Picturing protein disaggregation

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How chaperones clear protein aggregates is a notoriously impenetrable problem. A new study now shows how single-molecule movies of Hsp104 and Hsp70 chaperones acting on amyloid fibers are key to revealing their underlying cooperation in time and space.

Over time, proteins can lose their properly folded structure and expose sticky peptide segments that causes them to aggregate together[1]. Fibrillar aggregates called amyloids are associated with a range of neurodegenerative disorders, even as their causal nature remains unclear[2]. Many of the core mechanisms that allows aggregates to be recognized, and broken up into free monomers, remain poorly understood[3,4]. Within bacteria and yeast cells, the Hsp100 class of chaperones play an important role. Having a doughnut-like shape and central pore, Hsp100 chaperones were recently shown to processively translocate polypeptide chains[5], and are hence thought to 'extract' proteins from aggregates. Hsp100 chaperones (Hsp104 in yeast) work in concert with the Hsp70 system, which are known to bind both the aggregate and Hsp100, and possesses aggregation-prevention and disaggregation capabilities itself[6,7]. Elucidating how aggregates are ultimately cleared from cells has proven difficult, however, owing in part to the many possible ways that these factors could work together. Nakagawa *et al.* have now developed a microscopy-based single-molecule approach, which shows how fibril fracturing starts with Ssa1 (Hsp70) and Sis1 (Hsp40) binding the amyloid fibril jointly, and is then completed by repeated binding of Hsp104 at the same site.

The core assay performed by Nagakawa *et al.* is conceptually straightforward (Fig. 1). Amyloid fibrils (Sup35NM) of one of two conformations (Sc4 or Sc37) are deposited on a surface, and the three chaperones (Hsp104, Ssa1, and Sis1) are added in the surrounding solution. Up to three components are fluorescently labelled, and imaged by total internal reflection (TIRF) microscopy, which limits perturbing background fluorescence of non-bound chaperones. The resulting movies beautifully show how peaks of fluorescence intensity slowly build up along the fibril, with near-identical spatial profile and temporal development for Ssa1 and Sis1. A similar Hsp104 profile emerges about 70 seconds later, and finally the amyloid fluorescence decreasing right where the chaperones peak – indicating fibril fracturing. In these experiments, too many chaperones were bound to identify individual copies. Hence, fluorescent and non-fluorescent chaperones were mixed to lower the overall intensity. Now, single Hsp104 chaperones could now not only be seen binding the fibrils, but also leaving again after about 3 to 30 seconds, yielding repeated Hsp104 binding and unbinding. These data suggested an appealing model in which fragmentation is achieved by repeated extraction of individual Sup35NM monomers.

The data illustrate the power of tracking single molecules in space and time. The highly orchestrated nature of many cellular processes suggests pervasive hidden spatio-temporal dynamics, and methods like those advanced here will be key to elucidating them. Indeed, simply visualizing where and in which temporal order molecular activities take place can be incredibly revealing. Yet, simple these experiments

are not. Indeed, the work illustrates well how crucial features can be identified within a sea of molecular heterogeneity, leading to hypothesis that are subsequently carefully tested. For instance, the authors could distinguish the discrete fragmentation from a more global and gradual fibril dissolution, while gradual dissolution features were also observed in the fragmentation data, and additional controls provided further support of these two distinct modes.

The reported findings give rise to many fascinating questions. The data suggests an intriguing cooperativity, in which first extractions trigger subsequent ones. Elucidating how this works is challenging, as only a sub-population of Hsp104 can be followed, actively extracting Hsp104 are not directly identified, and the spatial resolution is obviously bound by diffraction. Freshly created fibril ends may be hotspots for chaperone recruitment, though pre-existing fibril ends did not appear preferentially targeted, and a strict Hsp70 first rule for new sites may raise consistency issues. Monomer extraction efficiency could depend on local fibril stability, which may be diminished by prior extractions. It is speculated that Hsp104 is targeted to Sup35NM monomers that are structurally remodeled by Hsp70. Hsp104 may also be recruited by first directly binding to amyloid-bound Hsp70, which also activates Hsp104 ATPase activity[4], and subsequently drives Sup35NM insertion. High local Hsp70 concentrations on the fibril surface may enable stable binding of Hsp104 to multiple Hsp70's, which may relate to how Hsp104 avoids erroneous targeting of native proteins. A glimpse of selectivity was observed here, as the Sc37 conformation recruited more Hsp70 and Hsp104 chaperones. Decoding this recruitment program is challenging. Amyloid aggregates offer a good model system to begin addressing this problem, given their large size and uniform structure, which contrasts with other aggregated and phase separated protein states. More generally, the study highlights key open questions regarding the substrate conformations, which is central to the process.

The approach presented here adds to a rapidly expanding experimental arsenal to study protein dynamics, including FRET, optical tweezers, and cryo-EM, and various others[8,9]. Indeed, with these technological capabilities we are entering a new phase in which we are finally able to visualize the spatio-temporal dynamics that underpins protein homeostasis within cells.

Figure: **Single-molecule observation of amyloid disaggregation by Hsp104.** Total internal fluorescence microscopy (TIRF) of Sup35NM amyloid fibrils and interacting chaperones (Ssa1, Sis1, and Hsp104). Ssa1 and Sis1 that are part of the Hsp70 system are found to bind the amyloid fibrils first, after which the disaggregase Hsp104, which can translocate protein chains through its central pore, and hence can cause fibril fracturing by extracting Sup35NM monomers.

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