Structural dynamics of Hsp90 in vitro and in cells

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Abstract

Heat shock protein 90 (Hsp90) is a central molecular chaperone that regulates a plethora of client proteins involved in processes such as cancer and neurodegeneration, making it an important target for therapeutic intervention. Hsp90 is a Mg(II)-dependent ATPase whose activity and conformational cycle are tightly regulated by co-chaperones. Structurally, Hsp90 is a homodimer, with each monomer composed of three domains: the N-terminal domain (NTD) bearing ATPase, the middle domain (MD) involved in client binding and ATP hydrolysis, and the C-terminal domain (CTD) which constitutes the dimerization interface and provides a binding platform for tetratricopeptide repeat (TPR) domain co-chaperones.

Hsp90 function relies on large-scale and subtle conformational rearrangements driven by nucleotide and co-chaperone binding and is thus unamenable to structural interrogation using conventional structural approaches. In this talk, I will present how electron paramagnetic resonance (EPR) spectroscopy combined with site-directed spin labeling has been used to resolve Hsp90 dynamics. EPR allowed us to (i) distinguish three distinct closed conformations at the NTDs, (ii) characterize how different co-chaperone binding reshapes the NTDs and CTDs, and (iii) reveal structural heterogeneity within the CTDs by comparing full-length Hsp90 with domain-truncated variants. Importantly, we have also extended these measurements inside living cells, enabling the first direct observation of co-chaperone-dependent structural changes in a native cellular environment. Together, these results demonstrate how EPR-based methods provide unique insights into the conformational landscape of Hsp90 and its regulation by co-chaperones.

Short CV

Dr. Angeliki Giannouli obtained her Diploma and MSc in Chemistry from the University of Ioannina, Greece, with focus in bioinorganic chemistry. She then completed her PhD at the University of St Andrews, UK, where she focused on EPR studies of inorganic complexes and organic radicals. She then joined the Weizmann Institute of Science (WIS), Israel, as a Postdoctoral Fellow and later as a Senior Postdoctoral Fellow, investigating protein conformations in vitro and in mammalian cells using spin-labeling and EPR techniques. She was then appointed Staff Scientist at the WIS, where she worked on NMR and DNP methodologies. She has been a visiting scientist at the United States National High Magnetic Field Laboratory Florida (NHMFL), the CNRS in

Marseille and the University of Frankfurt. She is an active member of several professional societies and organizations, including the Royal Society of Chemistry (MRSC).

Currently, Dr. Giannouli is an Assistant Professor of Biochemistry at the University of Crete, Greece. Her research focuses on the structural and biochemical characterization of chaperones and enzymes using EPR and complementary biophysical methods, as well as on developing synthetic biology approaches to exploit biomolecular condensates for whole-cell encapsulation.