Selective citrate-based fluorophore generation on N-terminal cysteine in proteins for bioimaging and histology

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Fluorescence detection has become widely used tools in various fields such as biochemistry. Since it has superior selectivity and sensitivity to absorbance detection. In biochemistry, fluorescence technique can be used as microarrays, fluorescence spectroscopy and fluorescence imaging. Especially, fluorescence microscopy has become promising technique for monitoring various cell organelles and tissues to the fore. To visualize the location of specific molecules in the cells, fluorescent labeling is usually accomplished by immunofluorescent staining technique. However, in this technique, undesired fluorescence can be emitted from the sample, when a fluorophore containing antigen improperly fixates, usually called non-specific binding, causing a loss in the probe's specificity. Blocking step using BSA containing buffer is the one of typical methods for minimizing the non-specific binding. Such efforts are time-consuming and sophisticated. We tried to develop the staining technique which can minimize the non-specific binding by simple and prompt manner. As a proof of concept, we investigated the De Novo formation of blue fluorophore (TPA), from citric acid and cysteine. Use of an amide coupling reagent (PyBOP) greatly facilitates the fluorophore formation on peptides and proteins with N-terminal cysteine.
Through series of experiments using amino acids, peptides, and proteins, we confirmed that only Cys with free amine and thiol groups can produce the blue fluorescence in the condition. Moreover, intracellular or extracellular peptides and proteins with N-terminal Cys were fluorescently labelled in a spatially-specific manner by the DNFC method. The DNFC method, which exhibits characteristic distributions of fluorescently labelled proteins in biosamples under various conditions. The DNFC staining is able to provide a new potential protocol for future cell imaging, histology and diagnosis. Moreover, it can provide information of N-terminal cysteine containing proteome based on N-degron rule.

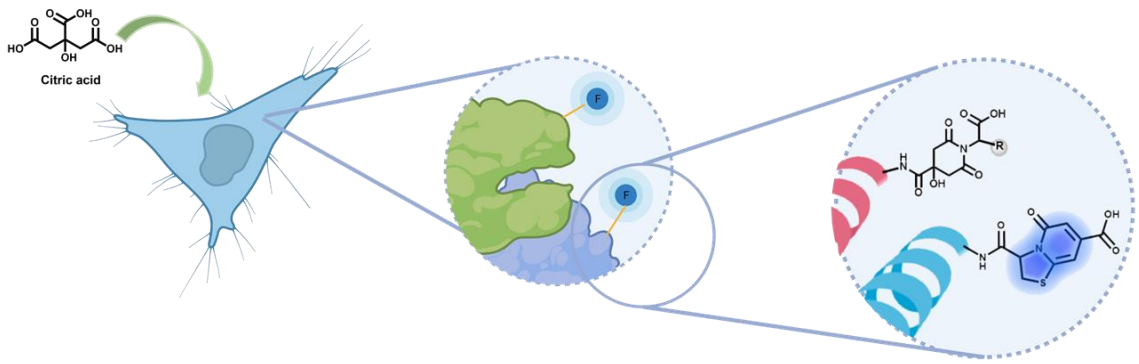

Figure 1. Scheme of *De novo* formation of citrate-based fluorophores on N-terminal proteins.