New flow cytometry tricks to view protein conformations in cells and their subcellular localization

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Defining how protein misfolding and aggregation relates to toxicity or cellular dysfunction remains a difficult challenge because of a dearth of approaches that can systematically examine different sized forms *in situ* and relate their presence to specific biological outcomes. This is particularly relevant in light of increasing evidence suggesting oligomers are more toxic than larger aggregates for many aggregating proteins. In addition, cells actively sort misfolded proteins into punctate reservoirs such as aggresomes or inclusions to protect themselves from toxicity caused by dispersed forms of the misfolded proteins. To better understand these processes we have developed a new framework to monitor the “aggregation kinetics” of polyglutamine expanded Huntingtin in cells. The first component employed pulse shape analysis (PulSA), which we recently developed to monitor protein localization changes in mammalian cells by flow cytometry (Ramdzan *et al.*, 2012). The second component combines new tetracysteine and FRET based biosensors to distinguish monomers from oligomers of Huntingtin (Ramdzan *et al.*, 2010). Collectively, these provide time dependent analysis of cells enriched with monomers, oligomers and inclusions and an exciting new platform to which we can assess the fundamental changes in cells enriched with specific aggregate forms of Huntingtin.

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