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EDITORIAL CONTROVERSY

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Alpha-synuclein oligomerization and aggregation: A model will always be a model

This is a response to "Monitoring alpha-synuclein oligomerization and aggregation using bimolecular fluorescen ce complementation assays: What you see is not always what you get".

Read the reply on "Alpha-Synuclein oligomerization and aggregation: All models are useful but only if we know what they model".

The articles are accompanied by a Preface "How good are cellular models?".

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Many different types of protein aggregates have attracted the minds and curiosity of countless *armies* of scientists, that is, laboratories, in the world for more than 100 years. For example, it was in 1912 that F. Lewy reported the presence of structures, eternalized as Lewy bodies (LBs) and Lewy neurites (LNs), in the brains of patients with Parkinson's disease (Lewy, 1912). Since then, tremendous efforts have been dedicated to figuring out how such deposits "cause" Parkinson's disease. In 1997, with the identification of alpha-synuclein (a-syn) as the majr protein component of LBs and LNs (Spillantini et al., 1997) gave us a culprit and, since then, numerous scientists have acted as prosecutors, trying to demonstrate the toxicity of the protein towards the "hard-working" and particularly "vulnerable" dopaminergic neurons from the substantia nigra.

Twenty-three years have passed, data have been collected, hypotheses have been tested, and the causality between LB pathology and disease is still a matter of intense debate.

The problem is that, shockingly, we still have not been able to define whether a-syn is the culprit (or one of the culprits) and, if it is, what forms of a-syn are the most toxic. Accumulating evidence suggests that LBs and LNs might not be the most toxic forms of a-syn and, instead, smaller assemblies, known as oligomeric species, might be more toxic and cause pathology (Outeiro et al. 2008; Karpinar et al. 2009; Winner et al. 2011). Time will tell how this dispute will end.

In the study recently published by Frey and colleagues (Frey et al. 2020), the authors put their skills to work in order to test the validity of an assay applied to the study of the process of a-syn aggregation in 2008 (Outeiro et al. 2008), known as bimolecular fluorescence complementation (BiFC). The assay is based on the use of award-winning proteins in cell biology, owing to their intrinsic fluorescent properties, known as fluorescent proteins. The issue with such assays is that they rely on the production of engineered proteins, that is, artificial, to try to study their subcellular distribution in living cells. Any type of engineered protein tagging, using shorter or longer polypeptide sequences, is likely to alter the normal behavior of the proteins being tagged. The question is not if but rather how much. The strongest argument supporting this unquestionable truth is that even a single point mutation may have dramatic consequences on

protein folding, structure, or localization. Therefore, no one is to be surprised if fusing a-syn to a fluorescent protein, such as GFP, Venus, or any other derivative including fragments of these proteins, may affect its normal behavior. In fact, this has been studied extensively by many in the field.

Frey and colleagues set out to highlight the limitations of the a-syn BiFC assay, and they do it with a critical eye and rigorous methods. They claim that (1) no reports validate the extent to which the BiFC fluorescence signal correlates with a-syn aggregation at the biochemical level; (2) no reports provide a structural characterization of the oligomers and aggregates formed by the BiFC fragments; and (3) no reports investigate the extent to which the oligomers of the fluorescent complex resemble oligomers formed on the pathway to a-syn fibrilization. The findings of this endeavour are important, but they are neither novel nor surprising. We and others have shown, not surprisingly, that the levels of the different fusion proteins used in the assay are different (e.g. Lázaro et al. 2014). In our original study in 2008, using a simpler assay, we already showed that the VN-aSyn fusion protein forms higher-order oligomers than the a-syn-VC protein. Finally, we have also shown in all our studies that, in cells, we cannot normally detect the formation of a-syn inclusions using the BiFC assay. However, we have identified genes that modify a-syn oligomerizatoin/aggregation using the BiFC assay, resulting in the formation of inclusions (Gonçalves et al. 2016).

While we agree with, and commend, the thorough characterization of the BiFC and other non-natural systems where modified versions of a-syn are used, we actually understand what it models and, importantly, what it does not model, and we still believe the merits of using in vitro, in cell, and in vivo models to study the biology and pathobiology of proteins presumed to be connected to human diseases, such as a-syn. No fusion protein or bacterially produced protein is the same as a protein produced in the human brain, and no mouse model is the same as a human patient. Nevertheless, scientific progress relies on, and is accelerated by the use of simplifications and approaches that attempt to mimic aspects deemed relevant in biology and pathological contexts. In synucleinopathies, for example, we need to understand where we are, and to define where we need to go (Brás et al. 2020). Also, we need to make peace with the fact that a model will always be just that—a model.

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