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State of aggregation

It is critical that studies examining the functional consequences of aggregated proteins clearly identify the exact source and aggregation state of the protein and critically discuss the implications of their approach.

The huge literature examining the functional consequences of disease-associated protein aggregates, especially those implicated in neurodegenerative diseases such as Alzheimer's disease, has relied, by necessity, on the application of pathogenic proteins into the biological model of choice. Manipulating clumps of proteins such as amyloid beta (Aβ), however, is a sticky proposition. When proteins are polymerized in a test tube to form multimers or even larger insoluble complexes, does the aggregation state accurately reflect its natural state in intact tissue? Does the aggregate purification process itself cause the formation of unnatural protein species? And even if the exact aggregation state of the protein at experiment onset is carefully determined, does the aggregate retain its state when placed in a more complex biological environment? All these issues are critical in our interpretation of the experimental results on how protein aggregates can cause cellular toxicity and dysfunction and can considerably muddy our attempts to reach consensus from different studies. As a first step toward confronting this issue, it is imperative that all papers reporting the effects of aggregated proteins clearly state the exact source of the protein, its state of aggregation when added to the system and discuss the potential caveats of their approach.

Alzheimer's disease is characterized by the aberrant aggregation of the cleaved products of the amyloid precursor protein (APP), most notably Aβ. Mutations in APP or APP-processing enzymes have been genetically linked to Alzheimer's disease susceptibility. Cleaved Aβ fragments can be of different lengths and can take many forms, all of which may behave differently in biological systems. Aβ in a monomeric form is relatively unstructured *in vitro.* Oligomerization can make the fragment more rigid while retaining its aqueous solubility. Further aggregation of Aβ can create an insoluble fibril structure, which is a key component of the amyloid plaques found in individuals with Alzheimer's disease.

Over the last decade, substantial effort has been invested in determining which form(s) of Aβ is primarily responsible for the neurodegeneration and neuronal dysfunction found in individuals with Alzheimer's disease. Although various *in vitro* and *in vivo* models have been devised to try and address this question, considerable discrepancies in the reported results remain. One problem in reconciling these reports comes from the source of Aβ and the degree of its aggregation. Even with the molecular purity of a synthetic Aβ fragment, its *in vitro* aggregation state may not reflect its natural *in vivo* aggregation state. When purified from the brains of individuals with Alzheimer's disease or animal models of Alzheimer's disease, Aβ aggregates may contain multiple species of various Aβ fragments. In such cases, the exact stochiometry of the Aβ aggregate (Aβ1–38, 1–40, etc.) may also influence the results.

As one example of how these issues can muddle the field, a recent study proposed that cellular prion protein (PrP^C) acts as a receptor for oligomeric Aβ[1]. The authors used biotinylated synthetic Aβ1–42 oligomers (mostly made of 50–100 monomers) that were aggregated *in vitro*. They reported that these synthetic Aβ oligomers, but not fibrils or monomeric Aβ1–42, impaired long-term potentiation in $Prnp^{-/-}$ mouse hippocampal slices. A different group, however, failed to replicate the long-term potentiation impairment when using virus-mediated overexpression of the C-terminal fragment of APP in organotypic brain slices (the aggregation state of the resulting Aβ is unclear)[2]. A third study was able to confirm synthetic Aβ1–42 oligomers binding to PrP^C (ref. 3), but found that the synthetic Aβ1–42 oligomers impaired recognition memory performance independently of PrP^C when injected into mice, calling into question the physiological relevance of the Aβ and PrP^C interaction.

Reconciling these disparate results is difficult. The interpretational caveat is made greater by not knowing whether these forms of Aβ are naturally found in the brains of individuals with Alzheimer's disease. Many of the Aβ studies used synthetic, *in vitro*–aggregated Aβ species that may differ greatly from the ones found in the brains of individuals with Alzheimer's disease, especially considering that Aβ oligomers found in these brains may be made up of multiple Aβ species. Although isoform- and conformation-specific antibodies could help address this issue, many of the available Aβ antibodies cross-react with other Aβ species to some extent, making it impossible to unambiguously differentiate between different Aβ states.

Even though assessing the exact state of exogenously applied proteins is no guarantee that their aggregation state is preserved when they interact with a complex biological system, a clear description of the initial state of the protein, its source and its stochiometry will at least ensure that other laboratories can try to precisely duplicate these results. It can also be critical when comparing results across different laboratories. Scientists must be encouraged to not only determine the state of the protein at the onset, but to also try and characterize its specific aggregation state in the context of the specific experimental set up they use. They must also be much more proactive about discussing the potential interpretational caveats of their studies; for example, whether the expression system used produces aggregate levels that are physiologically relevant and/or whether the form of Aβ they used is likely to be biologically relevant.

If we are ever to find ways to alleviate the symptoms of proteinopathy diseases such as Alzheimer's disease, it is crucial to know the pathogenic mechanisms involved. To do this, we need to be able to compare results across various laboratories and reproduce the findings. A clear explanation of the protein source, its state and how it was manipulated is critical. Not doing so is a disservice to both science and the millions affected by these diseases. ■

1. Laurén, J. *et al. Nature* **457**, 1128–1132 (2009).
2. Kessels, H.W. *et al. Nature* **466**, E3–4 (2010).
3. Balducci, C. *et al. Proc. Natl. Acad. Sci. USA* **107**, 2295–2300 (2010).