Delft University of Technology

Picturing protein disaggregation

Tans, Sander J.

DOI
10.1038/s41589-022-00977-w

Publication date
2022

Document Version
Final published version

Published in
Nature Chemical Biology

Citation (APA)
Tans, S. J. (2022). Picturing protein disaggregation. *Nature Chemical Biology*, *18*(3), 240-241.
https://doi.org/10.1038/s41589-022-00977-w

Important note
To cite this publication, please use the final published version (if applicable).
Please check the document version above.

Copyright
Other than for strictly personal use, it is not permitted to download, forward or distribute the text or part of it, without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license such as Creative Commons.

Takedown policy
Please contact us and provide details if you believe this document breaches copyrights.
We will remove access to the work immediately and investigate your claim.

This work is downloaded from Delft University of Technology.
For technical reasons the number of authors shown on this cover page is limited to a maximum of 10.

Green Open Access added to TU Delft Institutional Repository

'You share, we take care!' - Taverne project

https://www.openaccess.nl/en/you-share-we-take-care

Otherwise as indicated in the copyright section: the publisher
is the copyright holder of this work and the author uses the
Dutch legislation to make this work public.

degrade toxic compounds (Fig. 1b). Bacterial adhesion also improved the characteristics of the material as a bio-ink for 3D printing (Fig. 1c) and, most importantly, enhanced its ability to self-heal, because separated adhering bacteria spontaneously reconnect within minutes when put back in contact after damage. The engineered bacteria were also incorporated into a biohybrid material using alginate as a bulk polymer, creating size-tunable filaments that encapsulated bacteria alive for over 6 months. In this part of the research, LAMBA was used as conductive 'bio-wires' in a stretchable sensor in a wearable device, and demonstrated improved stretchability compared to the use of just gold wires instead.

LAMBA is an interesting step forward in the progress of ELMs and the first use of the exciting adhesion toolbox from Glass et al.[8]. So far LAMBA has been applied to only one of the adhesive pairs from this work, and using more pairs in various combinations would no doubt enable greater complexity, for example for the self-assembly of layers or other patterns (Fig. 1d). The simplicity of the approach also makes it attractive for others to build upon, especially as the modular component is *E. coli*, one of the most popular organisms for synthetic biology. It is likely that LAMBA can also be applied with other organisms, such as yeast engineered to surface-display similar adhesive pairs, or human cells engineered with cadherins. However, the downside is that the material formed by LAMBA is ultimately just a lot of cells stuck together, so in some ways it is more akin to a sticky bacterial colony, or a region of soft human tissue, than to what we normally think of as materials. The kind of strong, tough material properties that most other ELMs aim for are absent here, owing to the lack of an extracellular matrix around the cells. A next step, therefore, could be to bridge this gap and achieve the simplicity of self-organization seen in LAMBA, but do so with cells engineered to secrete and construct a material matrix that more strongly interconnects the cells it then surrounds. Indeed, this is the very strategy used by plant cells in wood and human cells in bone, showing once again how nature is the ideal source of inspiration for this kind of research. ❒

Joaquin Caro-Astorga and Tom Ellis ✉
Department of Bioengineering, Imperial College London, London, UK.
✉*e-mail: t.ellis@imperial.ac.uk*

Published online: 21 December 2021
https://doi.org/10.1038/s41589-021-00946-9

References
1. Nguyen, P. Q., Courchesne, N.-M. D., Duraj-Thatte, A., Praveschotinunt, P. & Joshi, N. S. *Adv. Mater.* **30**, 1704847 (2018).
2. Gilbert, C. et al. Living materials with programmable functionalities grown from engineered microbial co-cultures. *Nat. Mater.* **20**, 691–700 (2021).
3. Rivera-Tarazona, L. K., Campbell, Z. T. & Ware, T. H. *Soft Matter* **17**, 785–809 (2021).
4. Srubar, W. V. *Trends Biotechnol.* **39**, 574–583 (2021).
5. Chen, B. & Dai, Z. *Nat. Chem. Biol.* https://doi.org/10.1038/s41589-021-00934-z (2021).
6. Caro-Astorga, J., Walker, K. T., Herrera, N., Lee, K.-Y. & Ellis, T. *Nat. Commun.* **12**, 5027 (2021).
7. Schaffner, M., Rühs, P. A., Coulter, F., Kilcher, S. & Studart, A. R. *Sci. Adv.* **3**, eaao6804 (2017).
8. Glass, D. S. & Riedel-Kruse, I. H. *Cell* **174**, 649–658.e16 (2018).

Competing interests
The authors declare no competing interests.

PROTEOSTASIS

Picturing protein disaggregation

Just 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 the key to revealing their underlying cooperation in time and space.

Sander J. Tans

Over time, proteins can lose their properly folded structures, exposing 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 status remains unclear[2]. Many of the core mechanisms that allow aggregates to be recognized, and broken up into free monomers, remain poorly understood[3,4]. Within bacterial and yeast cells, the Hsp100 class of chaperones are known to play an important role. With a doughnut-like shape and central pore, Hsp100 chaperones were recently shown to processively translocate polypeptide chains[5], and they are hence thought to 'extract' proteins from aggregates. Hsp100 chaperones (Hsp104 in yeast) work in concert with the Hsp70 system, which is known to bind both the aggregate and Hsp100, and which itself possesses aggregation-prevention and disaggregation capabilities[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 showed that 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[8].

The core assay performed by Nagakawa et al. is conceptually straightforward (Fig. 1)[8]. Amyloid fibrils (Sup35NM) in 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 labeled and are 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 s later, and finally the amyloid fluorescence decreases — indicating fibril fracture — right where the chaperone levels peak. In these experiments, too many chaperones were bound to identify individual copies. Hence, the authors repeated the process but this time mixing fluorescent and non-fluorescent chaperones to lower the overall intensity. Now, single Hsp104 chaperones could be seen binding the fibrils, but also leaving again after about 3–30 s, yielding repeated Hsp104 binding and unbinding. These data suggested an

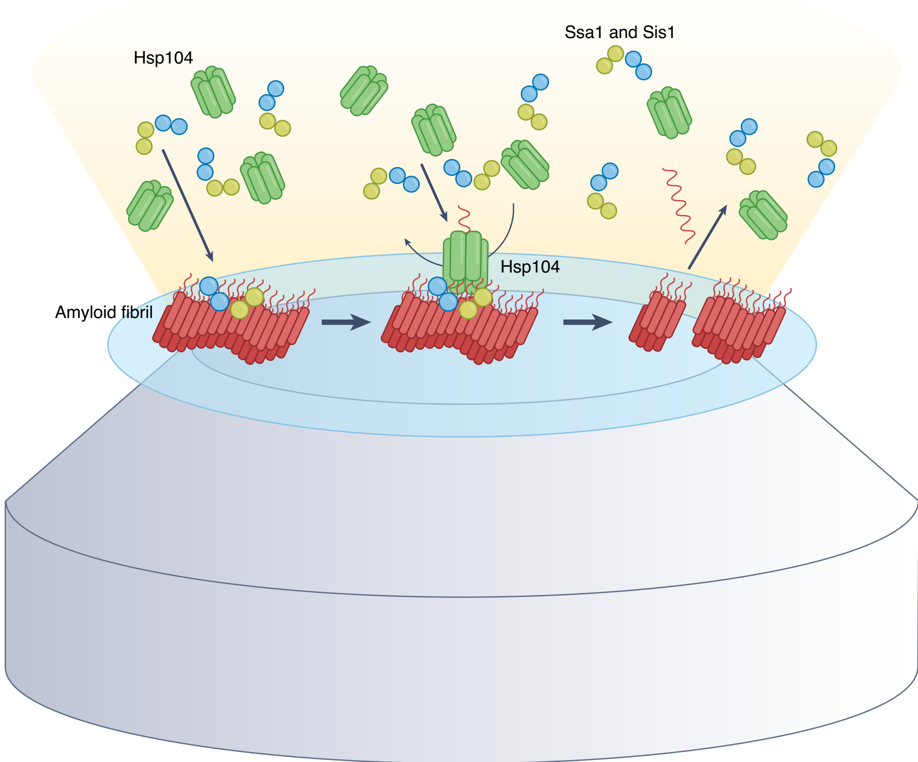

Fig. 1 | Single-molecule observation of amyloid disaggregation by Hsp104. Total internal fluorescence microscopy (TIRF) of Sup35NM amyloid fibrils and interacting chaperones (Ssa1 (Hsp70), Sis1 (Hsp40) and Hsp104). Hsp70 and Hsp40, which are part of the Hsp70 system, are found to bind the amyloid fibrils first, followed by the disaggregase Hsp104 (green), which can translocate protein chains through its central pore and hence cause fibril fracturing by extracting Sup35NM monomers.

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 the existence of pervasive, hidden spatiotemporal dynamics, and methods such as those advanced here will be key to elucidating them. Indeed, simply visualizing where and in what 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 hypotheses that are subsequently carefully tested. For instance, the authors could distinguish the discrete fragmentation from a more global and gradual fibril dissolution, while features of gradual dissolution were also observed in the fragmentation data, and additional controls provided further support for the existence 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 subpopulation of Hsp104 can be followed, actively extracting Hsp104 molecules are not directly identified and the spatial resolution is obviously bounded by the diffraction limit. Freshly created fibril ends may be hotspots for chaperone recruitment, though pre-existing fibril ends did not appear to be 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], which subsequently drives Sup35NM insertion. High local Hsp70 concentrations on the fibril surface may enable stable binding of Hsp104 to multiple Hsp70 molecules, which may relate to how Hsp104 avoids erroneous targeting of native proteins. A hint 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 in which 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 substrate conformations, which are central to the process.

The approach presented here adds to a rapidly expanding experimental arsenal for the study of protein dynamics, including FRET, optical tweezers, cryo-EM and various others[9,10]. Indeed, with these technological capabilities, we are entering a new phase in which we are finally able to visualize the spatiotemporal dynamics that underpins protein homeostasis within cells. ❒

Sander J. Tans[1,2] ✉
[1]*AMOLF, Amsterdam, the Netherlands.*
[2]*Bionanoscience Department, Kavli Institute of Nanoscience Delft, Delft University of Technology, Delft, the Netherlands.*
✉*e-mail: tans@amolf.nl*

Published online: 17 February 2022
https://doi.org/10.1038/s41589-022-00977-w

References

1. Dobson, C. M. *Nature* **426**, 884–890 (2003).
2. Valastyan, J. S. & Lindquist, S. *Dis. Model Mech.* **7**, 9–14 (2014).
3. Rosenzweig, R., Nillegoda, N. B., Mayer, M. P. & Bukau, B. *Nat. Rev. Mol. Cell Biol.* **20**, 665–680 (2019).
4. Tyedmers, J., Mogk, A. & Bukau, B. *Nat. Rev. Mol. Cell Biol.* **11**, 777–788 (2010).
5. Avellaneda, M. J. et al. *Nature* **578**, 317–320 (2020).
6. Nillegoda, N. B. et al. *Nature* **524**, 247–251 (2015).
7. Mashaghi, A. et al. *Nature* **539**, 448–451 (2016).
8. Nakagawa, Y. et al. *Nat. Chem. Biol.* https://doi.org/10.1038/s41589-021-00951-y (2022).
9. Avellaneda, M. J., Koers, E. J., Naqvi, M. M. & Tans, S. J. *Protein Sci.* **26**, 1291–1302 (2017).
10. Bai, X. C., McMullan, G. & Scheres, S. H. W. *Trends Biochem. Sci.* **40**, 49–57 (2015).

Competing interests

The author declares no competing interests.