Heat Shock Protein 90 (Hsp90) is a ubiquitous molecular chaperone that facilitates the folding and maturation of hundreds of “client” proteins, highly enriched for signaling and regulatory processes. Client maturation is assisted by an array of co-chaperones that sequentially interact with Hsp90. Despite their importance, how they function is largely unknown. Using cryo-EM, I determined atomic resolution structures for Hsp90 in complex with the ATPase-accelerating co-chaperone Aha1. The structures revealed that Aha1 catalyzes the Hsp90 cycle by breaking the barriers between 5 distinct and sequentially stabilized states. To dissect the mechanism of client maturation, I established the human succinate dehydrogenase B (SdhB) from respiratory complex II as the first mitochondrial Hsp90 (Trap1) client protein amenable to detailed biochemical and structural investigation. My cryo-EM structure of Trap1:SdhB complex elucidated the interplay between Hsp90 and a partially unfolded SdhB, demonstrating a highly conserved client interaction mechanism that transcends the need for co-chaperones.