Abstract Submitted
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Determining the Stoichiometry of Protein Complexes using V-Fusion and Single Molecule Imaging MARIA SIRENKO, Department of Biological and Environmental Engineering, Cornell University, Ithaca, NY, AVTAR SINGH, School of Applied and Engineering Physics, Cornell University, Ithaca, NY , ALEXANDER VAN SLYKE, Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY, USA, ALEXANDER SONG, School of Applied and Engineering Physics, Cornell University, Ithaca, NY, PAUL KAMMERMEIER, School of Medicine and Dentistry, University of Rochester Medical Center, Rochester, NY , WARREN ZIPFEL, Department of Biomedical Engineering, Cornell University, Ithaca, NY — The stoichiometry of subunits in protein complexes provides key insight into their molecular mechanisms but is often difficult to determine with existing methods. Although stepwise photobleaching has been used, it is often applied in vitro or cellular expression is intentionally kept low; both of these situations may lead to artifacts. We demonstrate a novel single molecule imaging method using cellular fusion between expressing and non-expressing cells to dilute the concentration of protein complexes without affecting their integrity. After dilution, single protein complexes can be resolved and photobleach step counting provides protein stoichiometric information. We determine the stoichiometry of ADRB2 and EGFR before and after addition of ligand as well as upon exposure to inhibitors.

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