Cell Stress and Chaperones (2024) 29:88–96
https://doi.org/10.1016/j.cstres.2024.01.003

EDITORIAL

Second international symposium on the chaperone code, 2023

Johannes Buchner[1,*] · Milad J. Alasady[2,3,4] · Sarah J. Backe[5,6] · Brian S.J. Blagg[7] · Richard L. Carpenter[8,9,10] · Giorgio Colombo[11] · Ioannis Gelis[12] · Daniel T. Gewirth[13] · Lila M. Gierasch[14,15] · Walid A. Houry[16,17] · Jill L. Johnson[18] · Byoung Heon Kang[19] · Aimee W. Kao[20] · Paul LaPointe[2,21] · Seema Mattoo[22,23,24] · Amie J. McClellan[25] · Leonard M. Neckers[26] · Chrisostomos Prodromou[27] · Andrea Rasola[28] · Rebecca A. Sager[5,6] · Maria A. Theodoraki[29] · Andrew W. Truman[30] · Matthias C. Truttman[31,32,33] · Natasha E. Zachara[34,35] · Dimitra Bourboulia[5,6,36] · Mehdi Mollapour[5,6,36,*] · Mark R. Woodford[5,6,36,]

The second chaperone code meeting was organized by Mark Woodford, Dimitra Bourboulia, and Mehdi Mollapour (State University of New York, Upstate Medical University, USA; https://www.cssimeeting.com,). It was held as a satellite event of the annual meeting of the Cell Stress Society. It featured a large number of speakers representing different aspects of the topic aiming to achieve a comprehensive overview of the field. In this context, there

* Johannes Buchner
johannes.buchner@tum.de

* Mehdi Mollapour
mollapom@upstate.edu

* Mark R. Woodford
woodform@upstate.edu

1 Department of Bioscience, Technical University of Munich, D85748, Garching, Germany

2 Department of Biochemistry and Molecular Genetics, Northwestern University Feinberg School of Medicine, Chicago, IL, 60611, USA

3 Simpson Querrey Center for Epigenetics, Northwestern University Feinberg School of Medicine, Chicago, IL, 60611, USA

4 Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, Chicago, IL, 60611, USA

5 Department of Urology, SUNY Upstate Medical University, Syracuse, NY, 13210, USA

6 Upstate Cancer Center, SUNY Upstate Medical University, Syracuse, NY, 13210, USA

7 Department of Chemistry and Biochemistry, Warren Family Research Center for Drug Discovery and Development, University of Notre Dame, Notre Dame, IN, 46556, USA

8 Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Bloomington, IN, 47405, USA

9 Medical Sciences, Indiana University School of Medicine, Bloomington, IN, 47405, USA

10 Simon Cancer Center, Indiana University School of Medicine, Indianapolis, IN, 47405, USA

11 Department of Chemistry, University of Pavia, 27100 Pavia, Italy

12 Department of Chemistry, University of South Florida, Tampa, FL, 33620, USA

13 Department of Biochemistry & Molecular Biology, University of Massachusetts Amherst, Amherst, MA, 01003, USA

14 Department of Chemistry, University of Massachusetts Amherst, Amherst, MA, 01003, USA

15 Hauptman-Woodward Medical Research Institute, Buffalo, NY, 14203, USA

16 Department of Biochemistry, University of Toronto, Toronto, Ontario, M5G 1M1, Canada

17 Department of Chemistry, University of Toronto, Toronto, Ontario, M5S 3H6, Canada

18 Department of Biological Sciences and the Center for Reproductive Biology, University of Idaho, Moscow, ID, 83844, USA.

Published online: 3 February 2024

was a focus on the molecular chaperone Hsp90. Accordingly, Johannes Buchner presented the keynote lecture opening the symposium. He showed an overview of the chaperone cycle of Heat shock protein-90 (Hsp90) and work from his lab demonstrating its modulation by diverse post-translational modifications (PTMs) including phosphorylation,[1] nitrosylation,[2] and lysine methylation.[3] In the examples presented, the PTMs had wide range allosteric effect on Hsp90 function. Thus, the sites affected can be seen as conformational switch points regulating chaperone activity.

Hsp90 chaperone code

Paul LaPointe (University of Alberta, Canada) continued the scheme on Hp90 PTMs. He presented a mechanism for how phosphorylation of Hsp90 inhibits the stimulatory effect of the co-chaperone Aha1 on the Hsp90 ATPase. Specifically, the phosphosimilar T22E mutation in Hsp90 neutralizes the conserved NxNNWHW motif of Aha1 and alters asymmetric stimulation and kinetic parameters of ATP hydrolysis. He also presented a model for how this modification can exert its effect from only one subunit of the Hsp90 dimer.[4] Brian Blagg (University of Notre Dame, USA) presented data on the development of Hsp90 isoform-selective inhibitors and their use against various disease states. In particular, he presented data regarding the development of human Hsp90β-selective inhibitors and included data that demonstrated that such compounds overcome several of the clinical detriments associated with Hsp90 pan-inhibitors, including cardio-, ocular-, and dose-limiting toxicities.[5,6] Together the data support the development of Hsp90β-selective inhibitors for the potential treatment of various cancers. Maria A. Theodoraki (Arcadia University, USA) presented data on gambogic acid (GBA) and DAP-19, two Hsp90β selective inhibitors.[7] The extraction of GBA from the gamboge of *Garcinia hanburyi* results in isomerization at the C2 center of the compound. The epimers, however, do not present significant differences in their biological profile as shown in cytotoxicity and induction of apoptosis studies using the MDA-MB-231 triple-negative breast cancer cell line.[8] DAP-19, a GBA derivative, showed potent antitumor effects in *in vitro* studies with breast cancer cell lines without affecting normal mammary epithelial cells. Additionally, treatment with DAP-19 decreased cancer cell migration and led to proteasomal degradation of Hsp90 clients. Dan Gewirth (Hauptman-Woodward Institute, USA) reported on the effect of glycosylation on Grp94, the Hsp90 paralog of the endoplasmic reticulum (ER). Using Grp94 variants that were engineered to be glycosylated at defined sites only, his group showed that the six glycosylation sites fall into two classes. One set of sites is structurally and functionally benign, while glycosylation at any of the other sites leads to Grp94s with shorter lifetimes, decreased stability, increased protein aggregation, and reduced client maturation, suggesting a mechanism whereby differential glycosylation tunes Grp94 activity and promotes new biological functions.[9] Grp94 glycosylation was also addressed by Giorgio Colombo (University of Pavia, Italy) who discussed the role of molecular

[19] Department of Biological Sciences, Ulsan National Institutes of Science and Technology (UNIST), Ulsan, 44919, South Korea
[20] Memory and Aging Center, Department of Neurology, University of California, San Francisco, CA, 94158, USA
[21] Department of Cell Biology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, T6G 2H7, Canada
[22] Department of Biological Sciences, Purdue University, West Lafayette, IN, 47907, USA
[23] Purdue Institute for Integrative Neuroscience, Purdue University, West Lafayette, IN, 47907, USA
[24] Purdue Institute for Inflammation, Immunology and Infectious Disease, Purdue University, West Lafayette, IN, 47907, USA
[25] Division of Science and Mathematics, Bennington College, Bennington, VT, 05201, USA
[26] Center for Cancer Research, National Cancer Institute, Rockville, MD, 20892, USA
[27] Genome Damage and Stability Centre, University of Sussex, Brighton, BN1 9RQ, UK
[28] Department of Biomedical Sciences, University of Padova, 35131 Padova, Italy
[29] Department of Biology, Arcadia University, Glenside, PA, 19038, USA
[30] Department of Biological Sciences, University of North Carolina at Charlotte, Charlotte, NC, 28223, USA
[31] Neuroscience Graduate Program, University of Michigan, Ann Arbor, MI, 48109, USA
[32] Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI, 48109, USA
[33] Geriatrics Center, University of Michigan, Ann Arbor, MI, 48109, USA
[34] Department of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD, 21205, USA
[35] Department of Oncology, The Johns Hopkins University School of Medicine, Baltimore, MD, 21205, USA
[36] Department of Biochemistry & Molecular Biology, SUNY Upstate Medical University, Syracuse, NY, 13210, USA.

Fig. 1 A Crystal Keynote Speaker Plaque was presented to Johannes Buchner (second from left) by meeting organizers Mehdi Mollapour (left), Dimitra Bourboulia (third from left), and Mark Woodford (right).

simulations in understanding the impact of post-translational modifications on the functional dynamics of chaperones and the implications for drug design.[10] Specifically, he found that aberrant glycosylation of Grp94[11] remodels the dynamic states of the chaperone and its interaction modes with drugs. Phosphorylation of co-chaperones, on the other hand, was shown to remodel the conformational landscape of these proteins, ultimately perturbing their engagement in the chaperone machinery.[12] Sarah Backe from the Mollapour group (State University of New York, Upstate Medical University, USA) showed that the autophagy-activating kinase Atg1/Ulk1 phosphorylates a conserved serine site, (yHsp90-S25/hHsp90α-S39), of Hsp90 inhibiting its ATPase activity and altering chaperone dynamics. Atg1/Ulk1-mediated phosphorylation of Hsp90 leads to dissociation of the Hsp90:Atg1/Ulk1 complex and activation of Atg1/Ulk1, which is essential for initiation of autophagy.[13] This work indicates a reciprocal regulatory mechanism between the chaperone Hsp90 and the autophagy kinase Atg1/Ulk1 and consequent maintenance of cellular proteostasis. Jill Johnson (University of Idaho, USA) discussed new results that take advantage of mutations in yeast Hsp90 that disrupt different steps within the folding pathway.[14] In prior studies, they were unable to identify client defects of one set of mutants that may explain the temperature-sensitive phenotype. However, recent studies suggest that mutations in that group selectively impact the folding of the ribosomal translocase, eEF2. Amie McClellan (Bennington College, USA) described preliminary findings on non-canonical ubiquitination, using yeast as a model system. Mutation of the three ubiquitinated lysine residues in the von Hippel Lindau (VHL) tumor suppressor protein to arginine prevents their ubiquitination by canonical iso-peptide linkages to lysine side chains. VHL lacking these lysine residues was still ubiquitinated and degraded by the proteasome with no observable differences in chaperone requirements such as Hsp90. This is the first evidence showing that, in addition to their well-established role in the quality control of lysine-bearing substrates, molecular chaperones also participate in the successful triage of misfolded proteins lacking lysine residues to the ubiquitin-proteasome system.[15–17]

Taken together, these presentations suggest that PTMs create unique subpopulations of Hsp90 chaperones which are fine-tuned to function in the folding, activation, and degradation of client proteins.

Heat shock response code

Another important element of chaperone regulation is the transcriptional tuning of chaperone expression by the specific heat shock transcription factor. Milad Alasady from Marc Mendillo's group (Northwestern University, USA) combined a genome-wide RNAi library with a heat shock response (HSR) reporter and identified JMJD6 as an essential mediator of HSF1 activity. In a positive feedback circuit, HSF1 binds and promotes JMJD6 expression, which in turn reduces HSP70 R469 mono-methylation to disrupt HSP70-HSF1 repressive complexes resulting in enhanced HSF1 activation.[18,19] Richard Carpenter (Indiana University, USA) developed an HSF1 activity signature and showed that HSF1 activity was negatively associated with antitumor immune cells in breast tumors, most notably CD8+ T cells. Knockdown of HSF1 in breast tumors decreased tumor volume by an influx of CD8+ T cells. An increase in expression and secretion of CCL5 was required to attract CD8+ T cells after knockdown of HSF1.[20] These findings indicate that HSF1 suppresses antitumor immune activity by reducing CCL5. Natasha Zachara (Johns Hopkins University, USA) presented unpublished work on the post-translational modification *O*-GlcNAc, which is one target of the cellular stress response. *O*-GlcNAc remodels cellular pathways to promote cellular survival, and elevating *O*-GlcNAc enhances cell and tissue survival in models of oxidative stress and ischemia-reperfusion injury.[21] One example of this remodeling by *O*-GlcNAc works via AMP-dependent protein kinase to regulate autophagy and cytoprotection.

The theme emerging from this section is that cellular responses to stress affect different aspects of biology,

Fig. 2 Group photo of meeting attendees at the Second International Symposium on the Chaperone Code outside the Hilton Old Town Alexandria, in Alexandria, VA, USA.

including transcription, metabolism, and antitumor immunity.

Hsp70 chaperone code

The other major player in the chaperone system of the eukaryotic cytosol is Hsp70. As expected for such a central factor, it is precisely regulated by PTMs as discussed in several presentations. Seema Mattoo (Purdue University, USA) described how AMPylation/adenylylation of the ER Hsp70 chaperone BiP by the Fic protein, HYPE/FicD could serve as a novel therapeutic nexus for the treatment of protein misfolding disorders.[22,23] Highlighting her lab's work on Parkinson's disease and the recent discovery of clinical mutations associated with HYPE, Dr Mattoo described the development and application of a high-throughput screen leading to the discovery of a small molecule inhibitor for HYPE. Matthias Truttman (University of Michigan, USA) continued the scheme on AMPylation of BiP and presented work describing a new mechanism linking protein AMPylation to developmental processes and protein aggregation stress in the nematode *C. elegans*.[24,25] Andy Truman (UNC Charlotte, USA) described the creation of the Hsp70 chaperone code collection, which allows high-throughput analysis of the phenotypic fingerprints of Hsp70 phospho-site mutants. Importantly, he reported that there was a lack of overlap between Hsp70 phospho-sites activated by a particular stress and those required for response to that stress. Taken together, his results suggest that Hsp70 acts as a major integrator of diverse cellular signals.[26,27] Lila Gierasch (University of Massachusetts-Amherst, USA) focused on the finding that some substrate peptide sequences bound to Hsp70 in two orientations, C- to N- or N- to C-, suggesting that the preferred binding mode is a result of the optimal positioning of substrate side chains in the binding cleft regardless of the backbone orientation.[28] She presented new findings on a peptide designed with a "palindromic sequence" that presents the same side chain sequences around the residue that occupies the "central pocket" regardless of the binding orientation. Strikingly, the palindromic peptide binds in both C- to N- or N- to C- orientations with nearly identical frequency, arguing that binding is based on the ability of the chaperone to optimize side chain fit regardless of backbone direction.

As evidenced by these presentations, manipulation of Hsp70 PTMs can provide valuable information on the molecular basis of the cellular stress response.

Mitochondrial chaperone code

Mitochondrial Hsp90 has gained increasing attention in recent years as it seems to play an important role in mitochondrial proteostasis. Andrea Rasola (University of Padova, Italy) presented new work on PTMs of the mitochondrial Hsp90 homolog TRAP1. In order to dynamically shape cell metabolism and tune it to the changing environmental conditions, TRAP1 undergoes a fine regulation by PTMs. He showed the influence of PTMs on TRAP1 activity in tumor cell models, which leads to adaptations that enhance their neoplastic features.[29] Byoung Heon Kang (UNIST, South Korea) presented data demonstrating that TRAP1 contributes to disease pathogenesis by regulating mitochondrial

function in non-cancerous cells, such as adipocytes in the tumor microenvironment and retinal cells in ischemic retinopathy, using knockout mice.[30] Additionally, he showed that TRAP1 inhibitors targeting its client binding site, such as mitoquinone and SB-U015, not only exhibit enhanced anticancer activity compared to ATP-mimetic inhibitors[31,32] but also show potential applications in treating human diseases caused by mitochondrial dysfunctions. Mark Woodford (State University of New York, Upstate Medical University, USA) presented unpublished work demonstrating phosphorylation of TRAP1 by a mitochondrial population of the *proto*-oncogene tyrosine kinase c-Abl. Phosphorylated TRAP1 showed increased binding to the apoptosis effector cyclophilin D, an interaction that blocks the induction of mitochondrial apoptosis. Mitochondrial c-Abl and subsequent TRAP1 phosphorylation is enriched in clear cell renal cell carcinoma cell lines, suggesting a potential mechanism driving cancer cell survival.[33]

Mitochondrial dysfunction underlies the pathogenesis of cancer and neurodegeneration, among others. Our nascent understanding of mitochondrial chaperone function has revealed that dysregulation of TRAP1-PTM plays a critical role in promoting oncogenesis, and targeting TRAP1 may provide clinical benefit in the context of different diseases.

Co-chaperone code

In addition to PTMs, the function of Hsp70s and Hsp90s is tuned by a suite of proteins known as co-chaperones. These co-chaperones are further regulated by PTMs, demonstrating the intricacies of the chaperone code. Len Neckers (National Cancer Institute, USA) shared his latest data on lactylation of a number of chaperones and co-chaperone proteins, including DnaJ/JDP proteins. He found that a unique set of DnaJs/JDPs are constitutively lactylated, as is the Hsp90 co-chaperone Aha1. These preliminary observations suggest that Hsp90 activity may be susceptible to metabolic regulation as are certain DnaJs/JDPs. Importantly, such metabolic regulation is likely more frequent in highly glycolytic tumor cells.[34,35]

Aimee Kao (UCSF, USA) presented work on TSC1/Hamartin, a co-chaperone for Hsp70 and Hsp90.[36,37] She showed that *Tsc1* haploinsufficiency is associated with an increased risk of the primary tauopathy, progressive supranuclear palsy, and the secondary tauopathy, Alzheimer's disease.[38,39] In *Tsc1* haploinsufficiency, tau becomes abnormally acetylated and stabilized, thereby increasing steady-state levels of this protein and leading to abnormal protein aggregation. Chris Prodromou (University of Sussex, UK) revealed the structure of the LA1011-Hsp90 C-terminal domain complex. LA1011 is a dihydropyridine that has been shown to improve the prognosis of Alzheimer's disease in mouse models,[40,41] LA1011 competes for binding with the Hsp90 co-chaperone FKBP51, an immunophilin known to be associated with Alzheimer's disease, defining the molecular target for LA1011 and providing structure-based drug design opportunities.[42] The mechanism by which LA1011 acts to improve Alzheimer's disease is suggested to be the restoration of the normal biology of tau, a Hsp90- and FKBP51-dependent protein. Ioannis Gelis (University of South Florida, USA) presented NMR and HDX-MS data that reveal essential dynamics during the Hsp90 chaperone cycle. Results from his group show that breathing motions in the client kinase domains expose otherwise buried segments utilized for Hsp90 binding, suggesting that inherent client dynamics poise for chaperone dependence. During kinase loading to Hsp90-Cdc37 complexes, this partially unfolded client conformation is stabilized by Cdc37 independently of Hsp90.[43] Dimitra Bourboulia (State University of New York, Upstate Medical University, USA) presented work on how c-Src tyrosine kinase regulates the extracellular Hsp90 (eHsp90) chaperone machinery. c-Src phosphorylates TIMP2, an eHsp90 co-chaperone, facilitating its interaction with MMP2, an eHsp90 client, and modulating extracellular matrix degradation.[44] In addition to targeting the TIMP2 co-chaperone, the studies demonstrate that active c-Src drives the generation of specific phospho-secretome enriched in cancer-promoting tyrosine-phosphorylated proteins, including eHsp90α and eHsp90β.[45] Considering that c-Src levels and activity are increased in human cancers, collectively, the data present a new concept for the regulation of the extracellular milieu and emphasize the importance of extracellular c-Src-mediated protein-protein interactions and tyrosine phosphorylation of the eHsp90 chaperone machinery.

Rebecca Sager from the Mollapour lab (State University of New York, Upstate Medical University, USA) presented unpublished data on the SUMOylation of protein phosphatase 5 (PP5). She demonstrated that phosphorylation of PP5-T362, shown previously to play a key role in PP5 activation in kidney cancer, is pre-requisite to SUMOylation of the phosphatase.[46,47] Together these modifications control PP5 activity and release of its substrates such as the glucocorticoid receptor in cells. Walid Houry (University of Toronto, Canada) presented data demonstrating that the R2TP chaperone complex is responsible for assembly of macromolecular complexes mainly acting through different adapters. Using proximity labeling mass spectrometry, the Houry group identified DPCD as a new adapter of R2TP. They demonstrated that R2TP-DPCD influences ciliogenesis initiation through a unique mechanism by interaction with Akt kinase to regulate its phosphorylation levels rather than its stability.[48–50]

As co-chaperones are themselves regulators of chaperone function, characterizing the "co-chaperone code" can provide critical information for understanding their impact on chaperone function.

Flash talks

Short talks by junior scientists covered a range of exciting topics expanding the scope of the chaperone code meeting. Asif Elahi from Ahmed Chadli's group (Augusta University, USA) reported that the Hsp90 co-chaperone UNC45A is highly overexpressed in prostate cancer patient samples and is correlated with a decrease in disease-free survival. UNC45A selectively localizes to the nucleus and regulates the proliferation of prostate cancer cells. He further showed that UNC45A silencing decreases the expression of the mitotic kinase NEK7 at both mRNA and protein levels in prostate cancer, suggesting a role for UNC45A in cell-cycle regulation in prostate cancer.[51] Vamsi Krishna Kommalapati from Ahmed Chadli's group (Augusta University, USA) reported results demonstrating that capsaicin inhibits Hsp90 by targeting its N-terminal domain without inducing HSR. It promotes the degradation of Hsp70 through the lysosome-autophagy pathway. Additionally, co-treatment of capsaicin enhances the antitumor activity of 17-AAG.[52] So-Yeon Kim from Byong-Heon Kang's group (UNIST, South Korea) showed that expression of mitochondrial TRAP1 was essential for pathological neovascularization and blood-retinal barrier breakdown in mouse models of ischemic retinopathies. He showed that TRAP1 inhibition alleviated retinal vascular pathologies by inducing HIF1α degradation mediated by activation of calpain-1. Furthermore, non-invasive ophthalmic solutions of TRAP1 inhibitors degraded HIF1α, simultaneously downregulating multiple angiogenic factors in ischemic retinopathy, decreasing expression levels to normal.[53] Katherine Meluni from the Mollapour lab (State University of New York, Upstate Medical University, USA) described the regulation of cyclin-dependent kinase 4 (CDK4), which regulates G1 to S phase transition.[54,55] CDK4 stability and activity depend on Hsp90 and the co-chaperone Cdc37. The E3 ubiquitin ligase VHL targets CDK4 for ubiquitination and degradation. Duhita Mirikar from Andy Truman's lab (UNC Charlotte, USA) presented work on RNR, a potential client of Hsp70, Hsp90 and DNAJA1. She reported a direct interaction of RNR subunits with molecular chaperones using cross-linking mass spectrometry and cryo electron microscopy.[56] Gianna Mochi from Mark Woodford's lab (State University of New York, Upstate Medical University, USA) presented a recently discovered metabolically derived PTM that impacts the function of TRAP1 and its client protein, driving mitochondrial respiration.[57] Siddhi Omkar from the Truman lab (UNC Charlotte, USA) described a screening approach to identify phosphorylation sites that are altered under heat shock and thus studying its cellular rationale that helps cells survive at high temperatures. She identified novel sites on Hsp70 that are modified under heat stress and result in a defective HSR. These data suggest that Hsp70 acts as an integrator of diverse stress signals to control the HSR via its PTMs.[26] Jenny Pessa from Lea Sistonen's lab (Åbo Akademi University, Finland) described recent data demonstrating that transforming growth factor-beta (TGF-β)-mediated loss of HSF2 is required for epithelial-mesenchymal plasticity as well as induced cellular invasion in breast cancer cells, and that ectopic expression of HSF2 counteracts the TGF-β-induced effects in both *in vitro* and *in vivo* models.[58] Pietro Poggio from Mara Brancaccio's group (University of Turin, Italy) reported that, in mice, the knockout of the Hsp90 co-chaperone Morgana caused accumulation of DNA damage and impaired proliferation of epithelial cells, leading to the destruction of intestinal integrity. Morgana-deficient cells also showed cell-cycle defects and mitotic abnormalities. His findings highlighted a crucial role for Morgana in maintaining homeostasis of actively proliferating tissues.[59] Francesca Zuppini from Mario Brancaccio's group (University of Turin, Italy) discussed the role of the extracellular Hsp90 co-chaperone Morgana in enhancing cancer cell migration through its interaction with specific cell surface receptors. Following this binding, Morgana facilitates the internalization of membrane-bound integrins, ultimately favoring their recycling over degradation.[60] Sarah Rolli from Emily Sontag's lab (Marquette University, USA) found that Hsp40, Hsp70, and Hsp90 alter the aggregation and toxicity of mutant huntingtin in a yeast model. Chaperone knockout increases the toxicity of mutant huntingtin and increases the amount of insoluble protein. Smriti Sangwan from the Walter Lab (UCSF, USA) reported on IRE1, a membrane protein in the ER which monitors the protein folding status of the ER in a process termed the unfolded protein response. She showed using single-particle cryoEM that IRE1 binds to approximately one-third of ER-localized ribosomes. This may be important for IRE1 to find its select mRNA substrates from the larger pool of ER-localized mRNAs. Leon Tang from Lin-fa Wang's group (National University of Singapore) described results of a study on the stress response of bats following heat shock. Bats are unique as they exhibit dampened inflammation and increased longevity, and they are the only mammal

capable of self-powered flight.[61] He uncovered enhanced heat resistance in their physiology coupled with an atypical molecular HSR. Valeria Uvarova from the van Oosten-Hawle lab (UNC Charlotte, USA) showed that Hsp90 directly contributes to remodeling of lipid metabolism via interacting with lipid catabolic enzymes in the gut of *C. elegans*, suggesting changes in free fatty acids. These changes induced by increased Hsp90 levels in the gut alleviate the toxicity of age-associated aggregative disease proteins expressed in the nervous system and muscle cells and extend *C. elegans* healthspan.[62] Nam Gu Yoon from Byoung-Heon Kang's lab (UNIST, South Korea) showed that the mitochondrial chaperone TRAP1 was essential for production of protumorigenic adipokines in cancer-associated adipocyte. Therapeutic targeting of TRAP1 has the potential of yielding beneficial effects for the treatment of breast tumor and its adipose-rich niche.[63]

Leon Tang (National University of Singapore), Nam Gu Yoon (UNIST, South Korea), and Gianna Mochi (State University of New York, Upstate Medical University, USA) received first, second, and third prizes, respectively, for their outstanding presentations.

Concluding remarks

Following the prolonged absence of in-person meetings resulting from the COVID-19 pandemic, the Second International Symposium on the Chaperone Code provided an opportunity to reconnect with friends and colleagues in Alexandria (Figures 1 and 2). The presentations offered a comprehensive overview of the intricate network of PTMs and regulatory mechanisms governing the chaperone machinery and delved into the diverse roles of major chaperones, such as Hsp90 and Hsp70, and their co-chaperones in cellular processes ranging from protein folding to disease pathogenesis. Notably, the focus extended to mitochondrial chaperones and their implications in cellular metabolism and disease. The meeting showcased cutting-edge research on the impact of PTMs on Hsp90 function, binding to isoform-selective inhibitors and the regulation of diverse cellular pathways. The integration of chaperones into the context of diseases, including cancer and neurodegenerative disorders, underscored the therapeutic potential of targeting chaperone networks. Moreover, the exploration of novel PTMs, such as lactylation, SUMOylation, and *O*-GlcNAcylation adds layers of complexity to the chaperone code. Future directions in the chaperone code field involve unraveling the intricate interplay between chaperones, co-chaperones, and their PTMs in various cellular contexts. Understanding how chaperone networks respond to different stresses, regulate immune responses, and contribute to cellular homeostasis will be crucial. The development of innovative techniques, as evidenced by proximity labeling mass spectrometry and structural studies, will further enhance our grasp of chaperone dynamics. Additionally, the implications of chaperone modifications in the extracellular milieu and their role in metabolic regulation present exciting avenues for exploration. The meeting set the stage for continued and new collaborations, emphasizing the need for interdisciplinary approaches and integration of emerging technologies. As the chaperone code field advances, it holds the promise of unveiling new therapeutic targets and strategies for diseases associated with protein misfolding and cellular stress.

The Third International Symposium on the Chaperone Code will be held in September 2025 in Syracuse, NY, USA.

Author contributions JB, MJA, SJB, BSJB, DB, RLC, GC, IG, DTG, LMG, WAH, JLJ, BHK, AWK, PL, SM, AJM, LMN, CP, AR, RAS, MAT, AWT, MCT, NEZ, MM, and MRW wrote the text. JB, MM, and MRW organized and assembled the manuscript. DB, MM, and MRW organized the Symposium.

Declarations of interest The authors declare no conflicts of interest.

Acknowledgments The authors thank Dr Ilse Ariadna Valtierra Gutierrez (Senior Editor, *Nature Communications*) and Danie Descoteaux (Executive Publisher, Molecular Biology, Elsevier) for their presentations and valuable insight. The authors acknowledge the Department of Urology at SUNY Upstate Medical University and the Cell Stress Society International, Inc for their generous support. The authors also express their gratitude to Eppendorf, Nicoya Lifesciences, Sino Biological, and StressMarq Biosciences for their meeting sponsorship.

References

1. Soroka J, Wandinger SK, Mäusbacher N, et al. Conformational switching of the molecular chaperone Hsp90 via regulated phosphorylation. *Mol Cell.* 2012;45:517–528. https://doi.org/10.1016/j.molcel.2011.12.031
2. Retzlaff M, Stahl M, Eberl HC, et al. Hsp90 is regulated by a switch point in the C-terminal domain. *EMBO Rep.* 2009;10:1147–1153. https://doi.org/10.1038/embor.2009.153
3. Rehn A, Lawatscheck J, Jokisch M-L, et al. A methylated lysine is a switch point for conformational communication in the chaperone Hsp90. *Nat Commun.* 2020;11:1219. https://doi.org/10.1038/s41467-020-15048-8
4. Mercier R, Wolmarans A, Schubert J, Neuweiler H, Johnson JL, LaPointe P. The conserved NxNNWHW motif in Aha-type co-chaperones modulates the kinetics of Hsp90 ATPase stimulation. *Nat Commun.* 2019;10:1273. https://doi.org/10.1038/s41467-019-09299-3
5. Khandelwal A, Kent CN, Balch M, et al. Structure-guided design of an Hsp90β N-terminal isoform-selective inhibitor.

Nat Commun. 2018;9:425. https://doi.org/10.1038/s41467-017-02013-1
6. Mishra SJ, Liu W, Beebe K, et al. The development of Hsp90β-selective inhibitors to overcome detriments associated with *pan*-Hsp90 inhibition. *J Med Chem.* 2021;64:1545–1557. https://doi.org/10.1021/acs.jmedchem.0c01700
7. Yim KH, Prince TL, Qu S, et al. Gambogic acid identifies an isoform-specific druggable pocket in the middle domain of Hsp90β. *Proc Natl Acad Sci U S A.* 2016;113:E4801–E4809. https://doi.org/10.1073/pnas.1606655113
8. Arevalo GE, Frank MK, Decker KS, Theodoraki MA, Theodorakis EA. Gambogic acid: multi-gram scale isolation, stereochemical erosion toward epi-gambogic acid and biological profile. *Front Nat Prod.* 2023;1:1018765. https://doi.org/10.3389/fntpr.2022.1018765
9. Yan P, Patel HJ, Sharma S, et al. Molecular stressors engender protein connectivity dysfunction through aberrant N-glycosylation of a chaperone. *Cell Rep.* 2020;31:107840. https://doi.org/10.1016/j.celrep.2020.107840
10. Colombo G. Computing allostery: from the understanding of biomolecular regulation and the discovery of cryptic sites to molecular design. *Curr Opin Struct Biol.* 2023;83:102702. https://doi.org/10.1016/j.sbi.2023.102702
11. Castelli M, Yan P, Rodina A, et al. How aberrant N-glycosylation can alter protein functionality and ligand binding: an atomistic view. *Structure.* 2023;31:987–1004.e8. https://doi.org/10.1016/j.str.2023.05.017
12. Castelli M, Bhattacharya K, Abboud E, Serapian SA, Picard D, Colombo G. Phosphorylation of the Hsp90 Co-chaperone hop changes its conformational dynamics and biological function. *J Mol Biol.* 2023;435:167931. https://doi.org/10.1016/j.jmb.2022.167931
13. Backe SJ, Sager RA, Heritz JA, et al. Activation of autophagy depends on Atg1/Ulk1-mediated phosphorylation and inhibition of the Hsp90 chaperone machinery. *Cell Rep.* 2023;42:112807. https://doi.org/10.1016/j.celrep.2023.112807
14. Hohrman K, Gonçalves D, Morano KA, Johnson JL. Disrupting progression of the yeast Hsp90 folding pathway at different transition points results in client-specific maturation defects. *Genetics.* 2021;217. https://doi.org/10.1093/genetics/iyab009
15. McClellan AJ, Scott MD, Frydman J. Folding and quality control of the VHL tumor suppressor proceed through distinct chaperone pathways. *Cell.* 2005;121:739–748. https://doi.org/10.1016/j.cell.2005.03.024
16. McClellan AJ, Laugesen SH, Ellgaard L. Cellular functions and molecular mechanisms of non-lysine ubiquitination. *Open Biol.* 2019;9:190147. https://doi.org/10.1098/rsob.190147
17. Melville MW, McClellan AJ, Meyer AS, Darveau A, Frydman J. The Hsp70 and TRiC/CCT chaperone systems cooperate in vivo to assemble the von Hippel-Lindau tumor suppressor complex. *Mol Cell Biol.* 2003;23:3141–3151. https://doi.org/10.1128/MCB.23.9.3141-3151.2003
18. Alasady MJ, Mendillo ML. The multifaceted role of HSF1 in tumorigenesis. *Adv Exp Med Biol.* 2020;1243:69–85. https://doi.org/10.1007/978-3-030-40204-4_5
19. Szewczyk MM, Ishikawa Y, Organ S, et al. Pharmacological inhibition of PRMT7 links arginine monomethylation to the cellular stress response. *Nat Commun.* 2020;11:2396. https://doi.org/10.1038/s41467-020-16271-z
20. Jacobs C, Shah S, Lu WC, et al. HSF1 inhibits antitumor immune activity in breast cancer by suppressing CCL5 to block CD8+ T cell recruitment. *Cancer Res.* 2023;84:276–290. https://doi.org/10.1158/0008-5472.CAN-23-0902
21. Fahie KMM, Papanicolaou KN, Zachara NE. Integration of O-GlcNAc into stress response pathways. *Cells.* 2022;11:3509. https://doi.org/10.3390/cells11213509
22. Sanyal A, Dutta S, Camara A, et al. Alpha-synuclein is a target of Fic-mediated adenylylation/AMPylation: possible implications for Parkinson's disease. *J Mol Biol.* 2019;431:2266–2282. https://doi.org/10.1016/j.jmb.2019.04.026
23. Camara A, George A, Hebner E, Mahmood A, Paluru J, Mattoo S. A fluorescence polarization-based high-throughput screen to identify the first small-molecule modulators of the human adenylyltransferase HYPE/FICD. *IJMS.* 2020;21:7128. https://doi.org/10.3390/ijms21197128
24. Hernandez-Lima MA, Champion M, Mattiola Z, Truttmann MC. The AMPylase FIC-1 modulates TGF-β signaling in Caenorhabditis elegans. *Front Mol Neurosci.* 2022;15:912734. https://doi.org/10.3389/fnmol.2022.912734
25. Truttmann MC, Pincus D, Ploegh HL. Chaperone AMPylation modulates aggregation and toxicity of neurodegenerative disease-associated polypeptides. *Proc Natl Acad Sci U S A.* 2018;115:E5008–E5017. https://doi.org/10.1073/pnas.1801989115
26. Nitika CM Porter, Truman AW, Truttmann MC. Post-translational modifications of Hsp70 family proteins: expanding the chaperone code. *J Biol Chem.* 2020;295:10689–10708. https://doi.org/10.1074/jbc.REV120.011666
27. Nitika ZB, Ruan L, Kline JT, et al. Comprehensive characterization of the Hsp70 interactome reveals novel client proteins and interactions mediated by posttranslational modifications. *PLoS Biol.* 2022;20:e3001839. https://doi.org/10.1371/journal.pbio.3001839
28. Clerico EM, Pozhidaeva AK, Jansen RM, Özden C, Tilitsky JM, Gierasch LM. Selective promiscuity in the binding of *E. coli* Hsp70 to an unfolded protein. *Proc Natl Acad Sci U S A.* 2021;118:e2016962118. https://doi.org/10.1073/pnas.2016962118
29. Faienza F, Lambrughi M, Rizza S, et al. S-nitrosylation affects TRAP1 structure and ATPase activity and modulates cell response to apoptotic stimuli. *Biochem Pharmacol.* 2020;176:113869. https://doi.org/10.1016/j.bcp.2020.113869
30. Kim S-Y, Yoon NG, Im JY, et al. Targeting the mitochondrial chaperone TRAP1 alleviates vascular pathologies in ischemic retinopathy. *Adv Sci (Weinh).* 2023;11:e2302776. https://doi.org/10.1002/advs.202302776
31. Yoon NG, Lee H, Kim SY, et al. Mitoquinone inactivates mitochondrial chaperone TRAP1 by blocking the client binding site. *J Am Chem Soc.* 2021;143:19684–19696. https://doi.org/10.1021/jacs.1c07099
32. Kang S, Kang BH. Structure, function, and inhibitors of the mitochondrial chaperone TRAP1. *J Med Chem.* 2022;65:16155–16172. https://doi.org/10.1021/acs.jmedchem.2c01633
33. Wengert LA, Backe SJ, Bourboulia D, Mollapour M, Woodford MR. TRAP1 chaperones the metabolic switch in Cancer. *Biomolecules.* 2022;12:786. https://doi.org/10.3390/biom12060786
34. Chen A-N, Luo Y, Yang YH, et al. Lactylation, a novel metabolic reprogramming code: current status and prospects. *Front Immunol.* 2021;12:688910. https://doi.org/10.3389/fimmu.2021.688910
35. Zhang D, Tang Z, Huang H, et al. Metabolic regulation of gene expression by histone lactylation. *Nature.* 2019;574:575–580. https://doi.org/10.1038/s41586-019-1678-1
36. Natarajan N, Shaik A, Thiruvenkatam V. Recombinant tumor suppressor TSC1 differentially interacts with *Escherichia coli* DnaK and human HSP70. *ACS Omega.* 2020;5:19131–19139. https://doi.org/10.1021/acsomega.0c02480
37. Woodford MR, Sager RA, Marris E, et al. Tumor suppressor Tsc1 is a new Hsp90 co-chaperone that facilitates folding of

kinase and non-kinase clients. *EMBO J.* 2017;36:3650–3665. https://doi.org/10.15252/embj.201796700
38. Alquezar C, Schoch KM, Geier EG, et al. TSC1 loss increases risk for tauopathy by inducing tau acetylation and preventing tau clearance via chaperone-mediated autophagy. *Sci Adv.* 2021;7:eabg3897. https://doi.org/10.1126/sciadv.abg3897
39. Hwang J-HL, Perloff OS, Gaus SE, et al. Tuberous sclerosis complex is associated with a novel human tauopathy. *Acta Neuropathol.* 2023;145:1–12. https://doi.org/10.1007/s00401-022-02521-5
40. Kasza Á, Hunya Á, Frank Z, et al. Dihydropyridine derivatives modulate heat shock responses and have a neuroprotective effect in a transgenic mouse model of Alzheimer's disease. *J Alzheimers Dis.* 2016;53:557–571. https://doi.org/10.3233/JAD-150860
41. Roe MS, Wahab B, Török Z, Horváth I, Vigh L, Prodromou C. Dihydropyridines allosterically modulate Hsp90 providing a novel mechanism for heat shock protein co-induction and neuroprotection. *Front Mol Biosci.* 2018;5:51. https://doi.org/10.3389/fmolb.2018.00051
42. Roe SM, Török Z, McGown A, et al. The crystal structure of the Hsp90-LA1011 complex and the mechanism by which LA1011 may improve the prognosis of Alzheimer's disease. *Biomolecules.* 2023;13:1051. https://doi.org/10.3390/biom13071051
43. Keramisanou D, Vasantha Kumar MV, Boose N, Abzalimov RR, Gelis I. Assembly mechanism of early Hsp90-Cdc37-kinase complexes. *Sci Adv.* 2022;8:eabm9294. https://doi.org/10.1126/sciadv.abm9294
44. Sánchez-Pozo J, Baker-Williams AJ, Woodford MR, et al. Extracellular phosphorylation of TIMP-2 by secreted c-Src tyrosine kinase controls MMP-2 activity. *iScience.* 2018;1:87–96. https://doi.org/10.1016/j.isci.2018.02.004
45. Backe SJ, Votra SD, Stokes MP, et al. PhosY-secretome profiling combined with kinase-substrate interaction screening defines active c-Src-driven extracellular signaling. *Cell Rep.* 2023;42:112539. https://doi.org/10.1016/j.celrep.2023.112539
46. Dushukyan N, Dunn DM, Sager RA, et al. Phosphorylation and ubiquitination regulate protein phosphatase 5 activity and its prosurvival role in kidney cancer. *Cell Rep.* 2017;21:1883–1895. https://doi.org/10.1016/j.celrep.2017.10.074
47. Sager RA, Dushukyan N, Woodford M, Mollapour M. Structure and function of the co-chaperone protein phosphatase 5 in cancer. *Cell Stress Chaperones.* 2020;25:383–394. https://doi.org/10.1007/s12192-020-01091-3
48. Houry WA, Bertrand E, Coulombe B. The PAQosome, an R2TP-based chaperone for quaternary structure formation. *Trends Biochem Sci.* 2018;43:4–9. https://doi.org/10.1016/j.tibs.2017.11.001
49. Lynham J, Houry WA. The role of Hsp90-R2TP in macromolecular complex assembly and stabilization. *Biomolecules.* 2022;12:1045. https://doi.org/10.3390/biom12081045
50. Seraphim TV, Nano N, Cheung YWS, et al. Assembly principles of the human R2TP chaperone complex reveal the presence of R2T and R2P complexes. *Structure.* 2022;30:156–171.e12. https://doi.org/10.1016/j.str.2021.08.002
51. Eisa NH, Jilani Y, Kainth K, et al. The co-chaperone UNC45A is essential for the expression of mitotic kinase NEK7 and tumorigenesis. *J Biol Chem.* 2019;294:5246–5260. https://doi.org/10.1074/jbc.RA118.006597
52. Patwardhan CA, Kommalapati VK, Llbiyi T, et al. Capsaicin binds the N-terminus of Hsp90, induces lysosomal degradation of Hsp70, and enhances the anti-tumor effects of 17-AAG (Tanespimycin). *Sci Rep.* 2023;13:13790. https://doi.org/10.1038/s41598-023-40933-9
53. Antonetti DA, Silva PS, Stitt AW. Current understanding of the molecular and cellular pathology of diabetic retinopathy. *Nat Rev Endocrinol.* 2021;17:195–206. https://doi.org/10.1038/s41574-020-00451-4
54. Woodford MR, Backe SJ, Wengert LA, Dunn DM, Bourboulia D, Mollapour M. Hsp90 chaperone code and the tumor suppressor VHL cooperatively regulate the mitotic checkpoint. *Cell Stress Chaperones.* 2021;26:965–971. https://doi.org/10.1007/s12192-021-01240-2
55. Sager RA, Backe SJ, Ahanin E, et al. Therapeutic potential of CDK4/6 inhibitors in renal cell carcinoma. *Nat Rev Urol.* 2022;19:305–320. https://doi.org/10.1038/s41585-022-00571-8
56. Truman AW, Kristjansdottir K, Wolfgeher D, et al. Quantitative proteomics of the yeast Hsp70/Hsp90 interactomes during DNA damage reveal chaperone-dependent regulation of ribonucleotide reductase. *J Proteomics.* 2015;112:285–300. https://doi.org/10.1016/j.jprot.2014.09.028
57. Kim S, Backe SJ, Wengert LA, et al. O-GlcNAcylation suppresses TRAP1 activity and promotes mitochondrial respiration. *Cell Stress Chaperones.* 2022;27:573–585. https://doi.org/10.1007/s12192-022-01293-x
58. Pessa JC, Joutsen J, Sistonen L. Transcriptional reprogramming at the intersection of the heat shock response and proteostasis. S1097276523009693, Dec *Mol Cell.* 2023;84. https://doi.org/10.1016/j.molcel.2023.11.024
59. Poggio P, Sorge M, Seclì L, Brancaccio M. Extracellular HSP90 machineries build tumor microenvironment and boost cancer progression. *Front Cell Dev Biol.* 2021;9:735529. https://doi.org/10.3389/fcell.2021.735529
60. Seclì L, Avalle L, Poggio P, et al. Targeting the extracellular HSP90 co-chaperone morgana inhibits cancer cell migration and promotes anticancer immunity. *Cancer Res.* 2021;81:4794–4807. https://doi.org/10.1158/0008-5472.CAN-20-3150
61. Irving AT, Ahn M, Goh G, Anderson DE, Wang L-F. Lessons from the host defences of bats, a unique viral reservoir. *Nature.* 2021;589:363–370. https://doi.org/10.1038/s41586-020-03128-0
62. Hodge F, Bajuszova V, van Oosten-Hawle P. The intestine as a lifespan- and proteostasis-promoting signaling tissue. *Front Aging.* 2022;3:897741. https://doi.org/10.3389/fragi.2022.897741
63. Zhao C, Wu M, Zeng N, et al. Cancer-associated adipocytes: emerging supporters in breast cancer. *J Exp Clin Cancer Res.* 2020;39:156. https://doi.org/10.1186/s13046-020-01666-z