel technique adds an additional dimension that enabled the discovery that ATP and Aha1 independently but synergistically promote closing of the nucleotide pocket but antagonistically affect subsequent reopening[4]. Katarzyna Tych from the laboratory of Matthias Rief (Technische Universität München (TUM)) described the dynamics of Hsp90 C-terminal dimerization by using single-molecule optical tweezers. Interestingly, Hsp90 C-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's group (TUM) 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 nucleotide-exchange factor 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 the lab of Sue Wickner (NIH) showed that Hsp90 and Hsp70 directly interact in both *Escherichia coli* and yeast. The region of interaction on Hsp90 involves residues in the middle domain that also interact with several cochaperones and clients, whereas the interaction region on Hsp70 utilizes residues in the J-protein-binding region of Hsp70 (ref. [5]).

Hsp90 phosphorylation and regulation

Hsp90 and its cochaperones are subject to post-translational modifications (PTMs) including phosphorylation. Ioannis Gelis (University of South Florida) demonstrated that during the chaperone cycle, Hsp90 phosphorylation occurs in a cochaperone-regulated manner. This finding is exemplified by the Hsp90–Cdc37 heterocomplex, in which the phosphorylation of Cdc37 primes Hsp90 for phosphorylation of Tyr197 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) revisited previous studies on PTMs of Hsp90 and how they fine-tune Hsp90 function. His database of yeast growth assays revealed that some phosphomimetic mutants of Hsp90 grow more efficiently than yeast with wild-type Hsp90 and more potently activate steroid-hormone receptors, apart from the GR. This finding 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) presented data on the structure of the preamino (pre-N) domain of Grp94, the 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 absent in the ER[7].

Olivier Genest (CNRS, Aix Marseille University) 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 between folding by Hsp90 and degradation by the protease HslUV finely regulates the level of TilS.

Modulation of Hsp90 by cochaperones

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

Kaushik Bhattacharya from Didier Picard's group (University of Geneva) addressed the importance of the HOP protein in eukaryotes[9]. He found that the complex Hsp70–HOP–Hsp90 physically interacts with the proteasome. Although in the absence of HOP, proteasome activity is decreased, he showed that proteostasis is maintained by a 'superchaperone' complex minimally consisting of Hsp90, Hsp70, a J protein, and a nucleotide-exchange factor, thus resembling the chaperone complex found in prokaryotes. Michael Reidy from Dan Masison's group (NIH) reported that Sti1 (HOP) has two distinct functions for yeast Hsp90. The first function is connecting Hsp70 to Hsp90 by directly binding both chaperones. The second function is facilitating 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) developed a screen to find inhibitors of the RuvBL2 protein. He successfully identified a compound that inhibits RuvBL2. Chris Prodromou (University of Sussex) described the 3D structure of the yeast and human R2TP complex that has recently been solved through X-ray crystallography and single-particle EM[11]. These structures reveal the importance of the Hsp90–cochaperone complex as a hexamer ring and that, in yeast, binding of a single Tah1 coupled to Pih1 promotes Rvb1p–Rvb2p ATPase activity. Philippe Meyer (Sorbonne Université, CNRS) 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, thus stimulating Hsp90 ATPase activity in synergy with Aha1. This stimulation activity is abolished if the N terminus of RPAP3 is deleted.

Markus Zweckstetter (DZNE) described the 3D structure of Hsp90 in complex with misfolded transthyretin, which is similar to the binding previously described for tau[12]. The effect of cochaperones on Hsp90 structure was also described by using the example of FKBP51, which binds all three domains of Hsp90 with varying affinities and results in decreased ATPase activity by stabilizing an open conformation of the N-terminal domain. When bound to Hsp90, the catalytic site of FKBP51 remains accessible, thus supporting the idea that Hsp90 probably acts as a scaffold supporting the functional interaction of misfolded proteins with cochaperones. Mehdi Mollapour (SUNY Upstate Medical University) described the cooperative function of the new cochaperones Tsc1 and FNIP1/2, which cooperatively decelerate the Hsp90 chaperone cycle and play a role in the chaperoning of both kinase and nonkinase clients[13]. He also showed how phosphorylation, SUMOylation, and ubiquitination of the cochaperone protein phosphatase-5 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), 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, thereby allowing them to interact with target-gene promoters and in turn directing actin-polymer formation and chromosome motion.

Gergely Lukacs (McGill University) showed that both eukaryotic (Hsc70 and Hsp90) and prokaryotic chaperone (DnaK) systems can reshape the conformational energetics of the mutant CFTR-channel final fold toward that of the wild type at the single-molecule level and in cells. This mechanism has implications for the regulation of metastable ABC transporters and other membrane proteins. Dragana Vidovic from Ineke Braakman's group (Utrecht University) 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 similarly to the pathogenic mutant CFTR ΔF508. Michael Heider and Vanesa Fernandez-Saiz from Florian Bassermann's lab (TUM) 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 the Hsp90–CRBN interaction is abrogated by 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) presented his work on the role of Hsp90 in myosin assembly in *Caenorhabditis elegans*. He showed that knockout or overproduction of Hsp90 and some of its cochaperones results in a loss of motility phenotypes due to myosin-filament disorganization. After examining chaperone localization patterns in the sarcomere, he proposed that the Hsp90 machinery is important for regulating myosin translocation across the sarcomere, thus enabling formation of proper myosin filaments. Yu-Chun Wang from Patrik Verstreken's lab (VIB) 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) reported that the effects of Hsp90 on the evolutionary rate of protein kinases is comparable to the effects of gene expression and protein interactions. She showed that a single mutation can render a nonclient 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) demonstrated that Aha1 increases tau aggregation. In contrast, cyclophilin 40 (CyP40) disrupts tau fibrils, thereby decreasing neurotoxicity in vivo[15]. These findings provide a strong rationale for developing therapeutics for tauopathies by targeting molecular chaperones. Aaron Voigt (RTWH Aachen University) demonstrated that mitochondrial Hsp90 TRAP1 is an important player in Parkinson's disease, and his data suggest that enhancing TRAP1 abundance or activity in neurons might be an avenue for future Parkinson's disease therapies.

Oliver Krämer (Johannes Gutenberg University) 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 toward HDAC6 and Hsp90 triggers apoptosis of AML cells, thus providing new promising ways to treat AML. Ramona Schulz-Heddergott (University Medical Center of Göttingen) reported that stabilization of a cancer-relevant Hsp90 client, p53 R248Q, contributes to STAT3 hyperactivity. Conversely, Hsp90 inhibition by 17AAG leads to degradation of the p53 mutant and thus to downregulation of p-STAT3. These data suggest that the cancer-relevant Hsp90 client p53 mutants may be an actionable drug target for treatment with Hsp90 inhibitors.

Stephanie Diezmann (University of Bristol) described how Hsp90 and a lack of the cochaperone Sti1 (HOP) enable 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) highlighted the importance of Hsp90 in the life cycle of a poxvirus family member, vaccinia virus. 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) discussed the translatome changes in *Leishmania donovani* after Hsp90 inhibition. This protozoan parasite showed increased synthesis of proteins involved in oxidative stress protection, proteolysis, chromatin assembly, and chaperoning after 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) 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. Unexpectedly, the activity of Hsp90α is independent of its ATPase activity and can be fulfilled by a short region of Hsp90α of only ~115 amino acids, called fragment-5 (ref. [16]). These properties of secreted Hsp90α can be exploited by tumor cells to gain invasion and metastasis, and thus may be an alternative target for antitumor therapeutics. Natasha Boel from Adrienne Edkins' group (Rhodes University) investigated the role of Hsp90 in the dynamics of the fibronectin matrix. She showed that Hsp90 interacts with fibronectin and that inhibition of Hsp90 by novobiocin results in fibronectin turnover via an LRP-1-receptor-mediated response[17].

Patricija van Oosten-Hawle (University of Leeds) explained how a proteotoxic stress perceived in one tissue induces a response in other tissues, thus 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 decreases the formation of toxic amyloid beta aggregates.

Small-molecule inhibitors of Hsp90

Hsp90 inhibitors that target the ATP-binding pocket in the N-terminal domain are currently being evaluated in cancer patients. Brian Blagg (University of Notre Dame) presented the design and development of two new scaffolds that selectively inhibit the Hsp90β isoform[19]. As a result of these studies, new isoform-dependent substrates were identified that can be selectively modulated via inhibition of Hsp90β. In addition, selective inhibition of Hsp90β has been observed to induce the degradation of HSF1 and thus prevent induction of the heat-shock response. Initial studies suggest that Hsp90β-selective inhibition may overcome some of the obstacles encountered with the pan-Hsp90 inhibitors that have struggled in clinical trials. Timothy Haystead (Duke University) presented data on tethered inhibitors of Hsp90 N-terminal domain causing aggregation and reinternalization of the Hsp90 protein, such that it accumulates intracellularly at high concentration. This observation has led to the development of a series of imaging agents for the detection of early metastatic disease as well as imaging of inflammatory responses to immunological challenge.

Understanding Hsp90 function through artificial intelligence

William E. Balch (The Scripps Research Institute) 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. On the basis of spatial covariance, genetic diversity contributing to misfolding disease found in the population can be displayed as high-dimensional phenotype landscapes. Application of these technologies has revealed 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) described the stepwise fashion in which the Hsp90–Cdc37 heterocomplex recognizes client kinases through differential stabilization of kinase lobes. This work has revealed that Hsp90 binds after Cdc37, has shed light on the unique dynamics signatures of protein kinase clients and nonclients that dictate chaperone addiction, and has explained divergences in the regulatory mechanisms among structurally similar kinases.

Recognition of colleagues in the Hsp90 field

For the past 16 years, two leaders in the field, Didier Picard (University of Geneva) and Johannes Buchner (TUM) (pictured), have organized the Hsp90 conference biannually. At this Ninth 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, postdoctoral fellows, and new group leaders. ❐

Laura J. Blair[1]*, Olivier Genest[2]* and Mehdi Mollapour[3,4,5]*

[1]*Department of Molecular Medicine, USF Health Byrd Institute, University of South Florida, Tampa,*

FL, USA. [2]*Aix Marseille Univ, CNRS, BIP UMR 7281 Marseille, France.* [3]*Department of Urology, SUNY Upstate Medical University, Syracuse, NY, USA.* [4]*Department of Biochemistry and Molecular Biology, SUNY Upstate Medical University, Syracuse, NY, USA.* [5]*Upstate Cancer Center, SUNY Upstate Medical University, Syracuse, NY, USA.*
**e-mail: lblair@health.usf.edu; ogenest@imm.cnrs.fr; mollapom@upstate.edu*

Published online: 7 January 2019
https://doi.org/10.1038/s41594-018-0177-7

References

1. Schopf, F. H., Biebl, M. M. & Buchner, J. *Nat. Rev. Mol. Cell Biol.* **18**, 345–360 (2017).
2. Schopper, S. et al. *Nat. Protoc.* **12**, 2391–2410 (2017).
3. Verba, K. A. & Agard, D. A. *Trends Biochem. Sci.* **42**, 799–811 (2017).
4. Götz, M., Wortmann, P., Schmid, S. & Hugel, T. *J. Vis. Exp.* https://doi.org/10.3791/56896 (2018).
5. Kravats, A. N. et al. *Proc. Natl Acad. Sci. USA* **115**, e2210–e2219 (2018).
6. Bachman, A. B. et al. *Nat. Commun.* **9**, 265 (2018).
7. Huck, J. D., Que, N. L., Hong, F., Li, Z. & Gewirth, D. T. *Cell Rep.* **20**, 2800–2809 (2017).
8. Honoré, F. A., Méjean, V. & Genest, O. *Cell Rep.* **19**, 680–687 (2017).
9. Bhattacharya, K., Bernasconi, L. & Picard, D. *Sci. Rep.* **8**, 2801 (2018).
10. Houry, W. A., Bertrand, E. & Coulombe, B. *Trends Biochem. Sci.* **43**, 4–9 (2018).
11. Rivera-Calzada, A. et al. *Structure* **25**, 1145–1152.e4 (2017).
12. Oroz, J. et al. *Nat. Commun.* **9**, 4532 (2018).
13. Sager, R. A., Woodford, M. R. & Mollapour, M. *Trends Biochem. Sci.* **43**, 935–937 (2018).
14. Lauwers, E. et al. *Mol Cell* **71**, 689–702.e9 (2018).
15. Baker, J. D. et al. *PLoS Biol.* **15**, e2001336 (2017).
16. Cheng, C. F. et al. *J. Clin. Invest.* **121**, 4348–4361 (2011).
17. Boel, N. M., Hunter, M. C. & Edkins, A. L. *Sci. Rep.* **8**, 11438 (2018).
18. O'Brien, D. et al. *Cell Rep.* **23**, 3905–3919 (2018).
19. Khandelwal, A. et al. *Nat. Commun.* **9**, 425 (2018).
20. Wang, C. & Balch, W. E. *Cell Rep.* **24**, 2013–2028.e6 (2018).