# STRUCTURAL BIOLOGY

# Ratchet-like polypeptide translocation mechanism of the AAA+ disaggregase Hsp104

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Hsp100 polypeptide translocases are conserved members of the AAA+ family (adenosine triphosphatases associated with diverse cellular activities) that maintain proteostasis by unfolding aberrant and toxic proteins for refolding or proteolytic degradation. The Hsp104 disaggregase from *Saccharomyces cerevisiae* solubilizes stress-induced amorphous aggregates and amyloids. The structural basis for substrate recognition and translocation is unknown. Using a model substrate (casein), we report cryo–electron microscopy structures at near-atomic resolution of Hsp104 in different translocation states. Substrate interactions are mediated by conserved, pore-loop tyrosines that contact an 80-angstrom-long unfolded polypeptide along the axial channel. Two protomers undergo a ratchet-like conformational change that advances pore loop–substrate interactions by two amino acids. These changes are coupled to activation of specific nucleotide hydrolysis sites and, when transmitted around the hexamer, reveal a processive rotary translocation mechanism and substrate-responsive flexibility during Hsp104-catalyzed disaggregation.

sp100 disaggregases are highly conserved stress responders that unfold and solubilize protein aggregates (*I*, 2). They hexameric ring complexes, which couple adenosine triphosphate (ATP) hydrolysis to polypeptide translocation through a central channel (*I*, 2). *Saccharomyces cerevisiae* Hsp104 is powered by two distinct AAA+ (ATPases associated with diverse cellular activities) domains per protomer and collaborates with the Hsp70 system to disaggregate and refold amorphous aggregates and amyloids such as Sup35 prions, thereby promoting stress tolerance and prion propagation (2).

Cooperative ATP hydrolysis by nucleotidebinding domains (NBD1 and NBD2) of Hsp104 requires conserved Walker A and B motifs, "sensor" residues, and an Arg finger from the adjacent protomer (2, 3). The coiled-coil middle domain (MD) of Hsp104 mediates Hsp70 interactions and allosteric functions during hydrolysis and disaggregation (4–6). Conserved substrate-binding "pore loops" in the NBDs line the axial channel and contain essential Tyr residues that mechanically

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couple hydrolysis to translocation (7, 8). A recently described structure of Hsp104 bound to the nonhydrolyzable ATP analog adenylyl-imidodiphosphate (AMP-PNP) identifies an "open," spiral conformation with a ~30-Å-wide channel and an unusual heteromeric NBD1-NBD2 interaction that forms a flexible seam (9). High-resolution structures of the active hexamer have remained elusive, and it is unknown how hydrolysis and conformational changes power disaggregation.

Here we establish how Hsp104 binds and mechanically translocates substrates. Using the slowly hydrolyzable ATP analog adenosine 5'-O-(3-thiotriphosphate) (ATP- $\gamma$ -S) (10) and the substrate casein, we determined Hsp104 structures to ~4.0 Å by cryo–electron microscopy (cryo-EM). We identify pore loop–substrate contacts and a rotary translocation mechanism involving a ratchetlike change that advances interactions along the substrate polypeptide by two amino acids. NBD1-ATP hydrolysis and substrate binding trigger a massive open-to-closed conformational change in the hexamer, thereby coupling substrate engagement and release to processive disaggregation.

### Substrate-bound architecture of Hsp104

To capture the substrate-bound state of Hsp104, binding was assessed with fluorescence polarization by using fluorescein isothiocyanate (FITC)–labeled casein, a model substrate that is actively translocated (4, 8). High-affinity interactions ( $K_{\rm d} \sim 20$  nM) between Hsp104 and casein were identified in the presence of ATP- $\gamma$ -S, but not ATP, AMP-PNP, or adenosine diphosphate (ADP) (Fig. 1A and fig. S1). Two-dimensional (2D) reference-free classification of purified Hsp104:casein-ATP- $\gamma$ -S complexes revealed that the hexamer

undergoes a large rearrangement compared to the AMP-PNP-bound open state (9) (Fig. 1B and fig. S2A). An initial 3D structure refined to ~3.9 Å resolution (fig. S2B and table S1). However, a two-protomer site was identified to be flexible (fig. S2C). Extensive 3D classification resolved these protomers and revealed two hexamer conformations: "closed" and "extended" (fig. S2D). Refinement of the closed state yielded a 4.0-Åresolution map with well-resolved protomers and axial channel (figs. S2B and S3, A to C). The AAA+ subdomains show side-chain features, enabling an atomic model to be built and refined by using a homology model from the bacterial ortholog ClpB (11) (Fig. 1C and fig. S3, D and E). The AAA+ domains (protomers designated P1 to P6) form a near-symmetric closed double ring (Fig. 1D). Protomers P2 to P5 are identical [root mean square deviation (RMSD) = 2 Å], whereas the two mobile protomers, P1 and P6, each adopt different conformations (Fig. 1E and fig. S3F). The outside hexamer diameter is ~115 Å (compared to 125 Å for the open state), and density for the AAA+ domains surround a ~10-Å-wide axial channel. Notably, the channel is partially occluded by a continuous, 80 Å strand of density that, on the basis of the molecular model, is an unfolded portion of the substrate, casein (Fig. 1F).

# Tyr pore-loop contacts along substrate

In the closed state, pore-loop strands from both AAA+ domains become ordered compared to Hsp104-AMP-PNP (9) and other structures (11, 12) and contact substrate in a right-handed spiral arrangement (Fig. 2A and fig. S4). These regions are among the most highly resolved (<4.0 Å, fig. S3C), indicating bona fide interactions critical for translocation. The casein sequence could not be determined from the density; therefore, a strand of 26 Ala residues was modeled. Hsp104 translocates unfolded polypeptides in the presence of ATP- $\gamma$ -S (8); therefore, the pore loop-substrate interactions likely adopt a fixed register during translocation. Alternatively, a specific region of casein may be uniformly trapped in the channel. Substrate contacts are made by five protomers (P1 to P5), whereas protomer P6 breaks the helical arrangement and makes no direct contact (Fig. 2A and fig. S4). Substrate density is not observed outside the channel, and thus, nontranslocated portions of casein are likely disordered.

Conserved pore-loop tyrosines 257 and 662 in protomers P1 to P5 directly contact substrate, potentially via the aromatic rings, which are positioned ~4 to 5 Å away from the backbone (Fig. 2B). Together with conserved V663 in NBD2 (V, valine), these residues contribute most of the substrate interactions in the channel (fig. S4). Additional loops (residues 291 to 297 for NBD1 and residues 645 to 651 for NBD2) are also ordered and adjacent to the substrate, and K649 and Y650 for P2 and P4 appear to make contact (K, lysine; Y, tyrosine) (Fig. 2, A and B).

The pore loops are separated by ~6 to 7 Å along the channel, making contact with approximately every second amino acid of the substrate (Fig. 2C). K256 and K258 in NBD1, which flank Y257,



**Fig. 1. Substrate-bound Hsp104:casein closed complex.** (**A**) FITC-casein binding analysis, measured by fluorescence polarization in the presence of: ATP- $\gamma$ -S (red), ATP (green), AMP-PNP (black), and ADP (blue) (values = mean ± SD, *n* = 3). (**B**) Representative top- and side-view 2D class averages comparing the Hsp104–ATP- $\gamma$ -S:casein closed state and Hsp104–AMP-PNP

open state (9) (scale bar equals 50 Å). (**C**) Atomic model and segmented map of the AAA+ small (NBD1, green) and large (NBD2, brown) subdomains. (**D**) Final reconstruction of Hsp104:casein segmented by protomers (P1 to P6) and substrate (yellow). (**E**) Side view of the mobile protomer face (P1 and P6). (**F**) Channel view showing substrate polypeptide density (yellow).

project toward neighboring loops, possibly stabilizing the spiral arrangement. Protomers P1 and P5 comprise the lowest and highest contact sites with the substrate and are separated by ~26 Å along the channel axis. Protomer P6 is between these sites but disconnected from the substrate; its NBD1 pore loop is 13 Å away, whereas its NBD2 pore loop was less resolved and unable to be modeled (Fig. 2C). Overall, this structure reveals that substrate interactions are mediated almost entirely by the conserved Tyr residues, establishing their direct role in coordinating substrate during translocation (1, 2).

#### **Ratchet-like states of protomers**

In addition to the closed state, our 3D-classification analysis identified an extended conformation of Hsp104:casein (fig. S2D). Further classification and refinement resulted in a 4.1 Å resolution map from which an atomic model was determined (Fig. 3A and figs. S2B and S5, A and B). The structure reveals a substrate-bound hexamer with a different arrangement of the mobile protomers P1 and P6, which show distinct flexibility in the NBD1 and NBD2, respectively (Fig. 3A and fig. S5, C and D). Protomers P2 to P5 are identical to the closed state (RMSD =  $\sim 0.8$  Å), and density for the polypeptide substrate is slightly extended at the top of the channel but is overall similar and localizes to the same region (fig. S5E).

The closed- and extended-state conformational differences for P1 and P6 are substantial (RMSD = 13.7 and 11.5 Å, respectively) and involve rotations

of P1-NBD1 and P6-NBD2 (Fig. 3B and fig. S6A). P6 rotates toward the channel axis, and the pore loops become well ordered and directly contact substrate. The P6 pore-loop tyrosines, Y257 and Y662, directly contact substrate similarly as the other protomers (Fig. 3C). Conversely, P1-NBD2 rotates counterclockwise, releasing its interaction with P6 to contact P2-NBD2 but maintaining contact with the substrate (Fig. 3B).

Notably, P6-Y257 becomes positioned at the topmost contact site along the polypeptide, advancing interactions by two amino acids (~7 Å) compared with P5-Y257 (Fig. 3D and fig. S6B). These changes bring P6 pore loops in register to form a two-turn right-handed spiral of contacts. Each pore loop rotates ~60° and rises ~6 to 7 Å, enabling evenly spaced Tyr-substrate interactions across a 74 Å length of the channel. Together, the extended and closed states reveal a ratchet-like conformational change of the hexamer that yields a two-amino acid translocation step (movie S1). Although other conformations may exist that were not resolved, the extended and closed states predominate the data set (fig. S2D); therefore, these changes are likely critical for orchestrating substrate-binding and -release steps during translocation.

## **Coordinated nucleotide pockets**

NBD1 and NBD2 nucleotide pockets were examined to determine how nucleotide state is coupled to substrate interactions. P3 to P5 nucleotide pockets are identical, with well-resolved density and a bound ATP- $\gamma$ -S (fig. S7A). In NBD1, R334

(R, arginine) from the clockwise neighboring protomer contacts the  $\gamma$ -phosphate, establishing this residue as the Arg finger (13). R333 is adjacent to the  $\alpha$ - and  $\beta$ -phosphates, acting potentially as a sensor residue considering that the NBD1 does not contain a cis sensor 2 motif (3). In the NBD2 pocket, the Arg finger, R765, interacts with the  $\gamma$ -phosphate, whereas the sensor 2, R826 in the cis protomer, is positioned adjacent to the  $\alpha$ - and  $\beta$ -phosphates (fig. S4A). Thus, for P3 to P5, which make well-defined contacts with the substrate in both states, NBD1 and NBD2 are primed for ATP hydrolysis.

Conversely, the mobile protomer (P6, P1, and P2) NBDs (fig. S7B) are in different active and inactive configurations based on the position of Arg fingers and nucleotide density. For the P6 protomer, both NBDs appear inactive in the closed state (Fig. 4A) with P5-R334 ~12 Å away and P5-R765 ~6 Å away from the respective NBD1 and NBD2  $\gamma$ -phosphate in P6. By contrast, both P5-R334 and P5-R765 in the extended state are identified to contact the respective  $\gamma$ -phosphates directly, indicating that these sites are in an active configuration. Notably, the closed-to-extended conformational change results in both nucleotide pocket activation and substrate contact by P6 (Fig. 3B).

P1 contacts substrate at the lowest position in the hexamer and appears inactive in both states (Fig. 4A). In the closed state, P6-R334 is ~11 Å away and P6-R765 is ~21 Å away from the respective  $\gamma$ -phosphate in P1. These Arg residues are



**Fig. 2. Structural basis for substrate binding in the axial channel.** (**A**) Channel view of the map showing the extended poly-Ala strand modeled as the substrate (mesh) and pore loops with residues indicated. The P6 pore-loop regions not contacting substrate are indicated (circles). (**B**) Model and cryo-EM density showing P4 pore loop–substrate interactions mediated by Y257 (green) in the NBD1 and Y662 (green) and V663 in the NBD2, as well as additional, noncanonical pore loops that include residues K649 and Y650. (**C**) Spiral arrangement of the NBD1 and NBD2 canonical pore loops for P1 to P5 contacting substrate and the disconnected position of the P6-NBD1 pore loop.

further separated from the nucleotide pockets in the extended state, with R333, R334, and R765 more than 30 Å away (Fig. 4A). For P2, NBD1 is in an active configuration in both states. However, P2-NBD2 switches from inactive in the closed state to active in the extended state because of the P1-NBD2 conformational change that brings R765 adjacent the  $\gamma$ -phosphate (Fig. 4A). Finally, on the basis of difference maps, density for nucleotide is present in all sites but appears reduced at certain sites: NBD1 in P6 and P1 for the closed state and NBD2 in P1 for both states, indicating partial occupancy or a posthydrolysis state (fig. S7C).

The NBD states, along with substrate interactions, are depicted in a schematic to explain how active-site rearrangements and the closedto-extended conformational changes drive substrate translocation (Fig. 4B). In the closed state, substrate is bound by five protomers with four NBD1 sites and three NBD2 sites in an active (ATP) configuration. By contrast, in the extended state, substrate is bound by six protomers with five NBD1 sites and five NBD2 sites in an active state. Importantly, the "off" protomer (P6) that is unbound to substrate in the closed state becomes active in the extended state and binds substrate at the next position. The protomer counterclockwise from this position is in the lowest "down" position (P1) and remains inactive in both states, but undergoes a rotation in the extended state that activates NBD2 of the neighboring protomer.

These results suggest a rotary-type translocation mechanism whereby four protomers remain bound to substrate in a similar configuration with the NBDs primed for hydrolysis, while two protomers at the transition site between the lowest and highest position undergo conformational changes that alter substrate interactions. Given the righthanded spiral of pore loops, position of the "up" and "down" protomers, and NBD1 to NBD2 direction of translocation, peptide movement could occur through a counterclockwise cycling of these closed and extended states. On the basis of this model, the inactive protomer in the down position could release substrate and reengage in the up position, thereby advancing translocation by a two-amino acid step. Transmitting these changes counterclockwise would enable the hexamer to advance processively along the polypeptide during translocation (movie S2). Some variability in the step size, potentially to accommodate bulky residues, could be achieved by conformational changes in the extended-state protomer in the up position that shift its pore-loop contact. These results parallel the right-handed substrate interactions and rotary-driven hydrolysis models for DNA and RNA helicases, including AAA+ (E1, DnaA, and MCM) (3, 14, 15) and RecA (Rho) (16) families. Thus, this ratcheting mechanism may be conserved among many ATP-driven translocases.

#### Allosteric control by the MD

The Hsp104-AMP-PNP (9) structure revealed an open-spiral conformation that is substantially different from the closed, substrate-bound states characterized here. Furthermore, a MD-NBD1 interaction was identified that suggested an allosteric control mechanism. To further explore the MD and the role of nucleotide in the Hsp104 conformational cycle, we determined the cryo-EM structure of Hsp104 incubated with ADP to 5.6 Å resolution (Fig. 5A and fig. S8, A to C). The reconstruction reveals an identical AAA+ arrangement compared to Hsp104-AMP-PNP (RMSD = 2.5 Å) involving a left-handed spiral architecture defined by a ~10 Å rise per protomer and a heteromeric AAA+ interaction between P6-NBD1 and P1-NBD2.

The MD is resolved for three protomers (P3 to P5) and identified to be in a crisscross equatorial arrangement stabilized by contacts between the first (L1) and third (L3) helices (Fig. 5B). This arrangement is similar to previous structures (11, 12, 17) but markedly different to the Hsp104-AMP-PNP structure, in which the same MD L1 region makes contact across the NBD1 of the clockwise protomer (Fig. 5C). Comparison of these ATP- and ADP-state MD conformations reveals a substantial, ~30° rotation around position 409 at the MD-NBD1 junction (fig. S8D and movie S3). Although hexamers exclusively bound to ATP or ADP are likely rare in vivo, these data reveal that the MD adopts two nucleotide-specific conformations that reflect pre- and posthydrolvsis states.

Sites that comprise the MD-MD interactions in Hsp104-ADP are critical for function (4, 5, 12, 13). However, the AMP-PNP-specific MD L1-NBD1 interaction has not been characterized. Therefore, single charge-reversal mutations were introduced to disrupt three putative L1-NBD1 salt bridges (9): E412-R194, E427-R353, and D434-R366 (E, glutamic acid; D, aspartic acid) (Fig. 5C). These mutants exhibit robust ATPase activity (fig. S8E). However, they are unable to reactivate

# Fig. 3. Hsp104:casein extended-state conformation advances substrate contacts.

(A) Cryo-EM reconstruction of Hsp104:casein identifying substrate (yellow) and an extended conformation of protomers P1 and P6. (B) Filtered map of P1 (red) and P6 (magenta) overlaid with the corresponding closed-state protomers (gray) after alignment to P4 in the hexamer. NBD conformational changes (arrows) resulting in extended-state interactions (black circles) with substrate (yellow) and the P2-NBD2 (orange) are shown. (C) Model and map of the P6-NBD1 and -NBD2 pore loops showing change in the pore-loop position (arrow) compared to the closed state (gray) for NBD1 and substrate contact by Y257 and Y662 (green). (D) Model and map of the NBD1 and NBD2 P1 to P6 spiral of pore loop-substrate interactions.



denatured firefly luciferase aggregates in vitro (Fig. 5D) or confer thermotolerance in vivo (fig. S8E), supporting a functional role for the L1-NBD1 interaction.

To determine MD conformations in the caseinbound complex, additional 3D subclassification analysis and refinement were performed on the Hsp104 closed-state data without applying a mask (fig. S9A). Three classes with distinct MD arrangements were identified: MD class 1, class 2, and class 3, which refined to 6.7 to 6.9 Å (Fig. 5E and fig. S9B). For these maps, the AAA+ core and substrate density are identical to the closed-state structure. In the MD class 1, density corresponding to the MD coiled coil is identified for four protomers (P1, P2, P5, and P6), revealing an ADP-state, crisscross arrangement around the P6 to P1 mobileprotomer face (Fig. 5E). Notably, the N-terminal domains (NTDs) for all protomers are also resolved in this class, revealing that they interact together in an alternating, triangular arrangement with the polypeptide strand oriented asymmetrically in the channel, toward the P3 and P5 NTDs (fig. S10).

For MD class 2, density corresponding to MD helices L1 and L2 is identified for protomers P3 to P5, revealing that L1 is positioned across the clockwise protomer, which indicates an ATP-state conformation (Fig. 5E). For class 3, both MD conformations are identified: P1, P5, and P6 adopt the ADP state, while P3 and P4 are in the ATP state (Fig. 5E). This classification captures specific MD conformations that are in specific agreement with our analysis of the nucleotide pockets (Fig. 4). Thus, in an actively translocating hexamer, MD conformational changes likely propagate around







the hexamer in accordance with nucleotide state. The MD could function to lock the ATP state for protomers that are in contact with substrate (P2 to P5) and transition to a posthydrolysis release state toward the mobile face, thereby allosterically tuning the closed and extended conformational changes that advance substrate.

### NBD1-driven rearrangement engages substrate

To determine how the open and closed states may function together, crvo-EM data sets of wild-type and mutant Hsp104 incubated with different nucleotides were analyzed by 2D and 3D classification methods (Fig. 6A and fig. S11A). As expected, AMP-PNP and ADP data sets classify with 100% of the data matching the open conformation. With ATP incubations, alone and with substrate, Hsp104 primarily adopts the open conformation (>80%); however, a notable fraction (10 to 20%) are in the closed state. A 3D reconstruction of the Hsp104-ATP structure was determined to 6.7 Å and is identical to the AMP-PNP- and ADP-bound structures (cross-correlation = 0.98) (fig. S11B). Thus, during active hydrolysis (fig. S8E), the open state is favored; however, both conformations exist in equilibrium. With ATP-\gamma-S, nearly 80% of hexamers are in the closed state (Fig. 6A), which increases to 100% with casein, demonstrating that ATP- $\gamma$ -S and substrate together trigger complete conversion to the closed state.

The sensor 1 ATPase mutants (*18*), T317A in NBD1 and N728A in NBD2 (T, threonine; A, alanine; N, asparagine), were investigated to determine the role of NBD1 and NBD2 function

(Fig. 6A). In contrast to wild type, T317A incubated with ATP or ATP-y-S classifies with 100% matching the open state, indicating that a hydrolysis-active NBD1 promotes the closed conformation. Conversely, ~80 and 60% of N728A hexamers match the closed state in the presence of ATP and ATP-7-S, respectively. In caseinbinding experiments, N728A binds with high affinity ( $K_{\rm d} \sim 33$  nM); in contrast, wild type and T317A show weak binding ( $K_d > 2 \mu M$ ) (Fig. 6B) in the presence of ATP. With ATP-\gamma-S, both wild type and N728A bind with a high affinity ( $K_d \sim 16$ to 20 nM), whereas T317A has a reduced affinity in comparison ( $K_d \sim 1.4 \ \mu M$ ) (Fig. 6C). Thus, Hsp104 exists in an open-to-closed conformational equilibrium that is differentially controlled by NBDs. Hydrolysis by NBD1 promotes the closed state, but hydrolysis by NBD2 favors the open state. Substrate binding and the open-to-closed conformational change are coupled and likely driven by NBD1 function, whereas NBD2 may be important for substrate-release steps.

Massive conformational changes are required to transition between the open and closed states (Fig. 6D and movie S4). In the open state, protomer P1 is in the topmost position, and the hexamer adopts a left-handed spiral with P6 ~ 50 Å below, along the axial channel. Upon conversion to the closed state, P6 shifts by ~65 Å and rotates toward the channel by ~60° (fig. S12), resulting in a right-handed spiral and a channel that has narrowed by ~10 Å. Considering that substrate interactions and hydrolysis by NBD1 are critical for the closed state (Fig. 6, A to C), we propose that this large conformational change drives substrate-binding and -release steps of the cycle (Fig. 6D). When the conformational change is modeled with substrate, nearly 30 residues can be translocated into the channel. Additionally, similar open "lock-washer" conformations are populated by other translocases (*19–22*), which may represent a conserved off state.

Disaggregation involves nonprocessive and processive mechanisms (23, 24). Cycling between the open and closed states may enable nonprocessive bind and release "pulling" events. Alternatively, the two different substrate-bound states suggest a processive mechanism whereby two protomers undergo ratchet-like conformational changes that enable substrate-binding and -release steps to occur while the hexamer remains engaged. This rotary-like mechanism could drive disaggregation when coupled to stepwise cycles of ATP hydrolysis around the ring (movie S2). Such a cooperative mechanism could enable dissolution of more stable aggregates or amyloids (23). Although this mechanism contrasts with stochastic models proposed for ClpX (1), Hsp104 may exhibit different conformational cycles tuned to different substrates (23). Notably, both extended and closed states reveal a precise 6 to 7 Å separation of the pore loop-substrate contacts. A two-amino acid step involving conformational changes at the spiral interface would continually maintain this register during translocation. This pore-loop spacing is observed in related AAA+ rings (25-27) and represents a conserved feature of translocases. Although additional states are likely involved, our structures reveal a substrate-dependent structural plasticity for Hsp104, which could enable



#### **Fig. 6. Nucleotide-state and NBD function in the open-closed conformations and model for the disaggregation cycle.** (**A**) The fraction of open and closed conformations, determined by 3D classification analysis, is shown for wild-type Hsp104 and sensor 1 ATPase mutants (T317A in NBD1 and N728A in NBD2) after incubation with indicated nucleotides and substrate (-PNP stands for AMP-PNP; - $\gamma$ -S stands for ATP- $\gamma$ -S). (**B** and **C**) FITC-casein (60 nM) binding to Hsp104 wild type (black) and mutants T317A (green) and N728A (red) in the presence of (2 mM) ATP (B) or ATP- $\gamma$ -S (C). Values represent mean ± SD (n = 3). (**D**) Proposed models for nonprocessive and processive modes of translocation involving open-to-closed conformational change upon substrate engagement and release and ratchet-like open-to-extended protomer changes that occur around the hexamer during cycles of ATP hydrolysis.

adaptable mechanisms of protein disaggregation (2, 23).

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#### SUPPLEMENTARY MATERIALS

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# Ratchet-like polypeptide translocation mechanism of the AAA+ disaggregase Hsp104

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Untangling aggregates one step at a time Conserved AAA+ protein complexes exploit adenosine triphosphate hydrolysis to unfold and disaggregate their substrates in response to cell stress, but exactly how they do this has been unclear. Gates et al. determined high-resolution cryo-electron microscopy structures of the Hsp104 disaggregase bound to an unfolded polypeptide substrate in its channel. The structures reveal substrate interactions and two different translocation states. Hsp104 undergoes conformational changes that drive movement along the substrate by two-amino-acid steps. These states help explain how this molecular machine can solubilize protein aggregates and amyloids. Science, this issue p. 273

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