A new way of D/Ealing with protein misfolding

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Summary

Huang et al. show that proteins containing aspartate- and glutamate-rich stretches represent a putative new class of ATP-independent molecular chaperones that operate on diverse client proteins *in vitro* and protect *bona fide* interactors against aggregation in cells.

Main Text

Functional protein folds often place proteins on the knife's edge between solubility and aggregation. Mutations or environmental stressors upset this balance and induce misfolding, or the appearance of non-native conformations. Misfolded proteins expose hydrophobic regions that promote formation of protein aggregates seen in many pathological states. To combat misfolding, cells arm themselves with chaperones to help fold proteins and maintain proteome solubility (Balchin et al., 2016). With few exceptions, chaperoning was typically considered the domain of heat-shock proteins and their constitutively expressed homologs. Much of what is known about chaperone mechanisms comes from studying this family, including the ATP-dependent workhorse chaperone Hsp70. Huang and colleagues now identify DAXX as a member of a new class of protein folders that behave unlike traditional chaperones (Huang et al., 2021).

DAXX plays roles in many processes including p53 tumor suppressor regulation, nucleosome deposition, and apoptotic signaling (Mahmud and Liao, 2019). Like 44 other human proteins, DAXX possesses an aspartate- and glutamate-rich region (polyD/E). Huang et al. show that DAXX and two similar proteins (ANP32A and SET) utilize polyD/E to chaperone client proteins by blocking aggregation, refolding misfolded intermediates, and even solubilizing pre-existing aggregates. While it is unlikely that the model clients tested in their experiments are physiological substrates, the authors demonstrate that DAXX exerts chaperone-like effects on likely physiological clients. For instance, depletion of DAXX reduces p53 solubility in cells, suggesting that the previously-established role of DAXX in p53 regulation may relate to DAXX's chaperone function. Surprisingly, polyD/E-containing proteins achieve these chaperone activities without traditional client-binding domains or hydrolyzing ATP.

To help clients fold, chaperones must discriminate between native and misfolded states. Native structures typically bury hydrophobic residues in the core of the fold. Exposure of hydrophobic residues on the protein's exterior is therefore a marker for protein misfolding. Chaperones exploit this marker to target misfolded proteins. For example, the substrate binding domain of

Hsp70 contains a groove that binds linear peptide stretches containing hydrophobic and basic residues (Mayer and Gierasch, 2019). Such a stretch is likely unavailable for binding on native proteins, accounting for how Hsp70 recognizes misfolded proteins.

DAXX too can discriminate between native and misfolded proteins, but likely uses a different mechanism. Huang and colleagues show that DAXX strongly prefers to bind basic lysine and arginine residues, with an additional, albeit weaker, preference for several hydrophobic residues. The function of DAXX depends on electrostatic interactions, prompting the hypothesis that polyD/E mediates client recognition by binding positively charged regions. Native states often expose charged residues like lysine and arginine to solvent, making the residues that DAXX preferentially binds equally accessible in native and misfolded structures. DAXX may therefore detect misfolded proteins using a compound motif, such as the border between hydrophobic and basic regions. Indeed, the ubiquitin ligase UBE2O, though lacking a polyD/E stretch, recognizes hydrophobic-basic borders when ubiquitylating components of unassembled protein complexes (Yanagitani et al., 2017). In case of DAXX, polyD/E alone does not suffice for chaperoning, indicating that other domains could contribute to the affinity for hydrophobic residues. Future work investigating DAXX client recognition will expand our knowledge about the structural markers cells use to detect misfolded proteins.

While simply binding to misfolded regions enables some chaperones to block aggregation, ATP-dependent chaperones like Hsp70 couple their activities to ATP hydrolysis to overcome energetic barriers in protein folding (Imamoglu et al., 2020; Mayer and Gierasch, 2019). The use of ATP is particularly salient in disaggregation of amyloid fibrils, protein polymers with hyperstable cross-β-sheet structures. *In vitro* work from Wentink et al. shows how Hsp70 disaggregates fibrils of the amyloidogenic protein α-synuclein in cooperation with the ATPase activator Hsp40 and the nucleotide exchange factor Hsp110 (Wentink et al., 2020). These two co-chaperones recruit and selectively recycle Hsp70 along the fibril by toggling Hsp70 between its low-affinity (ATP-bound) and high-affinity (ADP-bound) states. This ATP-cycling interplay results in clusters of Hsp70 clamped onto the fibril. Clustering of Hsp70 then generates an entropic penalty, creating a thermodynamic state where fibril disassembly becomes favorable.

DAXX also has the capacity to solubilize protein aggregates, including those of p53 and several neurodegenerative disease proteins, but not α-synuclein fibrils (Huang et al., 2021). Strikingly, DAXX mediates disaggregation alone without co-chaperones or ATP. How a standalone protein could mechanistically accomplish this feat is unknown. One possibility is that DAXX interacts with aggregates in a way that destabilizes monomer-monomer interactions, perhaps initially sampling exposed, positively-charged segments with polyD/E (Figure 1). In the case of p53, DAXX likely binds the basic C-terminus, which borders the hydrophobic core of the aggregate (Wang and Fersht, 2017). A subsequent interaction between a non-polyD/E domain and the hydrophobic core could stabilize the DAXX-aggregate complex while prying apart the contacts that buttress the aggregate. Furthermore, clustering of the globular N-terminus of DAXX in this region may introduce an entropic disturbance in the vicinity of the core, similar to Hsp70 acting on α-synuclein (Wentink et al., 2020). This “entropic crowbar” effect could then destabilize the aggregate. Biophysical studies will be needed to understand this mechanism and may illuminate different strategies that cells use to disassemble protein aggregates.

DAXX and the other polyD/E-containing proteins join a growing club of multifunctional proteins that facilitate protein folding without hydrolyzing ATP. For example, the nuclear import receptor karyopherins antagonize fibrillization of several amyloidogenic proteins (Guo et al., 2018). Cyclophilin 40, a chaperone with peptide-prolyl isomerase activity, can fracture amyloid fibrils by twisting them at proline residues (Baker et al., 2017). These atypical chaperones may play key

roles in cell biology that we are only beginning to appreciate. Like DAXX, some perform a variety of chaperone-like activities *in vitro*, protecting against aggregation, refolding proteins, and dissolving aggregates. Identifying which of these activities contribute to their effects on clients *in vivo* will be a crucial next step. A deeper mechanistic understanding will perhaps enable us to develop variants whose activities can be steered toward problematic proteins.

References

Baker, J.D., Shelton, L.B., Zheng, D., Favretto, F., Nordhues, B.A., Darling, A., Sullivan, L.E., Sun, Z., Solanki, P.K., Martin, M.D.*, et al.* (2017). Human cyclophilin 40 unravels neurotoxic amyloids. PLoS Biol *15*, e2001336.

Balchin, D., Hayer-Hartl, M., and Hartl, F.U. (2016). In vivo aspects of protein folding and quality control. Science *353*, aac4354.

Guo, L., Kim, H.J., Wang, H., Monaghan, J., Freyermuth, F., Sung, J.C., O'Donovan, K., Fare, C.M., Diaz, Z., Singh, N.*, et al.* (2018). Nuclear-Import Receptors Reverse Aberrant Phase Transitions of RNA-Binding Proteins with Prion-like Domains. Cell *173*, 677-692 e620.

Huang, L., Agrawal, T., Zhu, G., Yu, S., Tao, L., Lin, J., Marmorstein, R., Shorter, J., and Yang, X. (2021). DAXX represents a new type of protein-folding enabler. Nature *597*, 132-137.

Imamoglu, R., Balchin, D., Hayer-Hartl, M., and Hartl, F.U. (2020). Bacterial Hsp70 resolves misfolded states and accelerates productive folding of a multi-domain protein. Nat Commun *11*, 365.

Mahmud, I., and Liao, D. (2019). DAXX in cancer: phenomena, processes, mechanisms and regulation. Nucleic Acids Res *47*, 7734-7752.

Mayer, M.P., and Gierasch, L.M. (2019). Recent advances in the structural and mechanistic aspects of Hsp70 molecular chaperones. J Biol Chem *294*, 2085-2097.

Wang, G., and Fersht, A.R. (2017). Multisite aggregation of p53 and implications for drug rescue. Proc Natl Acad Sci U S A *114*, E2634-E2643.

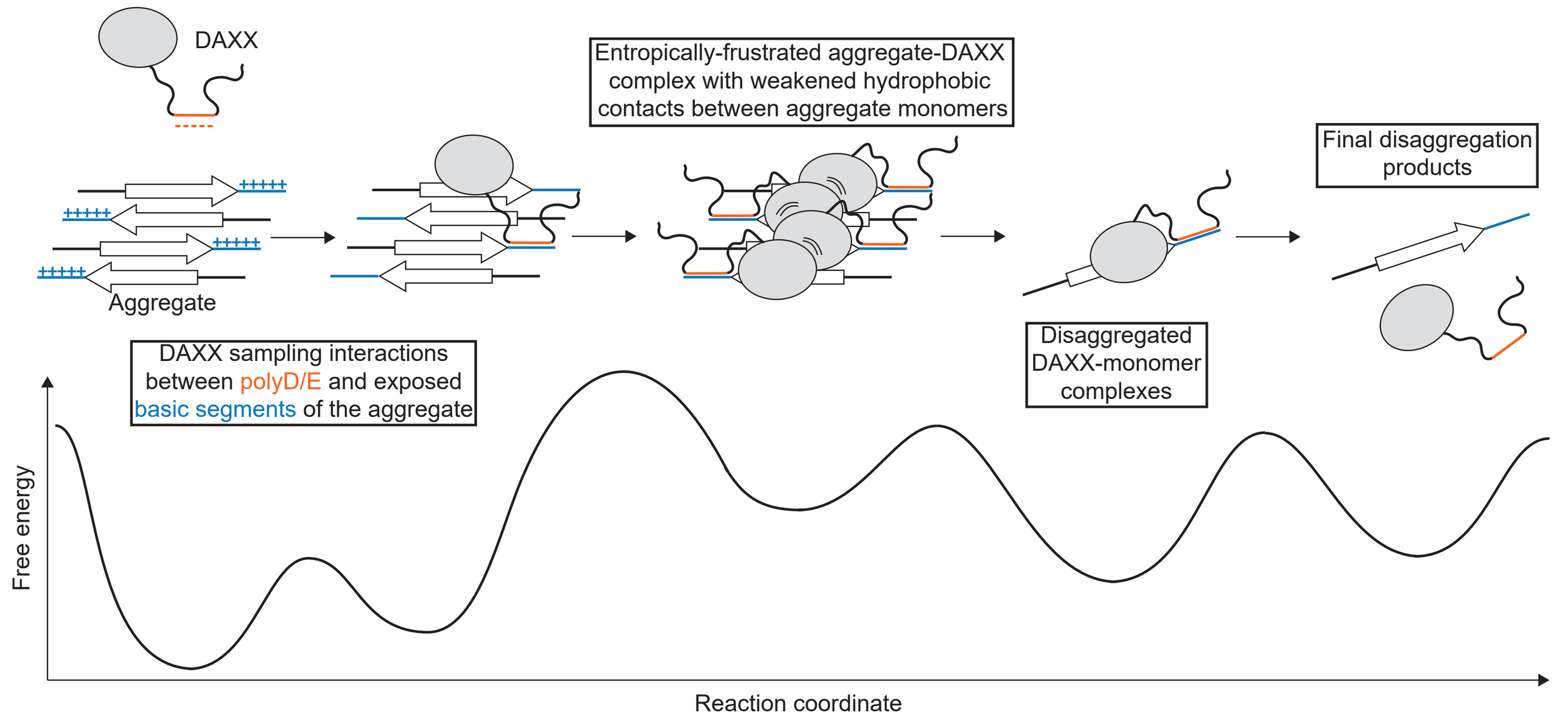
Wentink, A.S., Nillegoda, N.B., Feufel, J., Ubartaite, G., Schneider, C.P., De Los Rios, P., Hennig, J., Barducci, A., and Bukau, B. (2020). Molecular dissection of amyloid disaggregation by human HSP70. Nature *587*, 483-488.

Yanagitani, K., Juszkiewicz, S., and Hegde, R.S. (2017). UBE2O is a quality control factor for orphans of multiprotein complexes. Science *357*, 472-475.

Figure Legend

Figure 1. Model of DAXX-mediated disaggregation

In our proposed model, DAXX first binds to aggregates through interactions between its polyD/E stretch (red) and basic regions on the aggregate's exterior (blue). Subsequent binding of another DAXX domain (gray oval) to the hydrophobic interior of the aggregate (represented by arrows) has two effects: 1) destabilizing the hydrophobic interactions that hold the aggregate together, and 2) causing steric restrictions that reduce the entropy of the DAXX-aggregate complex. These two effects destabilize the aggregate and drive its disassembly, followed by dissociation of DAXX from the monomers.


DAXX
Aggregate
+++++
+++++
+++++
+++++
Entropically-frustrated aggregate-DAXX complex with weakened hydrophobic contacts between aggregate monomers
Disaggregated DAXX-monomer complexes
Final disaggregation products
DAXX sampling interactions between polyD/E and exposed basic segments of the aggregate
Free energy
Reaction coordinate