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Inter-subunit crosstalk synergistically regulates allosteric activation of proapoptotic serine protease HtrA2

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High-temperature requirement protease A2 (HtrA2) is a complex trimeric mitochondrial serine protease that primarily acts as a key player in apoptosis. It belongs to a large family of multi-domain serine proteases (S1, chymotrypsin family) that is found to be conserved from prokaryotes to humans [1]. There exist four human homologues (HtrA1-HtrA4), which reveal a common evolutionarily conserved structural architecture. They share an overall common polypeptide fold with an active site containing a catalytic triad, oxyanion hole and substrate specificity pocket. Despite sharing such structural integrity, low sequence identity exists between these serine protease homologues and the subtle conformational variations induced upon ligand binding provide the basis for their specificity and functional diversity. The presence of an N-terminal tetrapeptide AVPS motif in mature trimeric HtrA2 makes it the only unique IAP-binding protein amongst all the HtrA homologues that complements the proapoptotic activity of SMAC protein. Apart from mediating caspase-dependent apoptosis by deactivating XIAP (X-linked Inhibitor of Apoptosis Protein), the structural organization and domain plasticity have also enabled HtrA2 to promote caspase-independent apoptotic pathways via its intricate serine protease activity, the complex mechanisms of which are yet to be delineated [2, 3]. Deregulation in the functioning of a multitasking protein such as HtrA2 lays the foundation for a number of pathophysiological conditions, such as cancer, arthritis and neurodegenerative disorders [4]. However, the complex mechanism through which it promotes apoptotic cascades or regulates protein quality control still remains elusive.

HtrA2 comprises three important regions: a short N-terminal trimerization motif, a serine protease domain (SPD) and a C-terminal protein-protein interaction or PDZ domain. The available crystallographic data of HtrA2 provides a broad overview of the overall structural organization. However, the structural data is acquired from the mature unbound form of the inactive protease and hence fails to elucidate the intricate structural dynamics of PDZ-protease crosstalk that take place upon ligand binding. Previous studies from our lab have shown that activation of HtrA2 occurs allosterically, where the signal is relayed via a distal non-canonical substrate-binding site with subsequent opening up of the peptide-binding groove in PDZ.[5] Thus, the transition from basal to proteolytically active state seems to be predominantly governed by PDZs, owing to its specific interactions with numerous C-terminal binding partners. To expound on the role of regulatory PDZ domains in promoting synergistic coordination between HtrA2 subunits, we generated heterotrimeric HtrA2 variants comprising different numbers of PDZs and/or active-site mutations. Sequential deletion of PDZs from the trimeric

ensemble significantly affected its residual activity in a way that proffered a hypothesis advocating *intermolecular* allosteric crosstalk via PDZ domains in trimeric HtrA2. Furthermore, structural and computational snapshots affirmed the role of PDZs in secondary structural element formation and coordinated reorganization of the N-terminal region and regulatory loops. Therefore, apart from providing cues for devising structure-guided therapeutic strategies, this study establishes a working model of complex allosteric regulation through a multifaceted *trans*-mediated cooperatively-shared energy landscape.

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