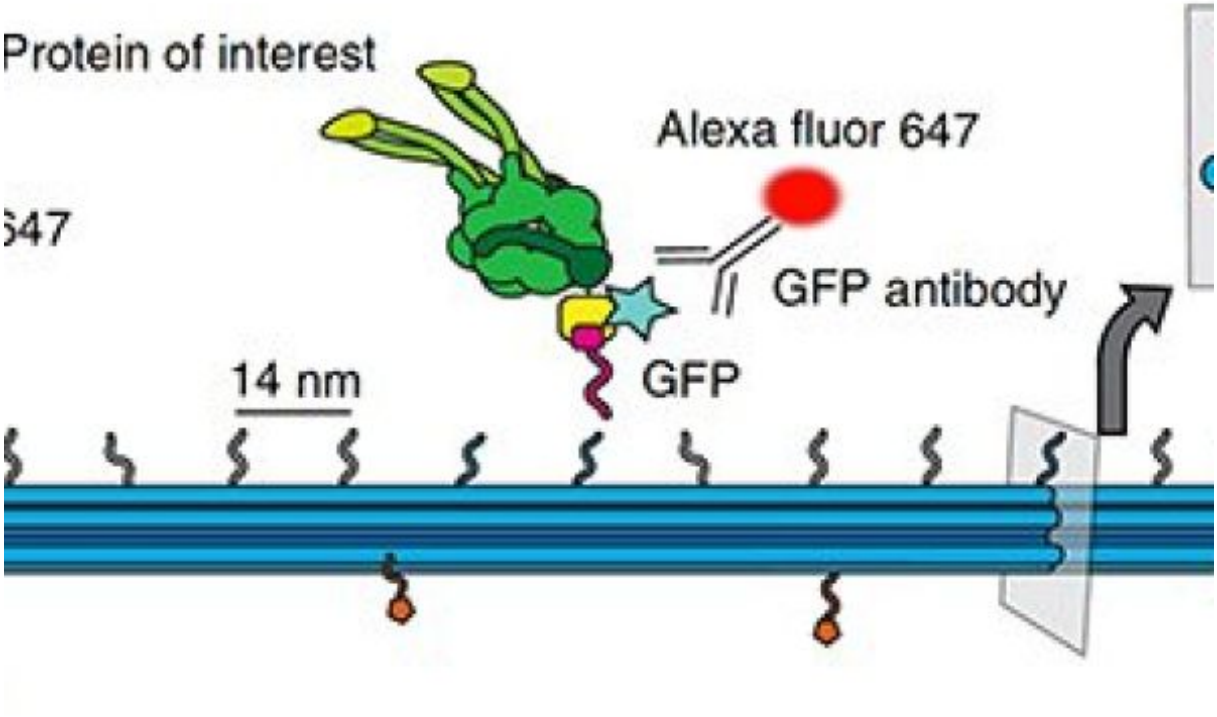

DNA origamis show their versatility for super-resolution microscopy

New methodology based on DNA origami platforms to quantify protein-copy numbers in super-resolution microscopy published in *Nature Methods*.

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Single-molecule-based super-resolution microscopy offers researchers with a unique opportunity to quantify protein copy number with nanoscale resolution. However, tools to truly determine the stoichiometry of complexes observed from super-resolution images have been lacking. Francesca Cella Zanacchi from the Advanced Fluorescence Imaging and Biophysics group led by Prof. Melike Lakadamyali and Carlo Manzo at the Single Molecule Biophotonics group led by ICREA Professor at ICFO Maria Garcia-Parajo have now developed a versatile platform based on DNA origami for calibrating fluorophore and antibody labeling efficiency. The method allows precise quantification of super-resolution images in cellular contexts. The work has been recently published in Nature Methods.

Single-molecule localization microscopy has evolved into an important tool to image biological processes on individual cells with nanometer scale resolution. As the field rapidly progresses, it is becoming increasingly important to extract truly quantitative information from super-resolution images. Unfortunately, determining the accurate stoichiometry of protein complexes and/or quantifying the protein copy number from super-resolution images constitute a major challenge. In general, the unknown stoichiometry of the labeled protein given by the stochasticity of the fluorophore-antibody and antibody-target binding, as well as the complex photophysics of the fluorescent probes, affects dramatically the precision of the final image quantification, jeopardizing the huge potential of super-resolution microscopy.

To break through these limitations, the research team devised a clever and versatile DNA-origami-based calibration method that determines and accounts for labeling stoichiometry and fluorophore photophysics. The DNA origami consisted of a 3D chassis formed by a 12-helix DNA structure. Handles projecting out from the chassis provide site- and sequence-specific attachment points for single fluorophores as well as for proteins of interest and allow testing of several labeling strategies. The researchers validated the entire structure and calibration capability of the DNA origamis under different experimental conditions, including individual fluorophores, and GFP-labeled dynein motors immunostained with primary and secondary antibodies. Importantly, a cluster-based algorithm together with robust data quantification allowed validation of the stoichiometry determination from the DNA origami. Finally, the team tested the method to quantify the copy-number distribution of the nuclear pore complex subunit Nup133 fused to GFP in fully intact cells.

The method reported in this paper can be used as a versatile calibration standard to quantify protein copy number in immunolabeled samples imaged with super-resolution to study a large number of proteins of interest in cellular contexts. Given its versatility, it is also applicable to antibodies against any endogenous protein as well as nanobodies and/or photoactivatable fluorescence proteins. Quantitative super-resolution microscopy is now within reach!