

Improved brightness-based oligomerization studies in living cells: Suitability of conventional fluorescent proteins

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Fluorescence fluctuation spectroscopy has become a popular toolbox for minimally invasive studies of molecular interactions and dynamics in living cells. The quantification of e.g. protein oligomerization and copy numbers in the native cellular environment is highly relevant for a detailed understanding of complex signaling pathways and biochemical reaction networks. Of particular interest in these measurements is the molecular brightness, which serves as a direct measure of molecular aggregation and can be easily extracted from temporal or spatial fluorescence fluctuations. However, fluorescent proteins (FPs) typically used in such studies suffer from complex photophysical transitions and limited maturation, potentially causing the existence of non-fluorescent states, which strongly affect molecular brightness measurements. Although these processes have been discussed already, a comprehensive study of this issue is missing.

Here, we investigate the suitability of the widely-used FPs EGFP, EYFP and mCherry for quantifying oligomerization based on the molecular brightness, obtained by Fluorescence Correlation Spectroscopy (FCS) and Number&Brightness (N&B) measurements in living cells. For all three FPs, we measured a lower than expected brightness of FP homodimers, allowing us to estimate, for each fluorescent label, the probability of fluorescence emission in a simple two-state model. By measuring higher FP homo-oligomers, we show that the oligomeric state of protein complexes can only be accurately quantified if this probability is taken into account. We verify this procedure by measuring the oligomerization of the Influenza virus Hemagglutinin (HA) protein in the plasma membrane. Further, we test different recently developed red FPs and provide strong evidence that mCherry2, an mCherry variant, possesses a superior fluorescence probability. We finally conclude that this leads to an improved quantification in fluorescence cross-correlation spectroscopy measurements and propose to use EGFP and mCherry2 as the novel standard pair for studying biomolecular hetero-interactions.

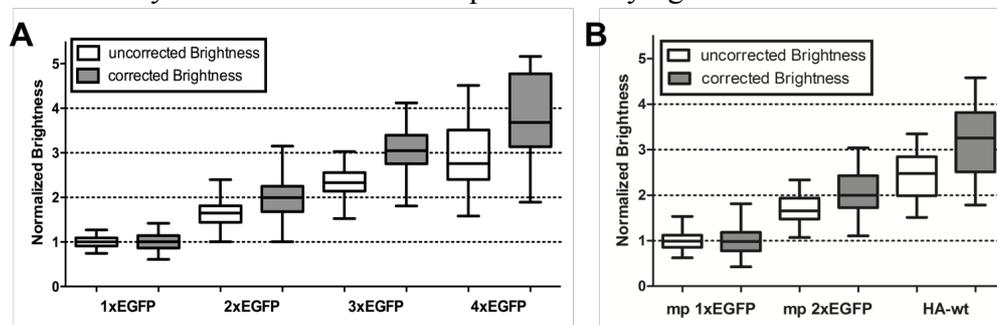


Figure 1: Oligomerization analysis of EGFP-homo-oligomers and Influenza A virus Hemagglutinin (HA). **A:** Normalized brightness of EGFP-homo-oligomers expressed in living A549 cells, measured with FCS. **B:** Normalized brightness of Influenza A virus HA, measured in living HEK 293T cells by scanning FCS. Obtained uncorrected brightness values were corrected for non-fluorescent labels with a fluorescence probability of $p=0.65$, calculated from the measured homo-dimer brightness.