

13. Takenaka, I.M., Leung, S.M., McAndrew, S.J., Brown, J.P. & Hightower, L.E. *J. Biol. Chem.* **270**, 19839–19844 (1995).
14. Mayer, M.P. *et al. Nat. Struct. Biol.* **7**, 586–593 (2000).
15. De Los Rios, P. & Barducci, A. *eLife* **3**, e02218 (2014).
16. Misselwitz, B., Staeck, O., Matlack, K.E. & Rapoport, T.A. *J. Biol. Chem.* **274**, 20110–20115 (1999).
17. Hagai, T. & Levy, Y. *Proc. Natl. Acad. Sci. USA* **107**, 2001–2006 (2010).
18. Goloubinoff, P. *Swiss Med. Wkly.* **146**, w14306 (2016).
19. Labbadia, J. & Morimoto, R.I. *Annu. Rev. Biochem.* **84**, 435–464 (2015).
20. Mattoo, R.U., Sharma, S.K., Priya, S., Finka, A. & Goloubinoff, P. *J. Biol. Chem.* **288**, 21399–21411 (2013).
21. Nillegoda, N.B. *et al. Nature* **524**, 247–251 (2015).

## Sending protein aggregates into a downward spiral

Steven E Glynn & Peter Chien

Cells deploy the Hsp100 family of ATP-dependent machines to work with cellular chaperones in dismantling dangerous protein aggregates. New studies reveal an unprecedented spiral structure that provides mechanistic insight into the protein disaggregase Hsp104.

Protein aggregation is a toxic consequence of protein misfolding that must be resolved. AAA+ disaggregases such as Hsp104 and ClpB capture the energy of ATP hydrolysis to facilitate the recovery of proteins sequestered in aggregates. Often these machines collaborate with the Hsp70 (DnaK) chaperone systems to refold the freed proteins<sup>1</sup>. Given that protein aggregates are heterogeneous in structure, how the Hsp104 family performs its critical functions on such diverse clients is an unanswered question. Prior lower-resolution electron microscopy studies have suggested that Hsp104 exists as a flattened closed ring<sup>2,3</sup>, similarly to other AAA+ enzymes, such as ClpX and ClpC<sup>4,5</sup>. In this issue, a new higher-resolution cryo-EM structure of Hsp104 by Yokom *et al.*<sup>6</sup> provides an extraordinary view of this crucial enzyme, showing that it forms an unprecedented double-ring asymmetric spiral structure.

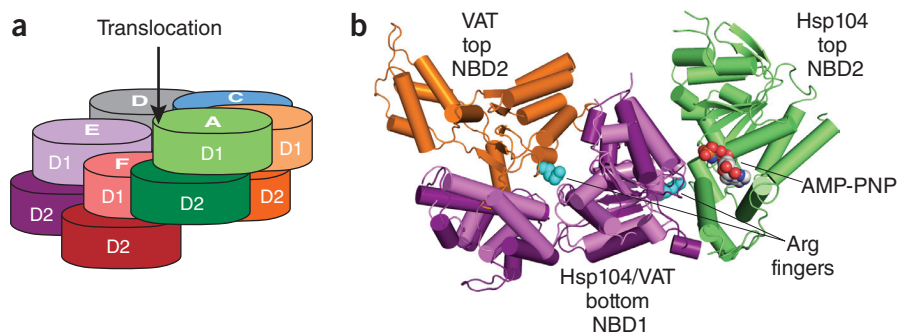
Structures of several assembled AAA+ enzymes have been determined by both crystallography and cryo-EM. Given that many AAA+ enzymes assemble from multiple identical subunits, they might be expected to form closed symmetric rings. However, considerable asymmetry is commonly observed in both the protein architecture and the varied interaction of the ATP-binding sites with nucleotide<sup>7–11</sup>. These structures exhibit diversity in the relative height of the subunits in the ring, the orientation of subdomains and the position of conserved substrate-contacting residues in the central channel. Attempting to reconcile this array of structures with a general mechanism for coupling ATP hydrolysis to the remodeling of

biomolecules is an ongoing challenge. In most cases, these structures form closed rings that limit the possible conformational changes that can be envisaged during the catalytic cycle.

Hsp104 assembles from six identical subunits, each containing two nucleotide-binding domains (NBDs). In the structure by Yokom *et al.*<sup>6</sup>, rather than adopting a closed arrangement of discrete stacked NBD rings, the AMP-PNP-bound Hsp104 assembles as a left-handed helix with individual subunits positioned at strikingly different heights in the plane of the ring (Fig. 1). The formation of the spiral is driven by subdomain rotations within NBDs that progressively alter the conformations of each successive subunit. This conformational change in turn opens the conventional central pore into an extended cleft and places the conserved substrate-contacting loops in a staircase arrangement that follows the path of the helix. Interestingly, the helical turn is extended by the formation of a seam that connects the NBD1 and NBD2 domains of two adjacent subunits found at the ‘bottom’ and ‘top’ positions of the helix, respectively (Fig. 1a). Density for AMP-PNP is observed at the binding site

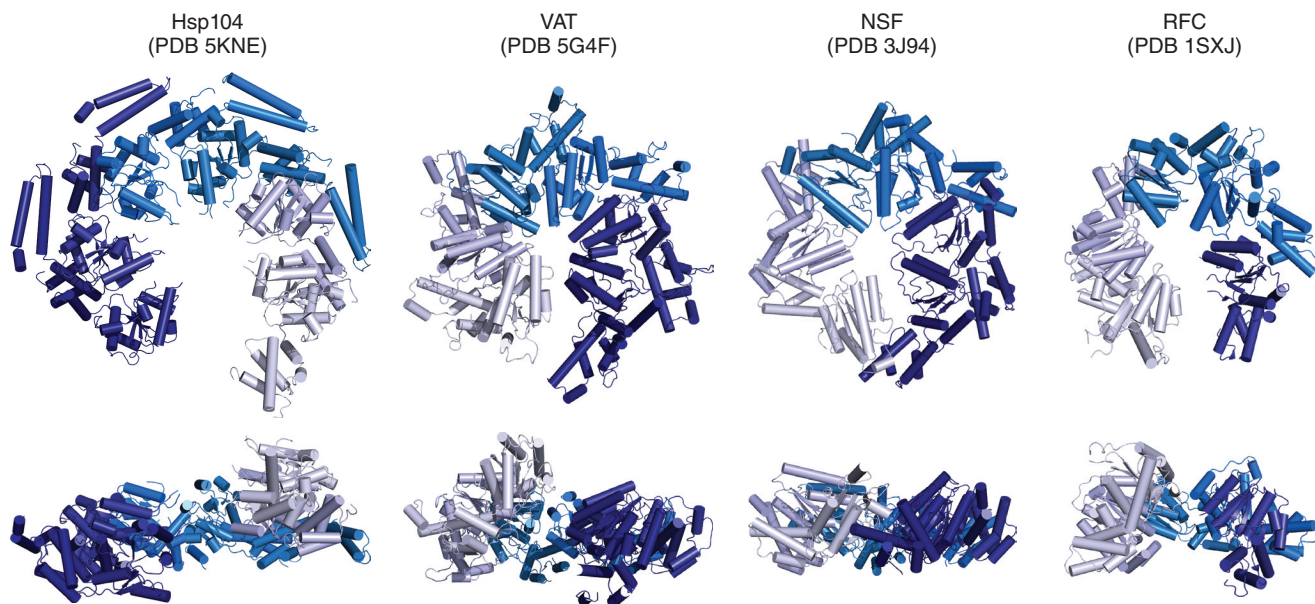
formed between these two domains, and close examination of the interactions at the seam reveals that arginine-finger side chains, which typically bridge the interface and regulate ATP hydrolysis, are displaced a short distance from their functional position.

Helical or split-ring structures have been observed in several studies on other AAA+ enzymes. The archaeal VAT, another double-NBD family member and a homolog of eukaryotic Cdc48 (p97), has recently been shown to form a right-handed spiral in the presence of ADP (Fig. 1b), whereas it can also adopt a stacked-ring configuration when bound to ATP- $\gamma$ S<sup>12</sup>. Importantly, the existence of this spiral in solution has been validated by chemical cross-linking and TROSY NMR experiments. As with Hsp104, the helical structure of VAT is also stabilized by an interface between the NBD1 and NBD2 domains of the subunits that flank the seam<sup>12</sup>. However, the difference in the handedness of the helix produces an interface distinct from that seen in Hsp104 (Fig. 1b). Split-ring conformations with less pronounced helical pitches have also been observed for the *N*-ethylmaleimide-sensitive factor (NSF)



**Figure 1** AAA+ enzymes can form diverse double-ring spirals. (a) Cartoon showing the left-handed helical architecture of Hsp104. Nucleotide-binding domains are colored light (NBD1) or dark (NBD2). This arrangement creates an interaction between the NBD1 of subunit F and the NBD2 of subunit A. (b) Overlay of Hsp104 NBD1 (light purple) and VAT NBD1 (dark purple) from subunits found in the bottom positions of their respective spirals, showing the relative position of the interacting Hsp104 (green) and VAT (orange) NBD2 domains from the adjacent top subunit. Side chain positions of putative arginine-finger residues (blue) from both interfaces and the position of bound AMP-PNP in Hsp104 are shown as space-filling models.

Steven E. Glynn is at the Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, New York, USA. Peter Chien is at the Department of Biochemistry and Molecular Biology, University of Massachusetts Amherst, Amherst, Massachusetts, USA. e-mail: [steven.glynn@stonybrook.edu](mailto:steven.glynn@stonybrook.edu) or [pchien@biochem.umass.edu](mailto:pchien@biochem.umass.edu)



**Figure 2** Examples of helical architectures across diverse AAA+ enzymes. Fitted cryo-EM or crystal structures of AAA+ domains of only the NBD1 rings are shown from Hsp104 (ref. 6), VAT<sup>12</sup>, NSF<sup>13</sup> and the eukaryotic clamp loader RFC<sup>16</sup>. Structures are shown from two views related by 90° and are colored light blue (top) to dark blue (bottom), reflecting their position in the spiral.

component of the SNARE complex<sup>13</sup> but without the connection of the two NBD rings that is so striking in Hsp104 and VAT.

What are the functional implications of this spiral structure in Hsp104? Several speculations emerge, given the nature of Hsp104's substrates. The open top cleft created by the spiral structure affords easy access to the continuous path of substrate-binding loops in the lumen. This feature is particularly useful, given the heterogeneity and size of aggregated substrates. Hsp104 family proteins have an M domain that interacts with the Hsp70 chaperones and facilitates substrate disaggregation<sup>1</sup>. On the basis of the M-domain positioning on other protomers, the M domains of the subunits surrounding the cleft are well poised to provide an entry point for Hsp70 clients to enter or exit the spiral binding cavity, although these specific domains are disordered in the reported structure. Once a substrate is engaged at the top end of this spiral, there is a natural continuous path for polypeptide-chain binding, stair stepping through the chamber.

The highly asymmetric nature of the structure reported by Yokom *et al.*<sup>6</sup> also helps to explain observations that the disaggregation activity of Hsp104 alone is highest if some of the protomers are not actively hydrolyzing ATP<sup>14</sup>. One interpretation is that the subunits flanking the seam are incapable of hydrolysis, and the other subunits provide energy

for conversion between ring conformations, thus leading to movement of protomers along the structural seam. These types of changes are highly similar to those of the replication clamp-loader complexes (Fig. 2), in which a pentameric AAA+ machine contains three active ATP-hydrolyzing subunits and two inactive subunits that cap the incomplete ring. In those systems, ATP binding induces formation of a short spiral ring structure that wrenches open the replication sliding clamp, and hydrolysis results in resetting the ring with subsequent release of the clamp<sup>15</sup>. If ATP-hydrolysis cycles in Hsp104 result in transitions between split- and stacked-ring conformations, as seen with the VAT structure<sup>12</sup>, then compression and expansion of the spiral substrate-binding sites may generate the force needed for disaggregation.

With these increasingly higher-resolution snapshots of protein-remodeling machines, we are edging closer to knowing how these enzymes restore proteins locked into aggregated states. There are many unresolved questions yet to address. How does Hsp70 functionally work with Hsp104, given these new structural insights? How does the unexpected double-ring-spiral nature of the substrate-binding sites contribute to protein disaggregation? What motion exerted after ATP hydrolysis allows for client remodeling? More generally, the identification of such an unexpected structure of Hsp104 prompts questions regarding how other AAA+ machines might be

organized at the atomic level. We eagerly await the answers to these questions as technical developments, particularly in cryo-EM, pave the way to understanding the molecular structures of these fascinating enzymes.

#### ACKNOWLEDGMENTS

The authors thank J. Rubenstein for sharing coordinates before release. Work in the authors' laboratories is supported by NIH grants R01GM111706 (P.C.) and R01GM115898 (S.E.G.).

#### COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

- Mogk, A., Kummer, E. & Bukau, B. *Front. Mol. Biosci.* **2**, 22 (2015).
- Wendler, P. *et al. Mol. Cell* **34**, 81–92 (2009).
- Lee, S., Sielaff, B., Lee, J. & Tsai, F.T. *Proc. Natl. Acad. Sci. USA* **107**, 8135–8140 (2010).
- Glynn, S.E., Martin, A., Nager, A.R., Baker, T.A. & Sauer, R.T. *Cell* **139**, 744–756 (2009).
- Wang, F. *et al. Nature* **471**, 331–335 (2011).
- Yokom, A.L. *et al. Nat. Struct. Mol. Biol.* **23**, 830–837 (2016).
- Matyskiela, M.E., Lander, G.C. & Martin, A. *Nat. Struct. Mol. Biol.* **20**, 781–788 (2013).
- Enemark, E.J. & Joshua-Tor, L. *Nature* **442**, 270–275 (2006).
- Schmidt, H. & Carter, A.P. *Biopolymers* **105**, 557–567 (2016).
- Stinson, B.M. *et al. Cell* **153**, 628–639 (2013).
- Tan, D., Blok, N.B., Rapoport, T.A. & Walz, T. *FEBS J.* **283**, 986–992 (2016).
- Huang, R. *et al. Proc. Natl. Acad. Sci. USA* **113**, E4190–E4199 (2016).
- Zhao, M. *et al. Nature* **518**, 61–67 (2015).
- Doyle, S.M. *et al. Nat. Struct. Mol. Biol.* **14**, 114–122 (2007).
- Kelch, B.A. *Biopolymers* **105**, 532–546 (2016).
- Bowman, G.D., O'Donnell, M. & Kuriyan, J. *Nature*, **429**, 724–730 (2004).