

“TRACKING PROTEINS INSIDE LIVE CELLS USING COORDINATION-BASED FLUORESCENCE APPROACH.”

by

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Abstract

We utilized two fluorescence-based approaches to track intracellular tagged proteins and monitor metal-protein interactions. We have developed coumarin-based fluorescent probe Ni-NTA-AC, which enters cells and traces intracellular His-tagged proteins in minutes, without perturbing the functions of target proteins. Significant fluorescence enhancement (~13-fold) was observed upon binding of Ni-NTA-AC to its protein of interest and formation of covalent linkage through acylazide photoactivation. Ni-NTA-AC is readily applicable to successfully visualize the subcellular localization of His-tagged proteins in various types of cells, including bacterial and mammalian cells and even the plant tissues. The probe could also be used in metalloproteomics. A series of probes with different fluorophores are under development in this laboratory. Ni-NTA system has revolutionized protein purification and immobilized metal affinity-chromatography subsequently has widely been used in proteomics for identification of protein phosphorylation. The new fluorescent probe represents another breakthrough of Ni-NTA system to visualize proteins directly in cells.

We also constructed two fluorescent sensors *CYHpnI* and *CYHpnI_1-48* (with C-terminus glutamine-rich sequence deleted) to elucidate the role of Hpn-like by FRET. Our FRET analysis confirmed the role of HpnI for Ni(II) storage and revealed the potential association of HpnI with Bi-based antiulcer drugs in cells. The potential of coordination-inspired fluorescence technique in biology and medicine will be discussed.

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All are welcome