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Keeping Cool Under Pressure: Hsp70's Role in Stabilising Proteins During Heat Stress

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The Hsp70 chaperone system plays key roles in the folding of nascent polypeptides and preventing their misfolding and aggregation during cellular stress, making it indispensable for the maintenance of proteostasis. However, it is not clear how chaperone engagement influences the conformation of individual clients during heat stress to maintain a functional ensemble. To address this, we used single-molecule fluorescence resonance energy transfer (smFRET) to temporally interrogate how the human Hsp70 system regulates the conformation of a heat-sensitive client protein, firefly luciferase, during heat stress. We found that Hsp70 recognises heat-induced misfolded states and resolves them by conformationally expanding the client to prevent aggregation. Release of the client by Hsp70 allows an opportunity for it to refold to its native state; interestingly, unsuccessful refolding results in a conformationally distinct misfolded state that is absent without Hsp70. The observed Hsp70 binding events are transient and continue during heat-stress, with ~ 30% of the clients populating the heat-induced misfolded state or transiently engaging with Hsp70. Collectively, this work visualises the mechanisms by which Hsp70 modulate client conformations during heat-stress to maintain a folded proteome.