Analysis of Endocytic Uptake and Retrograde Transport to the Trans-Golgi Network Using Functionalized Nanobodies in Cultured Cells

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Transport of proteins and membranes from the cell surface to the Golgi and beyond is essential for homeostasis, organelle identity and physiology. To study retrograde protein traffic, we have recently developed a versatile nanobody-based toolkit to analyze transport from the cell surface to the Golgi complex, either by fixed and live cell imaging, by electron microscopy, or biochemically. We engineered functionalized anti-green fluorescent protein (GFP) nanobodies - small, monomeric, high-affinity protein binders - that can be applied to cell lines expressing membrane proteins of interest with an extracellular GFP moiety. Derivatized nanobodies bound to the GFP reporters are specifically internalized and transported piggyback along the reporters' sorting routes. Nanobodies were functionalized with fluorophores to follow retrograde transport by fluorescence microscopy and live imaging, with ascorbate peroxidase 2 (APEX2) to investigate the ultrastructural localization of reporter-nanobody complexes by electron microscopy, and with tyrosine sulfation (TS) motifs to assess kinetics of trans-Golgi network (TGN) arrival. In this methodological article, we outline the general procedure to bacterially express and purify functionalized nanobodies. We illustrate the powerful use of our tool using the mCherry- and TS-modified nanobodies to analyze endocytic uptake and TGN arrival of cargo proteins.
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