Electron microscopy highlights the challenges of using fluorescently-tagged proteins for cellular biology

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Proteins drive every cellular process in living organisms - from DNA replication and repair, to metabolism and signalling. In order to understand these fundamental processes of life, we need to understand the role proteins play in the cell. To do this, we need to investigate not only the structure of proteins at a molecular level, but also their cellular distribution. Several types of microscopy are crucial to our understanding of protein biology, particularly electron microscopy and fluorescence microscopy. Transmission electron microscopy enables us to solve the 3-dimensional structure of proteins, giving insight into their mechanism. Fluorescence microscopy allows us to study the sub-cellular location of proteins, giving us an understanding of the roles they play in a cellular context. Typically, fluorescent proteins are fused to target proteins to allow visualization by fluorescence microscopy, and inferences on function can be made based on the localisation.

Here, we use electron microscopy to study the structure of fluorescently-tagged proteins. We studied two bacterial proteins which form large multimers, and show that adding fluorescent tags can dramatically perturb their 3-dimenstional structures. Furthermore, we show that the addition of fluorescent tags can block protein-protein interaction sites. The nature of these changes depends not only on the location of the fluorescent fusion, but also on the identity of the fluorescent protein chosen. Because of this, great care must be taken when interpreting fluorescence microscopy data from fluorescently-tagged oligomeric proteins. Any conclusions about the biological role of a protein or its sub-cellular location may be invalid, as the oligomeric state or binding to partners may be perturbed. Instead, we show that using labelled nanobodies (single-domain antibodies) for immunofluorescence is a much safer alternative when studying the localisation of oligomeric proteins, as this preserves both the oligomerisation state and protein-protein interactions. By using labelled nanobodies instead of fluorescent fusions, observations by fluorescence microscopy are more likely to be biologically relevant, allowing for greater confidence in the data.